Endocrinology
Series Editor: Andrea Lenzi
Series Co-Editor: Emmanuele A. Jannini

SPRINGER
REFERENCE

Enzo Bonora
Ralph A. DeFronzo *Editors*

Diabetes
Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment

Springer

Endocrinology

Series Editor
Andrea Lenzi
Department of Experimental Medicine
Section of Medical Pathophysiology
Food Science and Endocrinology
Sapienza University of Rome
Rome, Italy

Series Co-Editor
Emmanuele A. Jannini
Department of Systems Medicine
University of Rome Tor Vergata
Rome, Italy

Within the health sciences, Endocrinology has an unique and pivotal role. This old, but continuously new science is the study of the various hormones and their actions and disorders in the body. The matter of Endocrinology are the glands, i.e., the organs that produce hormones, active on the metabolism, reproduction, food absorption and utilization, growth and development, behavior control, and several other complex functions of the organisms. Since hormones interact, affect, regulate, and control virtually all body functions, Endocrinology not only is a very complex science, multidisciplinary in nature, but is one with the highest scientific turnover. Knowledge in the Endocrinological sciences is continuously changing and growing. In fact, the field of Endocrinology and Metabolism is one where the highest number of scientific publications continuously flourishes. The number of scientific journals dealing with hormones and the regulation of body chemistry is dramatically high. Furthermore, Endocrinology is directly related to genetics, neurology, immunology, rheumatology, gastroenterology, nephrology, orthopedics, cardiology, oncology, gland surgery, psychology, psychiatry, internal medicine, and basic sciences. All these fields are interested in updates in Endocrinology. The aim of the MRW in Endocrinology is to update the Endocrinological matter using the knowledge of the best experts in each section of Endocrinology: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreas with diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenals and endocrine hypertension, sexuality, reproduction, and behavior.

More information about this series at http://www.springer.com/series/14021

Enzo Bonora • Ralph A. DeFronzo
Editors

Diabetes

Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment

With 74 Figures and 38 Tables

Springer

Editors
Enzo Bonora
Division of Endocrinology, Diabetes and Metabolism
University and Hospital Trust of Verona
Verona, Italy

Ralph A. DeFronzo
Diabetes Division, Diabetes Research Unit
University of Texas Health Science Center
San Antonio, TX, USA

ISSN 2510-1927 ISSN 2510-1935 (electronic)
ISBN 978-3-319-45014-8 ISBN 978-3-319-45015-5 (eBook)
ISBN 978-3-319-73218-3 (print and electronic bundle)
https://doi.org/10.1007/978-3-319-45015-5

Library of Congress Control Number: 2018945915

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Preface

Is there an unmet need for a new MRW series in Endocrinology and Metabolism? It might not seem so! The vast number of existing textbooks, monographs and scientific journals suggest that the field of hormones (from genetic, molecular, biochemical and translational to physiological, behavioral, and clinical aspects) is one of the largest in biomedicine, producing a simply huge scientific output. However, we are sure that this new Series will be of interest for scientists, academics, students, physicians and specialists alike.

The knowledge in Endocrinology and Metabolism almost limited to the two main (from an epidemiological perspective) diseases, namely hypo/hyperthyroidism and diabetes mellitus, now seems outdated and closer to the interests of the general practitioner than to those of the specialist. This has led to endocrinology and metabolism being increasingly considered as a subsection of internal medicine rather than an autonomous specialization. But endocrinology is much more than this.

We are proposing this series as the *manifesto* for "**Endocrinology 2.0**", embracing the fields of medicine in which hormones play a major part but which, for various historical and cultural reasons, have thus far been "ignored" by endocrinologists. Hence, this MRW comprises "traditional" (but no less important or investigated) topics: from the molecular actions of hormones to the pathophysiology and management of pituitary, thyroid, adrenal, pancreatic and gonadal diseases, as well as less common arguments. Endocrinology 2.0 is, in fact, the science of hormones, but it is also the medicine of sexuality and reproduction, the medicine of gender differences and the medicine of wellbeing. These aspects of Endocrinology have to date been considered of little interest, as they are young and relatively unexplored sciences. But this is no longer the case. The large scientific production in these fields coupled with the impressive social interest of patients in these topics is stimulating a new and fascinating challenge for Endocrinology.

The aim of the **MRW in Endocrinology** is thus to update the subject with the knowledge of the best experts in each field: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreatic disorders, diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenal and endocrine hypertension, sexuality, reproduction and behavior. We are sure that this ambitious aim,

covering for the first time the whole spectrum of Endocrinology 2.0, will be fulfilled in this vast Springer MRW in Endocrinology Series

Andrea Lenzi, M.D.
Series Editor
Emmanuele A. Jannini, M.D.
Series Co-Editor

Volume Preface

The incidence of both type 1 and type 2 diabetes mellitus has increased dramatically over the last two decades. While it is clear that obesity is driving the epidemic of T2DM, the factors responsible for the increase in T1DM remain unclear. Despite major advances in our understanding of the pathophysiology of both T2DM and T1DM and the addition of many new therapeutic classes, over 50% of diabetic patients fail to achieve appropriate glycemic control (A1c $>$ 7.0%). Not surprisingly, the incidence of microvascular complications has failed to decrease significantly and, while therapeutic advances have reduced the incidence of macrovascular complications, diabetic patients still remain at twofold greater risk than nondiabetic patients for an adverse cardiovascular event. The treatment of diabetes and its associated micro- and macrovascular complications takes a heavy toll on the individual and places a heavy economic burden on society. In the current volume of the Springer Diabetes Textbook, world-renowned clinicians and scientists review the pathophysiology of diabetes, both type 1 and type 2, and obesity and their multiple associated clinical manifestations and provide timely updates about the most recent advances in diabetes therapy. The information provided herein will allow clinicians and investigators alike to advance to the frontiers of biomedical investigation and identify therapeutic milestones in the field of diabetes and related metabolic disorders.

Verona, Italy
San Antonio, TX, USA

Enzo Bonora
Ralph A. DeFronzo
Editors

Contents

About the Editors

Enzo Bonora M.D, Ph.D., Board Certified in Internal Medicine, Endocrinology, and Diabetes and Metabolic Diseases.

Full Professor of Endocrinology, University of Verona, Verona, Italy.

Director, School of Specialization in Endocrinology and Metabolic Diseases, University of Verona, Verona, Italy.

Coordinator, Doctorate in Clinical and Experimental Medical Sciences, University of Verona, Verona, Italy.

Coordinator, Integrated Course of Systematic Pathology II, School of Medicine, University of Verona, Verona, Italy.

Head, Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Verona, Verona, Italy.

Chief, Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University Hospital of Verona, Verona, Italy.

Scientific activities mainly in the fields of: (a) epidemiology of diabetes and related conditions; (b) risk factors of type 2 diabetes and its chronic complications; (c) pathophysiology of type 2 diabetes (impaired insulin secretion and insulin resistance) and related conditions (e.g., metabolic syndrome); (d) obesity and fat distribution in humans and their metabolic and cardiovascular impact; (e) therapy of diabetes and related conditions.

Author or coauthor of about 300 full-length papers in peer-reviewed journals (Impact factor: about 2,500; Citations: about 15,000; H-index: about 60), and further 150 papers, reviews, and chapters of books.

More than 500 invited lectures and seminars in international and national congresses and meetings as well as in public and private institutions.

"Outstanding Scientific Achievement Award" from the Italian Diabetes Society in 1992.

"Michaela Modan Memorial Award" from the American Diabetes Association in 1997.

President, Italian Diabetes Society 2014–2016. President Diabetes Research Foundation 2016–2018.

Ralph A. DeFronzo, M.D., is Professor of Medicine and Chief of the Diabetes Division at the University of Texas Health Science Center and the Deputy Director of the Texas Diabetes Institute, San Antonio, Texas. Dr. DeFronzo is a graduate of Yale University (BS) and Harvard Medical School (MD) and did his training in Internal Medicine at the Johns Hopkins Hospital. He completed fellowships in Endocrinology at the National Institutes of Health and Baltimore City Hospitals and in Nephrology at the Hospital of the University of Pennsylvania. Subsequently, he joined the faculty at the Yale University School of Medicine (1975–1988) as an Assistant/Associate Professor. From 1988 to present Dr. DeFronzo has been Professor of Medicine and Chief of the Diabetes Division at the University of Texas Health Science Center at San Antonio. He also serves as the Deputy Director of the Texas Diabetes Institute.

His major interests focus on the pathogenesis and treatment of type 2 diabetes mellitus and the central role of insulin resistance in the metabolic-cardiovascular cluster of disorders known collectively as the Insulin Resistance Syndrome. Using the euglycemic insulin clamp technique in combination with radioisotope turnover methodology, limb catheterization, indirect calorimetry, and muscle biopsy, he has helped to define the biochemical and molecular disturbances responsible for insulin resistance in type 2 diabetes mellitus.

For his work in this area, Dr. DeFronzo received the prestigious Lilly Award (1987) by the American Diabetes Association (ADA), the Banting Lectureship (1988) by the Canadian Diabetes Association, the Novartis Award (2003) for outstanding clinical investigation world wide, and many other national and international awards. He also is the recipient of the ADA's Albert Renold Award (2002) for lifetime commitment to the

training of young diabetes investigators. Dr. DeFronzo received the Banting Award from the ADA (2008) and the Claude Bernard Award from the EASD (2008). These represent the highest scientific achievement awards given by the American and European Diabetes Associations, respectively. In 2008, Dr. DeFronzo also received the Italian Diabetes Mentor Prize and the Philip Bondy Lecture at Yale. In 2009, he received the Presidential Award for Distinguished Scientific Achievement from the University of Texas Health Science Center at San Antonio. Dr. DeFronzo received the Outstanding Clinical Investigator Worldwide Award by CODHy (2012), the Outstanding Scientific Achievement Award from the American College of Nutrition (2014), the Samuel Eichold II Memorial Award for Contributions in Diabetes from the American College of Physicians (2015), the George Cahill Memorial Lecture from the University of Montreal (2015), and the Priscilla White Memorial Lecture from the Joslin Clinic & Brigham and Women's Hospital (2015). Most recently (2017), Dr. DeFronzo received the Hamm International Prize for his many seminal observations on the pathogenesis and treatment of type 2 diabetes and the Distinction in Endocrinology Award from the American College of Endocrinology. With more than 800 articles published in peer-reviewed medical journals, Dr. DeFronzo is a distinguished clinician, teacher, and investigator who has been an invited speaker at major national and international conferences on diabetes mellitus.

Contributors

Emma Ahlqvist Department of Clinical Sciences, Diabetes and Endocrinology Unit, Lund University Diabetes Centre, Malmo, Sweden

Robert M. Anderson Department of Learning Health Sciences, The University of Michigan Medical School, Ann Arbor, MI, USA

Cristina Bianchi Department of Clinical and Experimental Medicine, Section of Metabolic Diseases and Diabetes, University of Pisa, Pisa, Italy

Riccardo C. Bonadonna Department of Medicine and Surgery, University of Parma, Parma, Italy

Division of Endocrinology and Metabolic Diseases, Azienda Ospedaliera Universitaria of Parma, Parma, Italy

Linda Boselli Department of Medicine, University of Verona, Verona, Italy

Raffaella Buzzetti Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy

Avivit Cahn Diabetes Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel

Endocrinology and Metabolism Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel

Simona Cernea Department M3/Internal Medicine IV, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

Diabetes, Nutrition and Metabolic Diseases Outpatient Unit, Emergency County Clinical Hospital, Tîrgu Mureş, Romania

Roberto Codella Department of Biomedical Sciences for Health, IRCCS Policlinico San Donato Milanese, Università degli Studi di Milano, Milan, Italy

Metabolism Research Center, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy

Stephen Colagiuri The Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, Level 2 Charles Perkins Centre D17, University of Sydney, Sydney, NSW, Australia

Agostino Consoli Dipartimento di Medicina e Scienze dell'Invecchiamento e, Centro Scienze dell'Invecchiamento e Medicina Traslazionale, Università d'Annunzio di Chieti-Pescara, Chieti Scalo, Italy

Giuseppe Daniele Department of Clinical and Experimental Medicine, Section of Metabolic Diseases and Diabetes, University of Pisa, Pisa, Italy

Angela Dardano Department of Clinical and Experimental Medicine, Section of Metabolic Diseases and Diabetes, University of Pisa, Pisa, Italy

Richard David Leslie Centre of Diabetes, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Melanie J. Davies Diabetes Research Centre, University of Leicester, Leicester, UK

Ralph A. DeFronzo Diabetes Division, Diabetes Research Unit, University of Texas Health Science Center, San Antonio, TX, USA

Alessandra Dei Cas Department of Medicine and Surgery, University of Parma, Parma, Italy

Division of Endocrinology and Metabolic Diseases, Azienda Ospedaliera Universitaria of Parma, Parma, Italy

Andrew Farmer Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK

Ele Ferrannini CNR Institute of Clinical Physiology, Pisa, Italy

Department of Internal Medicine, University of Pisa, Pisa, Italy

Martha M. Funnell Department of Learning Health Sciences, The University of Michigan Medical School, Ann Arbor, MI, USA

Rosalba Giacco Institute of Food Science of National Research Council, Avellino, Italy

Laura J. Gray Department of Health Sciences, University of Leicester, Leicester, UK

Leif Groop Department of Clinical Sciences, Diabetes and Endocrinology Unit, Lund University Diabetes Centre, Malmo, Sweden

Finnish Institute of Molecular Medicine, Helsinki University, Helsinki, Finland

Chiara Guglielmi Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio Medico, Rome, Italy

Jens J. Holst The NNF Center for Basic Metabolic Research and the Department of Medical Sciences, Panum Instituttet, University of Copenhagen, Copenhagen, Denmark

Kamlesh Khunti Diabetes Research Centre, University of Leicester, Leicester, UK

William C. Knowler Diabetes Epidemiology and Clinical Research Section, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, AZ, USA

Crystal Man Ying Lee The Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, Level 2 Charles Perkins Centre D17, University of Sydney, Sydney, NSW, Australia

School of Public Health, Curtin University, Perth, WA, Australia

Sylvia H. Ley Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Livio Luzi Department of Biomedical Sciences for Health, IRCCS Policlinico San Donato Milanese, Università degli Studi di Milano, Milan, Italy

Metabolism Research Center, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy

Sten Madsbad Department of Endocrinology, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark

The NNF Center for Basic Metabolic Research and the Department of Medical Sciences, Panum Instituttet, University of Copenhagen, Copenhagen, Denmark

Paola Maffi Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

Clinical Transplant Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

James B. Meigs Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Rita Nano Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

Katharine R. Owen Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK

Kingshuk Pal Research Department of Primary Care and Population Health, University College London, London, UK

Gretchen A. Piatt Department of Learning Health Sciences, The University of Michigan Medical School, Ann Arbor, MI, USA

John C. Pickup Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Guy's Hospital, London, UK

Lorenzo Piemonti Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

Internal Medicine and Transplant Unit, Vita-Salute San Raffaele University, Milan, Italy

Paolo Pozzilli Department of Endocrinology and Diabetes, University Campus Bio-Medico, Rome, Italy

Centre of Diabetes, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio Medico, Rome, Italy

Rashmi B. Prasad Department of Clinical Sciences, Diabetes and Endocrinology Unit, Lund University Diabetes Centre, Malmo, Sweden

Stefano Del Prato Department of Clinical and Experimental Medicine, Section of Metabolic Diseases and Diabetes, University of Pisa, Pisa, Italy

Alberto Pugliese Department of Medicine, Division of Diabetes, Endocrinology and Metabolism, Department of Microbiology and Immunology, Diabetes Research Institute, Leonard Miller School of Medicine, University of Miami, Miami, FL, USA

Itamar Raz Diabetes Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel

Gabriele Riccardi Department of Clinical Medicine and Surgery, Università degli Studi di Napoli "Federico II", Naples, Italy

Antonio Secchi Internal Medicine and Transplant Unit, Milan, Italy

Internal Medicine and Transplant Unit, Vita-Salute San Raffaele University, Milan, Italy

Marta Seghieri Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Jay S. Skyler Diabetes Research Institute, University of Miami, Miami, FL, USA

Carlo Socci Unit of Transplant Surgery, IRCCS San Raffaele Scientific Institute, Milan, Italy

Ileana Terruzzi Diabetes Research Institute, Metabolism, Nutrigenomics and Cellular Differentiation Unit, San Raffaele Scientific Institute, Milan, Italy

Maddalena Trombetta Department of Medicine, University of Verona, Verona, Italy

Division of Endocrinology, Diabetes and Metabolic Diseases, Azienda Ospedaliero-Universitaria Integrata of Verona, Verona, Italy

Marilena Vitale Department of Clinical Medicine and Surgery, Università degli Studi di Napoli "Federico II", Naples, Italy

David Webb Diabetes Research Centre, University of Leicester, Leicester, UK

Andrew Willis Diabetes Research Centre, University of Leicester, Leicester, UK

Simona Zampetti Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy

Overview of Glucose Homeostasis

1

Ele Ferrannini and Marta Seghieri

Contents

Abstract

During fasting conditions, glucose metabolism is maintained through a fine balance between endogenous glucose production from the liver (80%) and kidney (20%) and glucose utilization by body tissues. After the ingestion of a meal, the rise in plasma glucose and insulin, together to gut factors, combine to suppress endogenous glucose production and stimulate glucose uptake in adipose tissue

E. Ferrannini (✉)
CNR Institute of Clinical Physiology, Pisa, Italy

Department of Internal Medicine, University of Pisa, Pisa, Italy
e-mail: ferranni@ifc.cnr.it

M. Seghieri
Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_1

and muscle. The liver (hepatic glucose production) is more sensitive to the inhibitory action of insulin than are peripheral tissues (glucose uptake) to the stimulatory action of insulin. Glucose metabolism is dependent upon the coordinate activation of the insulin signal transduction system, glucose transport/phosphorylation and oxidation by the pyruvate dehydrogenase complex and the mitochondrial chain. Insulin action on glucose metabolism is both direct (stimulation of glucose transport, glycolysis, and glycogen synthesis) and indirect (inhibition of lipolysis, lipid oxidation, and protein degradation). In insulin-sensitive tissues, the three major substrates (glucose, FFAs, amino acids) are in competition with one another. Glucagon plays a role in the tonic support of hepatic glucose production and is also the leading counterregulatory mechanism activated in the defense against hypoglycemia. The amplification of insulin secretion during an oral test is attributed to the nutrient-stimulated release of incretin hormones and their physiological actions, including potentiation of glucose-induced insulin secretion, suppression of glucagon release, inhibition of gastric emptying, and enhancement of satiety. GLP-1 directly, and indirectly by increasing insulin and inhibiting glucagon, augments hepatic glucose uptake and inhibits hepatic glucose production.

Keywords
Plasma glucose · Insulin · Endogenous glucose production · Glucose disposal · Insulin sensitivity and secretion · Incretin effect · Counterregulatory system

Glucose Distribution

Glucose is one of the most abundant biological molecules, representing the main fuel for most cells. It is also a structural component of living systems, to a lesser extent in animals than in plants. As a metabolic substrate, glucose is present in organisms in its simple monomeric form, α-D-glucopyranose, and as a branched polymer of α-glucose, namely, glycogen. Glucose is also a component of dysaccharides, which are quantitatively less important and include lactose, maltose, and sucrose. In overnight fasted individuals, plasma glucose concentration ranges between 3.6 and 5.5 mmol/L (65–99 mg/dL) and increases up to 8.9–10 mmol/L (160–180 mg/dL) in the fed state.

At the whole-body level, the first step in glucose metabolism is its uptake into tissues and organs. This step is effected by glucose transporters (GLUT, encoded by *SCL2A*), which are transmembrane proteins. Fourteen GLUT proteins are expressed in humans, and they include transporters for substrates other than glucose, such as fructose, myoinositol, and urate. The well-established glucose transporter isoforms, GLUTs 1–4, are known to have specific regulatory and/or kinetic properties that reflect their roles in cellular systems, thus regulating whole-body glucose homeostasis (Thorens and Mueckler 2010). These transporters exert their action by coupling with hexokinase (HK) enzymes, which catalyze the first intracellular step, i.e., phosphorylation of glucose to glucose-6-phoshate (G6P) (Colowick 1973; Rogers et al. 1975).

GLUT1 is a non-insulin-dependent transporter, facilitating diffusion of glucose from plasma water into red blood cells (RBC). It has a low K_m (~1 mmol/L) and is found in association with HKI. Because of the high density of GLUT1 in RBCs' membrane and the low rate of intracellular glucose utilization via glycolysis (~17,000 times slower than the rate of glucose transport), circulating plasma glucose is in rapid equilibration with intraerythrocyte concentration (Carruthers et al. 2009). Plasma proteins represent ~8% of plasma volume, whereas RBC proteins and ghosts occupy ~38% of the packed RBC volume (which, in turn, averages 40% of the total blood volume). Therefore, 20% (i.e., $0.38 \cdot 0.4 + 0.08 \cdot 0.6 = 0.2$) of the total blood volume is inaccessible to glucose. It follows that glucose concentration should be identical in plasma and RBC water under most circumstances and that a blood water glucose concentration of 5.0 mmol/L (90 mg/dL) translates into a plasma glucose concentration of 4.6 mmol/L (83 mg/dL) and a whole-blood glucose concentration of 4.0 mmol/L (72 mg/dL), that is, a 15% systematic difference between plasma and whole-blood glucose concentration under typical conditions of hematocrit, proteinemia, and erythrocyte volume.

Insulin-independent glucose transport, as occurs in the brain and placenta, is mediated by another non-insulin-dependent transporter (GLUT3) together with GLUT1. In these regions, glucose influx must be ensured independently of metabolic conditions (Haber et al. 1993). Another non-insulin-sensitive transporter, GLUT2, is widely expressed in pancreatic β-cells, in the basolateral membranes of intestinal and kidney epithelial cells and of hepatocytes. It has a uniquely high K_m for glucose (15–20 mM), resulting in a rapid equilibration of glucose between the extracellular space and the cell cytosol at all physiological or diabetes-associated concentrations. In these cells, glucose phosphorylation is promoted by HKIV, also referred to as glucokinase. In pancreatic β-cells, GLUT2 works as a glucose sensor, triggering insulin secretion in close phase with rises in blood glucose concentrations; in hepatocytes GLUT2 induces the expression of glycolytic and lipogenic genes (Mueckler and Thorens 2013). Studies in knockout mice show that GLUT2 is also required for glucose sensing in the hepatoportal vein area as well as in the central nervous system. In addition, these sensors appear to also play a role in glucagon and insulin secretion, peripheral tissue glucose uptake, and feeding behavior (Burcelin and Thorens 2001).

Glucose transport in insulin-dependent tissues (skeletal muscle and adipocytes) depends on GLUT4 (James et al. 1988) as disruption of its regulation results in prevalent insulin-resistant conditions, such as obesity and type 2 diabetes mellitus (T2DM). Unlike most other GLUT isoforms, in non-stimulated muscle and adipose cells, GLUT4 is largely excluded from the plasma membrane and is predominantly retained within specialized intracellular membrane compartments, GLUT4-containing vesicles. According to the translocation paradigm, following insulin stimulation, these vesicles translocate to the plasma membrane and fuse with it along a multistep pathway involving numerous docking and fusion proteins (Larance et al. 2008). GLUT4 has a K_m of ~5 mmol/L, which is close to fasting plasma glucose concentrations and is associated with HKII (Printz et al. 1993). The cellular regulation of GLUT4 seems to be basically similar in muscle and adipose tissue,

with a few differences. For example, acute physiologic hyperinsulinemia does not increase the total number of GLUT4 in the muscle, even though several studies have demonstrated an increase in GLUT4 mRNA. Furthermore, the intracellular signaling pathway mediated via the adaptor protein APS (adaptor protein with pleckstrin homology and Src homology domains) plays an important role in GLUT4 exocytosis in adipose tissue, whereas a role for the insulin-stimulated APS pathways has yet to be defined in myocytes (Govers 2014).

Apart from insulin-dependent tissues, where glucose uptake is gated by hormone stimulation and where under resting conditions glucose transport is limited, glucose distribution in blood water, interstitial fluid, and intracellular water compartment of insulin-independent body regions (liver, brain, kidney, intestine, placenta) has the following quantitative characteristics. First, glucose is distributed in body water, reaching a total amount of 80 mmol (14 g or 1.2 mmol/kg of body weight), of which one-fifth is in the blood volume. Second, glucose concentrations decrease from the intravascular water compartment through the interstitial space (both radially and axially) to a higher extent in those organs that consume glucose avidly, such as the brain; this gradient may be reversed in tissues where glucose is also produced, such as hepatocytes and renal tubular cells. As a result, in the vascular bed glucose levels gradually decrease as arterial blood turns into capillary blood and then runs back toward the right heart as venous blood. In addition, the regional distribution of glucose across organs depends on the A-V glucose gradient, which is the result of the specific tissue composition, blood flow rate, and capillary density (i.e., the average distance between the capillary axis and the cell surface).

With regard to intracellular distribution, glucose is largely stored as glycogen in cytoplasmic granules of hepatic and skeletal muscle cells. The liver contains 3–4 g of glycogen in each 100 g of parenchyma, whereas in striated muscle glycogen concentration is much lower (0.7–1.0% weight by weight). The resulting total is ~60 g glycogen in the normal liver (1.5 kg) and 250 g in skeletal muscle (28 kg). Thus, glycogen stores 25 times the amount of glucose that is dissolved as free glucose in body water.

Intracellular Pathways of Glucose Metabolism

Insulin Receptor Signaling

At the cellular level, the effect of insulin on glucose metabolism is mediated by the activation of specific receptors, which are present on the cell membrane of all insulin-sensitive tissues. After insulin has bound to and activated its receptor, second messengers are produced, which initiate a cascade of phosphorylation-dephosphorylation reactions that eventually result in the stimulation of glucose transport and of metabolic pathways (glycolysis, glucose oxidation, and glycogen synthesis) (Saltiel and Kahn 2001; Siddle 2011). More than alterations in insulin receptor affinity or number, a variety of post-binding defects, contribute to resistance to insulin action (Del Prato et al. 1993).

Insulin activates the insulin receptor tyrosine kinase (IR), a glycoprotein consisting of two α subunits and two ß subunits linked by disulfide bonds. The intracellular domains of ß subunits express insulin-stimulated kinase activity directed toward its own tyrosine residues. These phosphotyrosine docking sites are substrate for various enzymes and protein adapters, which include the insulin receptor substrates (IRS) 1, 2, 3, and 4 and the Shc proto-oncogene product. Tyrosine phosphorylation of IRS by IR generates binding sites for the regulatory subunits of class 1A phosphatidylinositol-3 kinase (PI3K), which recruits Akt kinase. Several altered patterns of IRS tyrosine residue phosphorylation have been associated with the emergence of insulin resistance and diabetes (Copps and White 2012). Both IRS-1 and IRS-2 but not Shc are involved in the PI3K/Akt signaling pathway affecting glucose uptake and metabolism; conversely, Shc binding directly to IR or indirectly via IRS-1 or IRS-2 initiates the Ras/MAP kinase pathway involved in gene expression and cell growth (Whitehead et al. 2000). Activated PI3K/Akt signaling induces protein synthesis via mTOR, fatty acid, and cholesterol synthesis via liver X receptor (LXR) and sterol response element regulators; cell survival is promoted by the inhibition of several pro-apoptotic agents (Bad, FoxO transcription factors, GSK-3, and MST1) (Wilson et al. 2007).

Insulin Regulation of Glucose Metabolism

In muscle and adipocytes, insulin stimulates glucose uptake by translocating GLUT4 vesicles to the plasma membrane through the PI3K/Akt pathway. Akt signaling is also involved in glycolysis, by activating the enzymes that catalyze the three rate-limiting steps in this pathway: the phosphorylation of glucose by HK, the phosphorylation of fructose-6-phosphate by phosphofructokinase (PFK), and the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP by pyruvate kinase (Mosca et al. 2012). Furthermore, insulin stimulates glucose oxidation by increasing the activity of the multienzyme complex, pyruvate dehydrogenase (PDH). This enzyme stimulates PDH phosphatase, thus converting the enzyme from its inactive phosphorylated to its active dephosphorylated form. The PDH complex enzyme is also inhibited by its products, acetyl-CoA and reduced nicotinamide adenine dinucleotide (NADH) (Holness and Sugden 2003). In addition to oxidative glucose pathways, insulin promotes glycogenesis. This process depends on insulin activating glycogen synthase, the enzyme that adds glucose units to the growing polysaccharide chain of glycogen, via Akt activation, which in turn phosphorylates GSK-3 and/or PKA thereby preventing them from inactivating glycogen synthase. Insulin increases the levels of activated glycogen synthase also by activating protein phosphatase 1 (PP1). PP1 is a Ser/Thr protein phosphatase which can dephosphorylate glycogen synthase.

Insulin is able to modulate the transcriptional expression of over 100 genes. At this level, the effects of insulin are widespread and involve crucial biological processes. In the liver, the PI3K/Akt pathway of insulin signaling modulates the carbohydrate-responsive element-binding protein (ChREBP) and the transcription factor sterol regulatory element-binding protein-1c (STREBP-1c), which in turn

activate the transcription of most of the genes encoding metabolic enzymes including glycolytic enzymes and lipogenic enzymes (Ferré et al. 2001; Wang et al. 2015). On the other hand, insulin inhibits the transcription of genes that encode mainly enzymes involved in hepatic glucose production (gluconeogenesis). Gluconeogenesis is the process by which glucose is synthesized from 3-carbon precursors such as pyruvate and lactate. Through phosphorylation and translocation of the transcription factor FoxO out of the nucleus, insulin suppresses gluconeogenesis by decreasing the expression of the three rate-limiting enzymes, phosphoenolpyruvate(PEP)-carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase (Carter and Brunet 2007).

Insulin also reduces the production of glucose by glycogenolysis. This process consists in the sequential removal of glucose monomers as glucose-1-phosphate from glycogen through the phosphorylated form of the enzyme glycogen phosphorylase (phosphorylase *a*). The glucose-1-phosphate is then converted to glucose-6-phosphate by the enzyme phosphoglucomutase. The phosphate group of glucose-6-phosphate is removed by G6Pase and the free glucose exits the cell. In fact, the outflow of glucose (derived by glycogenolysis and gluconeogenesis in the liver and only by the latter in the kidney) into the bloodstream takes place along the concentration gradient between internal and external side of the plasma membrane, is mediated by GLUT2 transporters, and is limited by the availability of G6P and of the G6Pase activity inside liver and kidney cells. On the contrary, in muscle cells G6Pase is absent, and the role of glycogen degradation is to provide an immediate source of glucose-6-phosphate for glycolysis to produce energy for muscle contraction.

Insulin suppresses glycogenolysis by activating PP1 and the enzyme phosphodiesterase. PP1 dephosphorylates glycogen phosphorylase *a*, reforming the inactive glycogen phosphorylase *b*, whereas the phosphodiesterase converts cAMP to AMP, thus inactivating PKA and blocking the phosphorylation cascade that would end with formation of active, phosphorylated glycogen phosphorylase *a* (Fong et al. 2000).

Glucose Fluxes: Methodological Approaches

The bulk part of glucose production occurs in the liver (~80%), the kidney contributing to the remaining ~20% (DeFronzo 2009). The most direct approach to measure net glucose balance is given by the product of blood flow and the arteriovenous glucose concentration difference across the organ, according to the Fick principle. For the liver, simultaneous sampling from arterial, portal, and hepatic venous blood is necessary, as tested in animal experimental models (Abumrad et al. 1982). Because in humans the portal vein is generally inaccessible, splanchnic balance may be assessed by measuring arterial and the hepatic venous blood flow as follows:

$$\text{Net glucose balance (netGB)} = (\text{Hepatic Blood flow}) \cdot (\text{A} - \text{V})$$

where A is arterial and V is hepatic venous glucose concentration. It should be recalled that the use of plasma flow rates and plasma glucose concentrations

systematically underestimates the net organ balance of glucose (and, for that matter, of any substance that is transported in plasma, e.g., lactate or some amino acids). As discussed earlier, because plasma flow is less than blood flow by an amount equal to the hematocrit (~40%), whereas plasma glucose is higher than whole-blood glucose by only 15% ($0.6 \cdot 1.15 = 0.69$), the use of plasma instead of blood measures leads to a 31% underestimation of net organ balance.

The use of a glucose tracer is required to separately measure uptake and release of glucose by the liver (or kidney). Radioactive ([^{3}H]) or stable ([^{2}H]) isotopes can be used to label native glucose. By combining the organ balance technique with tracer glucose, one can calculate the uptake of glucose by the liver according to the following equation:

$$\text{Tracer glucose uptake } (\text{GU}^*) \;=\; (\text{Hepatic blood flow}) \;\cdot\; (A^* - V^*)$$

where A^* and V^* represent the glucose specific activity in the artery and hepatic vein, respectively.

From of the central assumption of the kinetic/biochemical equivalence of tracer and tracee, it follows that:

$$\text{GU}^*/\text{GU} = A^*/\text{A or GU} \;=\; \text{GU}^* \;\cdot\; \text{A}/\text{A}^*$$

Therefore:

$$\text{Glucose output} \;=\; \text{netGB} \;-\; \text{GU}$$

The use of a glucose tracer also allows one to measure whole-body glucose (or other substrates) turnover (Ferrannini et al. 1986a). Over the last 10 years, the use of stable isotopes has grown because of several reasons: (i) major awareness about potentially harmful effects on environment and health associated with radioactive isotopes, (ii) reduced costs and increased ease of measurement of highly enriched stable isotope-labeled compounds, and (iii) recognition of the unique metabolic information derived from the use of stable isotope tracers (Coggan 1999).

The tracer can be given as a pulse injection or constant intravenous infusion, depending on the type of information that is required. For metabolic studies, a primed continuous infusion usually is employed. When a steady state is achieved – i.e., negligible time-related changes in measured concentrations or fluxes – glucose turnover rate (milligrams per minute) is simply calculated by dividing the tracer infusion rate by the equilibrium plasma glucose-specific activity. When the steady state is perturbed, e.g., following ingestion or infusion of glucose, one of two strategies can be employed. Either the tracer administration is repeated when the glucose system has reached a new, reasonably steady state, or tracer glucose infusion rates can be adjusted empirically to "clamp" the plasma glucose-specific activity constant close to the basal level. In both cases, the aim is to minimize the changes in glucose-specific activity, thereby meeting the conditions under which steady-state equations can be used reliably.

The Basal (Postabsorptive) State

Glucose Production

Basal state refers to the period – prevailing in the morning – after an overnight (10–14 h) fast, in which glucose metabolism is maintained through a fine balance between endogenous glucose release and glucose utilization by body tissues. The true value of basal endogenous (liver plus kidney) glucose production is the one that would be reproducibly measured with the use of an irreversible glucose tracer, which loses its label at the earliest possible intracellular step without ever getting it reincorporated into a circulating tracer molecule. Thus, if a glucose tracer reappears as part of "futile" metabolic cycle, estimates in glucose production are inaccurate. In this sense, [6,6-^{2}H]glucose has been generally considered the tracer of choice, because it appears very unlikely that both ^{2}H will recycle back into the C-6 position of glucose following glycolysis and subsequent gluconeogenesis (Wajngot et al. 1989).

In the fasting state, glucose output in healthy adults averages ~840 μmol/min (or ~12 μmol/min per kg of body weight) (Abdul-Ghani and DeFronzo 2010). In healthy subjects, endogenous glucose output shows a large variability which mostly reflects the amount of lean mass; the latter fully accounts for the differences due to sex, obesity, and age. Glycogenolysis and liver/renal gluconeogenesis contribute to glucose production, gluconeogenesis becoming increasingly prevalent as fasting proceeds. Following a 68-hour fast, gluconeogenesis represented around 64% of total glucose production during the first 22 h of fasting, 82% during the next 14 h, and 96% during the next 18 h. Insulin inhibits both glycogenolysis and gluconeogenesis, although with different dose-response characteristics, gluconeogenesis being less sensitive (Rothman et al. 1991). From a biological point of view, glycogen storage is limited as excessive cellular accumulation may impair vital organ functions (e.g., storage diseases).

Fasting glucose production and its contribution to fasting hyperglycemia in T2DM have been well characterized. Whereas in nondiabetic subjects glycemia is stable during a short-term fast, in diabetic patients glucose levels spontaneously rise through the night peaking early in the morning, being anticipated by similar changes in glucose production. The percent contribution of gluconeogenesis to glucose release after an overnight fast is independently and quantitatively related to the degree of overweight (by ~1% per body mass index unit) and the severity of fasting hyperglycemia (by ~3% per mmol/L above the normal range) (Gastaldelli et al. 2000). Furthermore, while in nondiabetic obese individuals reduced glycogenolysis ensures a normal rate of glucose output, in diabetic patients the rate of glucose output derived from glycogenolysis is also inappropriately elevated. Regarding the substrate supply for gluconeogenesis, the bulk of precursor comes from circulating amino acids, lactate, pyruvate, and glycerol; nevertheless measurements of the net uptake by transcatheterization of the splanchnic area showed that glucose production derived from circulating precursors accounts for less than a half of the total (Landau 1993). Other sources include precursors (alanine and pyruvate) released from the gut

(as detected in arterial and portal vein samples) and substrates derived from intrahepatic lipolysis, proteolysis, and glycolysis. In general, any given rate of glucose output is the net result of the inhibitory actions of insulin, hyperglycemia, parasympathetic nervous activity, and substrate shortage on the one hand and the stimulatory actions of counterregulatory hormones, hypoglycemia, sympathetic nervous activity, and gluconeogenic substrate load on the other. Among substrates, FFA have an added regulatory value for glucose output.

In the fasting state, insulin reduces hepatic glucose production by acting both directly and indirectly on the liver. The most common in vivo model to evaluate which effect of insulin prevails comes from tracer studies, in which a pancreatic clamp (somatostatin plus basal insulin and glucagon infusions) is used to control endocrine pancreas. Under these experimental conditions, when a selective increase in either peripheral or portal vein insulin was induced, it was shown that a similar rise in insulin concentration was associated with a fall in glucose output, to indicate that both hepatic and extrahepatic effect might play a role even if with different kinetics and extents (Sindelar et al. 1996). In its capacity as the inhibitory signal for glucose release, insulin is greatly favored by the anatomical connection between the pancreas and the liver, as secreted insulin reaches the liver at a concentration that in fasting humans is three to fourfold higher than the peripheral (arterial) concentration (Ferrannini and Cobelli 1987). Such portosystemic gradient is maintained by a high rate of insulin degradation by hepatic tissues (with a fractional extraction of about 50%). Thus, a small secretory stimulus to the β-cell primarily serves to increase portal insulin levels, thereby selectively acting upon glucose production rather than also enhancing peripheral glucose utilization. In addition to short-circuiting the systemic circulation, pancreatic insulin release is potentiated by several gastrointestinal hormones (e.g., glucose-dependent insulinotropic polypeptide [gastric inhibitory polypeptide] and glucagon-like peptide 1). Therefore, anatomical and physiologic connections in the gut-liver-pancreas circle ensure that the primary station for the handling of foodstuff, the liver, is under close control by a nearby, well-informed unit, the β-cell.

It is noteworthy that hyperglycemia per se exerts an inhibitory effect on hepatic glucose production. When it is created while maintaining basal insulinemia during a hyperglycemic clamp, hyperglycemia reduces hepatic glucose output by the same degree as insulin (DeFronzo et al. 1983).

FFA have been shown to exert an extrahepatic effect on endogenous glucose production. FFA and/or products of their oxidation (e.g., citrate and acetyl-CoA) activate key gluconeogenic enzymes such as pyruvate carboxylase, PEP kinase, and G6Pase (Friedman et al. 1967). In addition, raised FFA concentrations in vivo are accompanied by raised glycerol levels, resulting from hydrolysis of triglycerides. Therefore, accelerated lipolysis normally supplies both the stimulus (FFA) and the substrate (glycerol) for gluconeogenesis. Finally, the liver takes up FFA avidly (with an extraction ratio of ~30%) and oxidizes them efficiently (as indicated by the low respiratory quotient of the organ) (Wahren et al. 1975). Thus, there are all the requisites to consider FFA oxidation in the liver as the energy-providing process that is coupled to energy-requiring gluconeogenesis.

The stimulatory effect on glucose output is determined by neuroendocrine responses, which are collectively defined as "counterregulatory system," in the sense that are activated in the defense against hypoglycemia. Hypoglycemia can activate hepatic glucose production independent of neurohormonal influences; this effect occurs when plasma glucose concentrations decrease below 2.7 mmol/L and glucose production increases only up to 60% of rates observed in control experiments (Bolli et al. 1985).

Glucagon plays a major part in the tonic support of hepatic glucose release: in man suppression of glucagon release with preservation of basal insulin secretion causes a fall of glucose production of over one-third. The release of pancreatic glucagon is the first of the "regulatory" endocrine mechanisms, resulting in a rise in endogenous glucose production by glycogenolysis and later gluconeogenesis. In response to hypoglycemia, glucagon is additionally stimulated by intracellular communications between β-cells and α-cells, and any disruption of this paracrine connection results in an impaired glucagon-mediated response to hypoglycemia. The sympathetic nervous system is activated at lower glucose levels (<3.6 mmol/L) than is glucagon (4 mmol/L) (Boyle et al. 1988). An increase in adrenaline, by also stimulating lipolysis, plays an important role, as shown by the recovery from hypoglycemia in type 1 diabetes where the glucagon response may be impaired. Under these conditions, glucose recovery is mainly mediated through β2-adrenergic receptors, although they do not fully compensate for the blunted plasma glucagon responses (De Feo et al. 1983). It is noteworthy that in this control system a host of hormones is required to balance the action of only one agonist, insulin. This fact arises from the inhibitory nature of insulin's effect on the production of a fuel upon which brain cell viability depends in an obligatory manner. In fact, together with glucagon and the sympathetic system, cortisol, GH, and triiodothyronine act in concert in glucose counterregulation.

Glucose Disposal

Following an overnight fast, glucose is released into the systemic circulation primarily by the liver, with a smaller contribution coming from the kidney and by the intestine. Glucose leaves the systemic circulation through the non-insulin and insulin-dependent tissue uptake, which has been estimated by regional catheterization studies. For the measure in body areas, as the splanchnic bed, where glucose production and uptake occur simultaneously, the indwelling catheter technique has been combined with glucose tracers. In the basal state, roughly 70% of glucose disposal takes place in insulin-independent tissues (brain, liver, kidney, intestine, erythrocytes); the fractional glucose extraction is low in these organs (1.7–2.8%), the brain being the more avid (9%) (Ferrannini and DeFronzo 2015). Normalizing organ glucose disposal by regional blood flow and estimated organ weight, glucose clearance can also be estimated. By this index, resting muscle (~40% of the body weight) is 10 times less active than the liver and up to 50 times less active than the

brain. The intermediate rate of glucose clearance of myocardial muscle is likely accounted for by its working state. However, these proportions change according to the rise in insulin levels, so that muscle glucose clearance can increase tenfold over the basal rate, while in the brain, liver, and kidneys, glucose clearance is maintained at basal rates. Whereas raising the plasma insulin concentration does not accelerate glucose clearance, reduced insulin levels may impair the efficiency of glucose removal, even in insulin-independent tissues. Such is the case of the liver, when hyperglycemia combined with a somatostatin infusion reducing plasma insulin resulted in a reduction of both total glucose uptake and clearance (DeFronzo et al. 1983).

By combining indirect calorimetry with glucose tracer studies, it has been possible to quantitate the two major components of whole-body glucose disposal, that is, glucose oxidation and nonoxidative glucose disposal. The latter is primarily (>90%) represented by glycogen synthesis, the remainder being accounted for by anaerobic metabolism, i.e., net lactate production. When using glucose tracers, muscle glycogen oxidation is not measured because G6Pase is absent in the muscle and the tissue therefore does not contribute free glucose to the plasma. In contrast, whole-body carbon dioxide production, as measured with indirect calorimetry, includes oxidized glycogen together with all the other oxidized substrates.

In general, indirect calorimetry assesses the amount of heat generated according to the amount and pattern of substrate use. In fact, carbon-based nutrients are converted into carbon dioxide (CO_2), water (H_2O), and heat in the presence of oxygen (O_2). Energy expenditure can be calculated from the amounts of O_2 used and CO_2 released. Oxygen consumption (VO_2) and CO_2 release (VCO_2) by the cells can be estimated by measuring the concentration of these gases in arterial and central venous blood and cardiac output (e.g., using Fick's equation) or by measuring pulmonary gas exchange, which is the principle of indirect calorimetry (Ferrannini 1988). The respiratory quotient (RQ), defined as the ratio between VCO_2 and VO_2, reflects the substrate mix that is oxidized. In fact, the complete oxidation of glucose yields an RQ value of 1, while that of either fat or protein an RQ of 0.7 and 0.8, respectively.

In the basal state and under ordinary nutritional circumstances, VO_2 averages ~250 mL/min, while VCO_2 is ~200 mL/min, yielding a whole-body RQ of 0.8. From simple equations, whole-body net carbohydrate oxidation is estimated to account for about 60% of total glucose uptake. Because 46% of glucose turnover occurs in the brain, which rapidly utilizes roughly all the transported glucose by oxidation, it follows that three-quarters (i.e., 46/60 = 77%) of basal glucose oxidation takes place in the brain. Therefore, other tissues preferentially derive their metabolic energy from the oxidation of fatty substrates and return most of the glucose to the liver after conversion into lactate (Cori cycle). Skeletal muscle, for example, has a respiratory quotient of 0.75 and relies on fat oxidation for the production of 80% of the energy it needs in the resting state (Natali et al. 1990). Thus, the basal state is characterized by parsimonious usage of glucose as fuel, which is selectively channeled to organs that cannot rely on alternative energy sources.

Insulin regulates energy metabolism by setting the competition between the two chief substrate fuels, glucose and FFA. When insulin concentrations fall, their inhibitory effect on lipolysis is diminished, and more fatty substrates are made available to oxidation; conversely, a rise in insulin levels determines a shift toward carbohydrate metabolism, restricting both lipolysis and protein breakdown (which contributes about 15% to energy metabolism).

The part that counterregulation plays in basal glucose uptake is less well defined, but probably is centered upon maintenance of lipolysis, since all the anti-insulin hormones are more or less potent lipolytic stimuli.

The Fed (Postprandial) State

The fed state is the period that intervenes after meals. Normally carbohydrates are assumed in a mixed meal with protein and fat and comprise 40–60% of dietary intake. The rate of absorption of carbohydrates is primarily regulated by gastric emptying and insulin response, which are in turn affected by a number of factors including the nutrient chemical form (e.g., refined sugars or complex carbohydrates), physical properties (e.g., solid or liquid), the timing (pre-load or co-ingestion of fat and protein), and the individual glucose tolerance status. Gastric emptying accounts for about 35% of the glycemic variance of the oral glucose response in healthy and T2DM subjects and is determined by the integrated motor activity of the interstitial cells of Cajal, which generate slow-wave currents working as pacemakers of stomach and small intestine muscle cells. The proximal stomach receives a meal by adapting its volume, with a slight increase in intragastric pressure. To proceed across to the duodenum, the solid components of the meal are triturated by the peristaltic contractions of the antrum; thus only particles $<$1–2 mm in size are pumped across the pylorus. The presence of nutrients in the small intestine triggers an inhibitory feedback to slow gastric emptying by neuronal (vagus nerve stimulation) and humoral mechanisms (the incretin hormone GLP-1, peptide YY, and CCK) (Holst et al. 2016). The sodium glucose transporter SGLT-1 then mediates glucose absorption from the intestinal lumen into enterocytes. Sodium flux is facilitated by a concentration gradient while glucose is actively transported. This system is coupled with GLUT1 transport at the basolateral membrane, which allows glucose transfer into the bloodstream; the co-transport is powered by a Na^+/K^+-ATPase pump which creates the sodium gradient that is required for SGLT activity.

Gastrointestinal endocrine cells (L and K cells) use SGLT and ATP-sensitive K^+ channels to sense intestinal glucose levels. Electrical activity transduces glucose sensing to calcium-stimulated release of the enterohormones. Vagal afferent activity, triggered by a number of enteroendocrine hormones including GLP-1 and GIP, provides a further level of control of glucose entry into the bloodstream.

Because of the complexity of the response to glucose or meal ingestion, intravenous glucose is also employed to investigate glucose metabolism in the fed state.

Testing Insulin Sensitivity and Insulin Secretion

The glucose clamp technique (especially in its euglycemic version) is generally accepted as the gold standard measurement of insulin action in vivo. This methodology – derived by analogy with the voltage clamp procedure in the neurosciences – has been widely studied and developed by DeFronzo et al. (1979). It uses a primed continuous insulin infusion to obtain a preset hyperinsulinemic plateau (approximately 70–80 μU/mL); after a few min an intravenous 20% glucose solution is administered at a variable rate that is dynamically adjusted to clamp the glucose concentrations at the normal fasting (5 mmol/L) or pre-existing level (isoglycemic version). When a steady state is reached, the exogenous glucose infusion rate equals the glucose disposal by all the tissues in the body, thus quantifying the overall amount of glucose metabolized (M). The time course of glucose infusion rates during an insulin/glucose clamp of nondiabetic subjects shows a quick rise within about 40 min of starting the insulin infusion and then a gentle upward trend. Even though in strict terms the glucose infusion rate never reaches a steady state, its average value during the final 40 min is a reliable index of insulin sensitivity. While in healthy subjects hyperinsulinemia is sufficient to completely suppress hepatic glucose output, whence glucose infusion rate represents the whole-body glucose disposal, in obese individuals or with lower insulin infusion rates, hepatic glucose release is not completely inhibited and must therefore be separately quantified by a glucose tracer technique.

To adequately compare clamp-derived data, some adjustments are generally requested. Firstly, the insulin infusion should be administered per unit of body surface area to avoid over-insulinization of obese individuals so that the most common clamp dose (1 $mU \cdot min^{-1} \cdot kg^{-1}$) should rather be calculated as 0.24 $nmol \cdot min^{-1} \cdot m^{-2}$ (40 $mU \cdot min^{-1} \cdot m^{-2}$). Secondly, the M value can be normalized by several variables, such as fat-free mass (M_{ffm}) according to the principle that glucose uptake occurs in lean tissues or by the resting rate of expenditure energy (M_{ree}) when indirect calorimetry is combined with the clamp; all the expressions of M can be also divided by the insulin plateau (M/I). Finally, glucose uptake can be adjusted by the steady-state plasma glucose concentration, thereby indexing the metabolic clearance rate of plasma glucose (MCR), which namely represents the fraction of plasma that is completely cleared of glucose by the virtue of the specific ability of the tissue to extract the substrate from the arterial side.

The disadvantages of the insulin/glucose clamp are the need for technical apparatus (two intravenous lines, calibrated pumps, and real-time plasma glucose level determinations) and trained personnel. The advantage that no other technique offers is that the clamp protocol can establish any desired combination of plasma glucose and insulin levels, thus investigating various domains of the glucose/insulin system. In addition, it can be combined with numerous other techniques, as indirect calorimetry, isotope turnover methodology, magnetic resonance imaging/spectroscopy, and muscle biopsy. Moreover, infusing tracers of non-esterified fatty acids (or glycerol) and amino acids, one can assess the influence of insulin per se on lipolysis and protein degradation, respectively. Finally, by limb catheterization (forearm and

leg), regional in comparison with whole-body glucose metabolism can be measured (Ferrannini and Mari 1988).

In its hyperglycemic version, the clamp allows to test insulin secretion. When β-cells are exposed to a square wave of hyperglycemia, a biphasic pattern of insulin release can be easily detected, in which a prompt initial surge (lasting approximately 10 min) is followed by a progressively increasing insulin secretory phase (10–120 min). While the first-phase insulin is the result of the release of hormone stored in readily releasable granules in the ß-cell cytoplasm, the release of insulin packaged in immature granules or newly synthesized insulin accounts for the second phase. The first-phase insulin secretion is similarly elicited in a more simple protocol, by intravenous glucose bolus administration (as in the intravenous glucose tolerance test, IVGTT) (Ferrannini and Pilo 1979). In this test, the burst of insulin release occurring from 2 to 10 min after glucose injection, called acute insulin response (AIR), has become the most common empirical index of insulin secretion. However, because correlation between in vivo tests of insulin secretion is generally unsatisfactory and AIR falls short of quantifying insulin secretion in type 2 diabetic subjects usually overestimating β-cell incompetence (Warram et al. 1996), several efforts have been made to develop a mathematical model of β-cell function. Pivotal work (Grodsky 1972; Licko 1973; Cerasi et al. 1974) identified the basic principles of this construct. The first parameter of the model, namely, "β-cell glucose sensitivity," originates from the straightforward evidence that insulin secretion rates follow glucose concentrations proportionally; for example, when the insulin secretion rates measured during an OGTT are plotted against the plasma glucose levels, each increment in glucose is associated with an increment in secretion; thus β-cell glucose sensitivity is merely the average slope of this function (Mari et al. 2002a). The second parameter is the "rate sensitivity," by which β-cells respond not only to the level of glucose but also to the rate of change of glucose levels. Finally, "potentiation" of glucose-induced insulin release is a well-characterized feature of β-cell function (Nesher and Cerasi 1987; Mari et al. 2002b). This emerges during an hyperglycemic clamp, where insulin release increases despite constant glucose levels, or during the OGTT, where insulin levels at similar glycemias are higher at later than earlier times (glucose-induced potentiation) during the test; furthermore, with nutrient ingestion the incretin effect participates to the potentiation (incretin-induced potentiation).

The Incretin Effect

The "incretin effect" refers to the incremental difference in insulin secretory response between oral and intravenous glucose administration at matched plasma glucose concentrations (Elrick et al. 1964). The greater stimulation of insulin secretion during an oral test is attributed to the nutrient-stimulated release of incretin hormones and their physiological actions, including potentiation of glucose-induced insulin secretion, suppression of glucagon release, inhibition of gastric emptying, and enhancement of satiety. The enterohormones classically involved in the incretin

effect are GLP-1 and GIP, which are respectively produced by L cells located in the distal small intestine and colon and K cells mainly distributed throughout the upper small intestine (duodenum and jejunum) (Holst 2007). Since nutrients are mostly absorbed in the upper gut, indirect mechanisms – perhaps neurally mediated – might be involved in the stimulation of L cells. Newly explored factors have been linked to the release of incretins, such as the gut microbial composition: bile acid receptors, such as the farnesoid X receptors, have been shown to reduce insulin resistance and hepatic glucose production and increase GLP-1 concentrations (Brighton et al. 2015).

Once secreted, incretin hormones diffuse across the basal lamina into the lamina propria and are taken up into a capillary, only to be broken down by dipeptidylpeptidase IV (DPP-IV), located on the luminal surface of the endothelial cells, such that only 25% of the secreted amount reaches the portal circulation. In the liver, a further 40–50% is cleared so that only 10–15% enters the systemic circulation and reaches the pancreas (perhaps even less because of the continued proteolytic activity of soluble DPP-IV present in plasma). Both incretin hormones bind to G-protein-coupled receptors, thus stimulating adenylate cyclase resulting in the formation of cAMP. In the β-cell, GLP-1 binding results in changes in ion channel activity, elevation of intracellular calcium concentrations, and enhanced exocytosis of insulin-containing granules (Mayo et al. 2003). Recent findings have suggested the presence of GLP-1 receptors on other tissues, thereby raising the possibility that GLP-1 may exert direct actions independent of the pancreatic hormonal changes. For example, it has been shown that GLP-1 has a direct effect on the liver to inhibit endogenous glucose production (Seghieri et al. 2013).

Numerous studies have established that in type 2 diabetic subjects the incretin effect is reduced; this defect seems to be partially independent of incretin concentrations, which vary widely between individuals depending on factors such as hyperglucagonemia, levels of FFA, and presence of obesity. Other favorable effects of incretin hormones such as the reduction of food intake and gastrointestinal motility are diminished in diabetes. Moreover, in diabetic subjects, GIP action on insulin response, even at pharmacological doses, has been shown to be much reduced. Conversely, GIP is thought to mainly contribute to the incretin effect in healthy subjects: at high glucose levels, it helps to promote insulin secretion, whereas at low glucose levels, it enhances glucagon secretion without any effects on insulin secretion, thus stabilizing plasma glucose levels within a narrow interval (Nauck et al. 1993).

Although it is believed that hyperglycemia is an important determinant in the impairment of the incretin effect in type 2 diabetes, glucose-lowering drugs (e.g., metformin) are not able to fully improve this defect (Vardarli et al. 2014). Achieving near normoglycemia by intensified insulin regimens increases β-cell responsiveness to exogenous GIP and GLP-1, but the resulting insulin secretory responses remain far lower than in nondiabetic subjects. Even incretin-based therapies (e.g., the DPP-IV inhibitor sitagliptin) do not change incretin effect, because they raise the insulin response to intravenous and oral glucose in the same degree (Muscelli et al. 2012).

Bariatric surgery by altering the nutrient transit or by other less immediate mechanisms (e.g., neuronal afferents, effects on other gut-derived factors, changes

in microbiota) is the most efficient intervention to ameliorate the altered incretin pattern. Studies are under way to explore the role of other gut peptides (gastrin, ghrelin, cholecystokinin) and additional targets (peptide YY, islet amyloid polypeptide, leptin receptors) or aimed at understanding the loss of the GIP insulinotropic effect will likely increase our knowledge on the incretin hormones and facilitate the development of incretin-based therapies.

Relationship Between Insulin Sensitivity and Insulin Secretion

Insulin sensitivity and insulin secretion are generally considered the two main determinants of glucose tolerance. Insulin action may be considered a relatively stable function. In fact, although it is affected by a number of factors, the most important of which are age, adipose tissue mass, fat topography, and degree of physical fitness, the intraindividual variation is limited in the daytime, and lifestyle and pharmacological interventions can at most double it. In contrast, β-cell function varies widely in the same person even within minutes or seconds to cope with dynamic glycemic changes as occurs with a large mixed meal, or over years, as happens with weight gain (Ferrannini and Mari 2014).

The relationship between insulin action and insulin secretion is well exemplified by obesity, the most prevalent condition associated with insulin resistance, where a stable, proportionate increase in insulin secretion maintains glucose tolerance. The minimal model paradigm hinges upon this feedback relationship (Kahn et al. 1993). It is based on a hyperbolic function describing the relationship between insulin sensitivity (Si from a frequently sampled IVGTT) and insulin secretion (variably defined by fasting insulin concentrations, AIR derived from the IVGTT or AIR_{max} from arginine-glucose potentiation according to Ward's protocol (Ward et al. 1984). The product of these two parameters, termed disposition index, is taken to represent whole body in vivo glucose disposition. This construct holds valid for static indices of ß-cell function – such as the fasting insulin secretion rate and the total insulin output in response to glucose or a mixed meal – because they do represent the chronic adaptive compensation for insulin resistance. However, the dynamics, rather than the absolute value, of insulin response appears to be more predictive of deteriorating glucose tolerance. In fact, glucose sensitivity, which reflects the ability of the ß-cell to increase insulin release in response to changing glucose levels over a time frame of minutes or hours, is poorly related to insulin sensitivity (Ferrannini and Mari 2004). Indeed, in multiple cross-sectional and longitudinal studies, glucose sensitivity is the single best descriptor of ß-cell dysfunction over a continuum from NGT to IGT to T2DM (Gastaldelli et al. 2004; Mari et al. 2010). In a time scale of months or years, obesity and insulin resistance provide compensation by expanding the ß-cell mass and raising the secretory set point, while weight loss reestablishes a normal functional capacity, possibly also by reducing on ß-cell mass. In diabetic subjects, ß-cell mass may be reduced – especially in long-standing disease – and ß-cell function is unequivocally and uniformly impaired. Whether the result of genetic predisposition, glucose toxicity (the detrimental effects of chronic hyperglycemia),

or a combination of both, diabetic ß-cells fail to sense glucose changes appropriately, thereby causing hyperglycemia (Ferrannini 2010). In overt diabetes, complete recovery of ß-cell glucose sensitivity is rare even with the most effective combination of lifestyle intervention and antihyperglycemic drugs (including insulin). Sustained remission is only seen in very obese diabetic patients following bariatric surgery.

Free Fatty Acid and Amino Acid Interactions

A high glucagon/insulin ratio, characteristic of the fasted state, stimulates adipose tissue lipolysis and hepatic glucose production to preserve glucose supply to those tissues that rely exclusively on glucose. Activation of lipolysis supplies tissues with FFA, which become the preferred fuel for respiration. In the liver, the excess FFA oxidation may promote ketogenesis. By inhibiting glucose oxidation, FFA and ketone bodies contribute to a glucose-sparing effect, an essential survival mechanism for the brain during starvation. In addition, inhibition of glucose oxidation preserves intermediate metabolites, like pyruvate and lactate, both of which are gluconeogenic precursors. Conversely, during the absorption of a high-fat meal, or during exercise, when FFA or ketone body concentrations are increased, part of glucose is not oxidized but reconverted to glycogen and stored in the muscle. Similarly, pyruvate in excess of the mitochondrial oxidative capacity (suggested by high levels of acetyl-CoA) is carboxylated and used by the anaplerotic route to form oxaloacetate. This FFA-glucose cycle, first described in heart and diaphragm muscle by Randle and coworkers in the early 1960s (Randle et al. 1963), has established the general concept of substrate competition, whereby the increased supply and use of a nutrient inhibits the use of the other directly, i.e., without hormonal mediation. In Randle cycle, the mechanism of inhibition of glucose utilization by fatty acid oxidation is exerted at the level of key glycolytic enzymes. The extent of inhibition in the glycolytic pathway is primarily exerted at the level of mitochondrial PDH complex; increased FFA oxidation leads to a rise in acetyl-CoA and NADH, which in turn activate PDH kinase, thus inhibiting the PDH complex through phosphorylation. Moreover, deactivation of PDH results in a rise of cytosolic citrate, which is a potent inhibitor of phosphofructokinase (PFK). As a consequence of decreased glycolysis, intracellular glucose-6-phosphate increases and may deactivate hexokinase. Another important site of competition is the direct inhibition of glucose transport via GLUT4 and GLUT2, respectively, in the muscle and liver (Roden et al. 1996). Interestingly, long-chain acyl-CoA derivatives directly inhibit glucokinase but do not inhibit the other hexokinases, thus offering a plausible mechanism for inhibition of glucose uptake by FFA in the liver (Hue and Taegtmeyer 2009).

In humans, evidence for effects of FFA and ketone bodies on glucose metabolism, supporting the inhibitory effects of lipid fuels on whole-body glucose utilization and glucose oxidation and the involvement of cardiac and skeletal muscles, derives mostly from studies utilizing intravenous lipid administration (Intralipid or liposome), usually in conjunction with heparin (to stimulate the release of FFA from triglycerides through the activation of lipoprotein lipase). The first study by Felber

and Vanotti (Felber and Vannotti 1964) reported that elevation of plasma FFA by Intralipid resulted in impaired oral glucose tolerance while raising plasma insulin levels. In particular, Intralipid decreased uptake, oxidation, and storage of glucose. Studies measuring fluxes across the forearm during euglycemic hyperinsulinemia confirmed that lipid infusion reduced forearm glucose uptake, whole-body glucose disposal, and oxidation (Yki-Jarvinen et al. 1991). Moreover, studies with [^{18}F] fluorodeoxyglucose/positron-emitting tomography combined with the euglycemic clamp found that elevation of FFA reduced whole-body glucose uptake by 31%, heart glucose uptake by 26%, femoral muscle glucose uptake by 29%, and arm muscle glucose uptake by 31%; thus, FFA decreased glucose uptake in the body and the three muscle groups to approximately the same extent (Nuutila et al. 1992). Separate estimates of the oxidative and nonoxidative pathways of glucose metabolism showed that, while inhibition of glucose oxidation is present within the first hour of lipid-heparin infusion, the reduction of the rate of nonoxidative utilization became apparent 2–4 h later (Bonadonna et al. 1989).

Amino acids too can participate in a substrate competition cycle with glucose, although somewhat less effectively than FFA. By using the clamp, it was found that the exogenous infusion of a mixture of crystalline amino acids was associated with a significant inhibition of whole-body glucose uptake despite significant stimulation of endogenous insulin secretion. The amino acid-induced decrement in glucose disposal was fully accounted for by inhibition of glucose oxidation, presumably caused by the concomitantly increased rate of protein oxidation (Ferrannini et al. 1988). On the other hand, in insulin-deficient states, increased amino acid provision enhances glucose production. Moreover, there is evidence that FFA themselves may have some protein-sparing property. In healthy humans, a lipid infusion has a hypoaminoacidemic effect independent of insulin both during fasting conditions and in the insulinized state, with or without hyperglycemia (Ferrannini et al. 1986b).

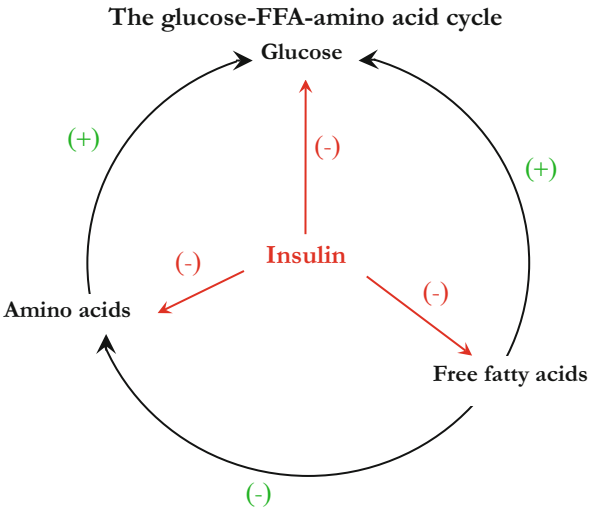


Fig. 1 Insulin lowers the circulating concentrations of glucose, free fatty acids, and amino acids. In addition, and independently of insulin, these three substrates are in mutual competition, such that an increased provision of amino acids raises plasma glucose levels and an increased provision of free fatty acids raises plasma glucose but also exerts a hypoaminoacidemic effect. (+) and (−) refer to stimulation and inhibition, respectively

The existence of substrate competition between amino acids and glucose makes it possible to expand the Randle cycle into a glucose-FFA-amino acid cycle, which integrates control of substrate disposition at the whole-body level (Fig. 1).

References

Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. J Biomed Biotechnol. 2010;2010:476279. https://doi.org/10.1155/2010/476279.

Abumrad NN, Cherrington AD, Williams PE, Lacy WW, Rabin D. Absorption and disposition of a glucose load in the conscious dog. Am J Phys. 1982;242(6):E398–406.

Bolli G, De Feo P, Perriello G, De Cosmo S, Ventura M, Campbell P, Brunetti P, Gerich JE. Role of hepatic autoregulation in defense against hypoglycemia in humans. J Clin Invest. 1985;75(5):1623–31. https://doi.org/10.1172/JCI111869.

Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA. Time dependence of the interaction between lipid and glucose in humans. Am J Phys. 1989;257(1 Pt 1):E49–56.

Boyle PJ, Schwartz NS, Shah SD, Clutter WE, Cryer PE. Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. N Engl J Med. 1988;318(23):1487–92. https://doi.org/10.1056/NEJM198806093182302.

Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, Gribble FM, Reimann F. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. Endocrinology. 2015;156(11):3961–70. https://doi.org/10.1210/en.2015-1321.

Burcelin R, Thorens B. Evidence that extrapancreatic GLUT2-dependent glucose sensors control glucagon secretion. Diabetes. 2001;50(6):1282–9. https://doi.org/10.2337/diabetes.50.6.1282.

Carruthers A, DeZutter J, Ganguly A, Devaskar SU. Will the original glucose transporter isoform please stand up! Am J Physiol Endocrinol Metab. 2009;297(4):E836–48. https://doi.org/10.1152/ajpendo.00496.2009.

Carter ME, Brunet A. FOXO transcription factors. Curr Biol. 2007;17(4):R113–4.

Cerasi E, Fick G, Rudemo M. A mathematical model for the glucose induced insulin release in man. Eur J Clin Investig. 1974;4(4):267–78.

Coggan AR. Use of stable isotopes to study carbohydrate and fat metabolism at the whole-body level. Proc Nutr Soc. 1999;58(4):953–61. https://doi.org/10.1017/S0029665199001263.

Colowick SP. The hexokinases. In: Boyer PD, editor. The enzymes, vol. IX. 3rd ed. New York: Academic; 1973. p. 1–48. https://doi.org/10.1016/S1874-6047(08)60113-4.

Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. Diabetologia. 2012;55(10):2565–82. https://doi.org/10.1007/s00125-012-2644-8.

De Feo P, Bolli G, Perriello G, De Cosmo S, Compagnucci P, Angeletti G, Santeusanio F, Gerich JE, Motolese M, Brunetti P. The adrenergic contribution to glucose counterregulation in type I diabetes mellitus. Dependency on A-cell function and mediation through beta 2-adrenergic receptors. Diabetes. 1983;32(10):887–93. https://doi.org/10.2337/diab.32.10.887.

DeFronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58(4):773–95. https://doi.org/10.2337/db09-9028.

DeFronzo RA, Tobin J, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Phys. 1979;237(3):E214–23.

DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wharen J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. Diabetes. 1983;32(1):35–45. https://doi.org/10.2337/diab.32.1.35.

Del Prato S, Bonadonna R, Bonora E, Gulli G, Solini A, Shank M, DeFronzo RA. Characterization of cellular defects in insulin action in type 2 (non-insulin-dependent) diabetes mellitus. J Clin Invest. 1993;91(2):484–94. https://doi.org/10.1172/JCI116226.

Elrick H, Stimmler L, Hlad CJ Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. J Clin Endocrinol Metab. 1964;24:1076–82. https://doi.org/10.1210/jcem-24-10-1076.

Felber JP, Vannotti A. Effect of the level of free fatty acids (NEFA) in the plasma on glycemia and insulinemia. Helv Physiol Pharmacol Acta. 1964;22:C13–5.

Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism. 1988;37(3):287–301.

Ferrannini E. The stunned beta cell: a brief history. Cell Metab. 2010;11(5):349–52. https://doi.org/10.1016/j.cmet.2010.04.009.

Ferrannini E, Cobelli C. The kinetics of insulin in man, II: role of the liver. Diabetes Metab Rev. 1987;3(2):365–97. https://doi.org/10.1002/dmr.5610030202.

Ferrannini E, DeFronzo RA. Insulin actions in vivo: glucose metabolism. In: DeFronzo RA, Ferrannini E, Zimmet P, Alberti G, editors. International textbook of diabetes mellitus. Chichester: Wiley-Blackwell; 2015. p. 211–33. https://doi.org/10.1002/9781118387658.ch14.

Ferrannini E, Mari A. How to measure insulin sensitivity. J Hypertens. 1988;16(7):895–906.

Ferrannini E, Mari A. Beta cell function and its relation to insulin action in humans: a critical appraisal. Diabetologia. 2004;47(5):943–56. https://doi.org/10.1007/s00125-004-1381-z.

Ferrannini E, Mari A. β-cell function in type 2 diabetes. Metabolism. 2014;63(10):1217–27. https://doi.org/10.1016/j.metabol.2014.05.012.

Ferrannini E, Pilo A. Pattern of insulin delivery after intravenous glucose injection in man and its relation to plasma glucose disappearance. J Clin Invest. 1979;64(1):243–54.

Ferrannini E, Del Prato S, DeFronzo RA. Glucose kinetics: tracer methods. In: Clarke WL, Larner J, Pohl SL, editors. Methods in diabetes research, Clinical methods, vol. II. New York: Wiley; 1986a. p. 107–42.

Ferrannini E, Barrett EJ, Bevilacqua S, Jacob R, Walesky M, Sherwin RS, DeFronzo RA. Effect of free fatty acids on blood amino acid levels in human. Am J Phys. 1986b;250(6 Pt 1):E686–94.

Ferrannini E, Bevilacqua S, Lanzone L, Bonadonna R, Brandi L, Oleggini M, Boni C, Buzzigoli G, Ciociaro D, Luzi L, DeFronzo RA. Metabolic interactions of amino acids and glucose in healthy humans. Diabetes. Nutr Metab. 1988;1:175–86.

Ferré P, Foretz M, Azzout-Marniche D, Bécard D, Foufelle F. Sterol-regulatory-element-binding protein 1c mediates insulin action on hepatic gene expression. Biochem Soc Trans. 2001;29(Pt 4):547–52.

Fong NM, Jensen TC, Shah AS, Parekh NN, Saltiel AR, Brady MJ. Identification of binding sites on protein targeting to glycogen for enzymes of glycogen metabolism. J Biol Chem. 2000;275(45):35034–9. https://doi.org/10.1074/jbc.M005541200.

Friedman B, Goodman EH Jr, Weinhouse S. Effects of insulin and fatty acids on gluconeogenesis in the rat. J Biol Chem. 1967;242(16):3620–7.

Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, Landau BR, Ferrannini E. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. Diabetes. 2000;49(8):1367–73.

Gastaldelli A, Ferrannini E, Miyazaki Y, DeFronzo RA. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. Diabetologia. 2004;47:31–9. https://doi.org/10.1007/s00125-003-1263-9.

Govers R. Molecular mechanisms of GLUT4 regulation in adipocytes. Diabetes Metab. 2014;40(6):400–10. https://doi.org/10.1016/j.diabet.2014.01.005.

Grodsky GM. A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. J Clin Invest. 1972;51(8):2047–59.

Haber RS, Weinstein SP, O'Boyle E, Morgello S. Tissue distribution of the human GLUT3 glucose transporter. Endocrinology. 1993;132(6):2538–43. https://doi.org/10.1210/endo.132.6.8504756.

Holness MJ, Sugden MC. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem Soc Trans. 2003;31(Pt 6):1143–51. https://doi.org/10.1042/BST0311143.

Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87(4):1409–39.

Holst JJ, Gribble F, Horowitz M, Rayner CK. Roles of the gut in glucose homeostasis. Diabetes Care. 2016;39(6):884–92. https://doi.org/10.2337/dc16-0351.

Hue L, Taegtmeyer H. The Randle cycle revisited: a new head for an old hat. Am J Physiol Endocrinol Metab. 2009;297(3):E578–91. https://doi.org/10.1152/ajpendo.00093.2009.

James DE, Brown R, Navarro J, Pilch PF. Insulin-regulatable tissues express a unique insulin sensitive glucose transport protein. Nature. 1988;333(6169):183–5. https://doi.org/10.1038/333183a0.

Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes. 1993;42:1663–72. https://doi.org/10.2337/diabetes.42.11.1663.

Landau BR. Estimating gluconeogenic rates in NIDDM. Adv Exp Med Biol. 1993;334:209–20.

Larance M, Ramm G, James DE. The GLUT4 code. Mol Endocrinol. 2008;22(2):226–33. https://doi.org/10.1210/me.2007-0282.

Licko V. Threshold secretory mechanism: a model of derivative element in biological control. Bull Math Biol. 1973;35(1):51–8.

Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for the assessment of β-cell function: modeling analysis in normal subjects. Am J Phys. 2002a;283(6):E1159–66.

Mari A, Tura A, Gastaldelli A, Ferrannini E. Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. Diabetes. 2002b;51(Suppl 1):S221–6.

Mari A, Tura A, Natali A, Laville M, Laakso M, Gabriel R, Beck-Nielsen H, Ferrannini E, RISC Investigators. Impaired beta cell glucose sensitivity rather than inadequate compensation for insulin resistance is the dominant defect in glucose intolerance. Diabetologia. 2010;53(4):749–56. https://doi.org/10.1007/s00125-009-1647-6.

Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, Drucker DJ. International union of pharmacology. XXXV. The glucagon receptor family. Pharmacol Rev. 2003;55(1):167–94. https://doi.org/10.1124/pr.55.1.6.

Mosca E, Barcella M, Alfieri R, Bevilacqua A, Canti G, Milanesi L. Systems biology of the metabolic network regulated by the Akt pathway. Biotechnol Adv. 2012;30(1):131–41. https://doi.org/10.1016/j.biotechadv.2011.08.004.

Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. Mol Asp Med. 2013;34(2–3):121–38. https://doi.org/10.1016/j.mam.2012.07.001.

Muscelli E, Casolaro A, Gastaldelli A, Mari A, Seghieri G, Astiarraga B, Chen Y, Alba M, Holst J, Ferrannini E. Mechanisms for the antihyperglycemic effect of sitagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab. 2012;97(8):2818–26. https://doi.org/10.1210/jc.2012-1205.

Natali A, Buzzigoli G, Taddei S, Santoro D, Cerri M, Pedrinelli R, Ferrannini E. Effects of insulin on hemodynamics and metabolism in human forearm. Diabetes. 1990;39(4):490–500. https://doi.org/10.2337/diab.39.4.490.

Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest. 1993;91(1):301–7. https://doi.org/10.1172/JCI116186.

Nesher R, Cerasi E. Biphasic insulin release as the expression of combined inhibitory and potentiating effects of glucose. Endocrinology. 1987;121(3):1017–24.

Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teras M, Haaparanta M, Bergman J, Solin O, Voipiopulkki LM, Wegelius U, Yki-Jarvinen H. Glucose–free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest. 1992;89:1767–74. https://doi.org/10.1172/JCI115780.

Printz RL, Koch S, Potter LR, O'Doherty RM, Tiesinga JJ, Moritz S, Granner DK. Hexokinase II mRNA and gene structure, regulation by insulin, and evolution. J Biol Chem. 1993;268 (7):5209–19.

Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;1(7285):785–9.

Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 1996;97(12):2859–65. https://doi.org/10.1172/JCI118742.

Rogers PA, Fisher RA, Harris H. An electrophoretic study of the distribution and properties of human hexokinases. Biochem Genet. 1975;13:857–66. https://doi.org/10.1007/BF00484416.

Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. Science. 1991;254 (5031):573–6.

Saltiel AR, Kahn CR. Insulin signaling and the regulation of glucose and lipid metabolism. Nature. 2001;414(6865):799–806. https://doi.org/10.1038/414799a.

Seghieri M, Rebelos E, Gastaldelli A, Astiarraga BD, Casolaro A, Barsotti E, Pocai A, Nauck M, Muscelli E, Ferrannini E. Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia. 2013;56(1):156–61. https://doi.org/10.1007/s00125-012-2738-3.

Siddle K. Signalling by insulin and IGF receptors: supporting acts and new players. J Mol Endocrinol. 2011;47(1):R1–10. https://doi.org/10.1530/JME-11-0022.

Sindelar DK, Balcom JH, Chu CA, Neal DW, Cherrington AD. A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. Diabetes. 1996;45(11):1594–604. https://doi.org/10.2337/diab.45.11.1594.

Thorens B, Mueckler M. Glucose transporters in the 21st century. Am J Physiol Endocrinol Metab. 2010;298(2):E141–5. https://doi.org/10.1152/ajpendo.00712.2009.

Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and metformin treatment on incretin hormone and insulin secretory responses to oral and "isoglycemic" intravenous glucose. Diabetes. 2014;63(2):663–74. https://doi.org/10.2337/db13-0805.

Wahren J, Hagenfeldt L, Felig P. Splanchnic and leg exchange of glucose, amino acids, and free fatty acids during exercise in diabetes mellitus. J Clin Invest. 1975;55:1303–14.

Wajngot A, Chandramouli V, Schumann WC, Kumaran K, Efendi S, Landau BR. Testing of the assumptions made in estimating the extent of futile cycling. Am J Phys. 1989;256(5 Pt 1): E668–75.

Wang Y, Viscarra J, Kim SJ, Sul HS. Transcriptional regulation of hepatic lipogenesis. Nat Rev Mol Cell Biol. 2015;16(11):678–89. https://doi.org/10.1038/nrm4074.

Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Invest. 1984;74(4):1318–28. https://doi.org/10.1172/JCI111542.

Warram JH, Sigal RJ, Martin BC, Krolewski AS, Soeldner JS. Natural history of impaired glucose tolerance: follow-up at Joslin Clinic. Diabet Med. 1996;13(9 Suppl 6):S40–5.

Whitehead JP, Clark SF, Urso B, James DE. Signalling through the insulin receptor. Curr Opin Cell Biol. 2000;12(2):222–8. https://doi.org/10.1016/S0955-0674(99)00079-4.

Wilson C, Vereshchagina N, Reynolds B, Meredith D, Boyd CA, Goberdhan DC. Extracellular and subcellular regulation of the PI3K/Akt cassette: new mechanisms for controlling insulin and growth factor signalling. Biochem Soc Trans. 2007;35(Pt 2):219–21. https://doi.org/10.1042/BST0350219.

Yki-Jarvinen H, Puhakeinen I, Koivisto VA. Effect of free fatty acids on glucose uptake and non-oxidative glycolysis across human forearm tissues in the basal state and during insulin stimulation. J Clin Endocrinol Metab. 1991;72:1266–77. https://doi.org/10.1210/jcem-72-6-1268.

Diagnostic Criteria and Classification

2

Crystal Man Ying Lee and Stephen Colagiuri

Contents

Abstract

Diabetes is an important contributor to global burden of disease. The number of people with diabetes has increased substantially since the first global estimates were published in 2000. Nevertheless, diabetes prevalence estimates are highly

C. M. Y. Lee
The Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, Level 2 Charles Perkins Centre D17, University of Sydney, Sydney, NSW, Australia

School of Public Health, Curtin University, Perth, WA, Australia

S. Colagiuri (✉)
The Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, Level 2 Charles Perkins Centre D17, University of Sydney, Sydney, NSW, Australia
e-mail: stephen.colagiuri@sydney.edu.au

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_3

dependent on factors such as data sources and quality, method used to diagnose diabetes, diagnostic criteria, and modelling assumptions. This chapter includes a review of the development of the current diagnostic criteria for diabetes and considers classification systems for diabetes.

Keywords
Diabetes · Intermediate hyperglycemia · Fasting plasma glucose · Glycated hemoglobin

Diabetes is recognized as an important contributor to global burden of disease and consequently the 2025 global goals arising from the 2011 United Nations High-Level Meeting on Noncommunicable Diseases (NCDs) include halting the rise in age-standardized adult prevalence of diabetes at 2010 levels (World Health Organization 2013b).

The number of people with diabetes has almost tripled since the first global estimates were published by the International Diabetes Federation in 2000 (International Diabetes Federation 2000). The latest figures suggest that 415 million people aged 20–79 years had diabetes in 2015 with almost half of these having undiagnosed diabetes (International Diabetes Federation 2015). This figure is remarkably similar to the NCD Risk Factor Collaboration (NCD-RisC) estimate of 422 million adults with diabetes in the world in 2014 (NCD Risk Factor Collaboration (NCD-RisC) 2016).

Diabetes prevalence estimates are highly dependent on a number of factors including data sources and quality, method used to diagnose diabetes, diagnostic criteria, and modeling assumptions. Studies used to estimate global diabetes prevalence and numbers have used a variety of methods to diagnose diabetes including diabetes biomarkers (fasting glucose, post-load glucose, and glycated hemoglobin (HbA1c)), self-reported diabetes, medical records, use of blood glucose-lowering therapies, and occasionally urine glucose. The International Diabetes Federation method preferentially selects data sources according to a prespecified set of criteria and on quality judged by an expert panel. The NCD-RisC estimate included a modeled conversion to a consistent definition of diabetes based on fasting plasma glucose to adjust for differences in diabetes biomarker data. Given these differences in methodologies, it is remarkable that both of these studies produced very similar and consistent results.

Over the years there have been a series of consensus expert meetings to consider the diagnosis and classification of diabetes in order to achieve international harmonization not only to compare epidemiological information but also to provide uniformity in diagnosing an individual with diabetes and the considerable impact of such a diagnosis. The World Health Organization published its first report on the diagnosis and classification of diabetes in 1965 (World Health Organization 1965). Since then, several modifications have been made to both the diagnostic criteria and classification by the World Health Organization (1980, 1985, 1999; World Health Organization & International Diabetes Federation 2006; Report of a World Health Organization Consultation 2011) and the American

Diabetes Association (National Diabetes Data Group 1979; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997 and 2003; American Diabetes Association 2010).

In addition to diagnosing people with diabetes, different forms of diabetes have been recognized and described for many years. This chapter reviews the development of the current diagnostic criteria for diabetes and considers classification systems for diabetes.

Diagnostic Criteria for Diabetes

Uniform and agreed diagnostic criteria for diabetes are essential for individual health and clinical care and epidemiological studies and monitoring population changes over time such as progress against the United Nations' targets. A diagnosis of diabetes has important implications for the individual not only for health but also as a result of labeling including employment, health and life insurance, driving, and social opportunities and has potential cultural, ethical, and human rights consequences. While we focus on biomedical criteria for establishing the presence of diabetes, diagnosing and labeling an individual with diabetes has far broader implications.

Drawing the line between normal and abnormal is difficult when a population biomarker such as glucose is a continuum without a self-evident cut point. The evolution of glucose based on the World Health Organization diagnostic criteria is summarized in Table 1. It is interesting to note that the 1965 World Health Organization technical report stated that the "committee recognized the difficulties posed by attempting to make world-wide recommendations on laboratory tests, particularly with respect to the glucose tolerance test blood-sugar values," a situation which has remained largely unchanged for the past 50 years. Consequently while the many expert consultations over a long period have produced the current universally accepted diagnostic criteria for diabetes, some aspects continue to be debated and may well be revised in the future.

Current Diagnostic Criteria

Diabetes

Diabetes can be associated with classical symptoms of hyperglycemia which include polyuria, polydipsia, polyphagia, and weight loss. The presence of these symptoms and an unequivocally elevated random plasma glucose are sufficient to make a diagnosis of diabetes. However many people with diabetes can remain asymptomatic for many years and blood tests are required for diagnosis. Diagnostic tests currently accepted by the World Health Organization and the American Diabetes Association include the measure of fasting plasma glucose, 2-h post-load plasma glucose during an oral glucose tolerance test (OGTT), and HbA1c. Asymptomatic people with

Table 1 Summary of WHO glucose-based diagnostic criteria for diabetes and intermediate hyperglycemia

	1965	1980	1985	1999	2006
Normal		Not defined	Not defined		Not defined
FPG	Not specified			<6.1 mmol/L (110 mg/dl)	
2hPG	<6.1 mmol/L (110 mg/dl)			Not specified but <7.8 mmol/L (140 mg/dl) implied	
Diabetes					
FPG	Not specified	≥8.0 mmol/L (144 mg/dl)	≥7.8 mmol/L (140 mg/dl)	≥7.0 mmol/L (126 mg/dl)	≥7.0 mmol/L (126 mg/dl)
		AND/OR	OR	OR	OR
2hPG	≥7.2 mmol/L (130 mg/dl)	≥11.0 mmol/L (199 mg/dl)	≥11.1 mmol/L (200 mg/dl)	≥11.1 mmol/L (200 mg/dl)	≥11.1 mmol/L (200 mg/dl)
IGT	Referred to as borderline state				
FPG		<8.0 mmol/L (144 mg/dl)	<7.8 mmol/L (140 mg/dl)	<6.1 mmol/L (110 mg/dl)	<6.1 mmol/L (110 mg/dl)
		AND	AND	AND	AND
2hPG	6.1–7.1 mmol/L (110–128 mg/dl)	≥8.0 and <11.0 mmol/L (145–199 mg/dl)	≥7.8 and <11.1 mmol/L (140–199 mg/dl)	≥7.8 and <11.1 mmol/L (140–199 mg/dl)	≥7.8 and <11.1 mmol/L (140–199 mg/dl)
IFG	Not defined	Not defined	Not defined		
FPG				≥6.1 and <7.0 mmol/L (110–125 mg/dl)	≥6.1 and <7.0 mmol/L (110–125 mg/dl)
				AND	AND
2hPG				<7.8 mmol/L (140 mg/dl) (if measured)	<7.8 mmol/L (140 mg/dl) (if measured)

FPG fasting plasma glucose, *2hPG* 2-h plasma glucose during an oral glucose tolerance test, *IGT* impaired glucose tolerance, *IFG* impaired fasting glucose

fasting plasma glucose ≥7.0 mmol/L (126 mg/dl), 2-h post-load plasma glucose ≥11.1 mmol/L (200 mg/dl), and/or HbA1c ≥6.5% (48 mmol/mol) are considered to have diabetes (Table 2). For asymptomatic people, repeat testing, preferably with the same test, is recommended to confirm the diagnosis.

Over the years there have been four major changes related to diagnostic criteria for diabetes:

Standardization of the Glucose Load Used in the OGTT

Since 1979/1980 the accepted glucose dose for an OGTT to diagnose diabetes in nonpregnant adults has been standardized to 75 g. This decision basically represented a compromise between the 50 g dose used in Europe and the 100 g used in the USA at that time.

Table 2 Current diagnostic criteria for diabetes and intermediate hyperglycemia

	World Health Organization (2006, 2011)			American Diabetes Association (2015)	
		Intermediate hyperglycemia			
	Diabetes	Impaired glucose tolerance	Impaired fasting glucose	Diabetes	Intermediate hyperglycemia (prediabetes)
Fasting plasma glucose	≥7.0 mmol/L (126 mg/d)	<7.0 mmol/L (126 mg/dl)	6.1–6.9 mmol/L (110–125 mg/dl)	≥7.0 mmol/L (126 mg/dl)	5.6–6.9 mmol/L (100–125 mg/dl)
	AND/OR	AND	AND	OR	OR
2-hr plasma glucose during an oral glucose tolerance test	≥11.1 mmol/L (200 mg/dl)	7.8–11.0 mmol/L (140–199 mg/dl)	<7.8 mmol/L (140 mg/dl) (if measured)	≥11.1 mmol/L (200 mg/dl)	7.8–11.0 mmol/L (140–199 mg/dl)
	AND/OR			OR	OR
Glycated hemoglobin	≥6.5% (48 mmol/mol)			≥6.5% (48 mmol/mol)	5.7–6.4% (39–47 mmol/mol)
				OR	
Random plasma glucose				≥11.1 mmol/L in patients with classic symptoms of hyperglycemia	

2-h Post-Load Glucose Levels

The original World Health Organization criterion for diagnosing diabetes was based solely on a 2-h post-load plasma glucose ≥7.2 mmol/L (130 mg/dl) (World Health Organization 1965). This was changed in 1979/1980 with the diagnostic cut point set at ≥11.1 mmol/L (200 mg/dl). Despite the evidence on which this is based not being particularly strong, this level has remained unchanged because no convincing new evidence has emerged to indicate that this should be changed.

Fasting Plasma Glucose

There have been a number of changes in relation to fasting plasma glucose levels. Initially no diagnostic level was set for fasting glucose. In 1979 the National Diabetes Data Group set a diagnostic level for fasting plasma glucose ≥7.8 mmol/L (140 mg/dl) on the basis of a bimodal distribution in some populations (National Diabetes Data Group 1979). In 1980, the World Health Organization recommended a fasting plasma glucose ≥8.0 mmol/L (145 mg/dl) (World Health Organization 1980) and revised this to ≥7.8 mmol/L (140 mg/dl) in 1985 (World Health Organization 1985). In 1997/1998 the diagnostic fasting plasma glucose was lowered to ≥7.0 mmol/L (126 mg/dl) (the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997; World Health Organization 1999). This was based on achieving a better alignment of fasting and 2-h post-load glucose and was largely

based on the point where prevalence of diabetes-specific microvascular complications increases.

HbA1c Included as a Diagnostic Criterion

HbA1c was adopted as a diagnostic criterion for diabetes by the American Diabetes Association in 2010 (American Diabetes Association 2010) and the World Health Organization in 2011 (Report of a World Health Organization Consultation 2011). This was also based on the point where prevalence of diabetes-specific microvascular complications increases (see below).

Intermediate Hyperglycemia

It has long been recognized that lesser degrees of hyperglycemia below diabetes levels are associated with an increased risk of progression to diabetes and with increased risk of cardiovascular events. There is also an increased focus on identifying these people in order to implement interventions to reduce this risk, particularly to decrease risk of developing diabetes. Intermediate hyperglycemia is often referred to as "prediabetes," a somewhat controversial term since the development of diabetes is not invariable and can only accurately be applied retrospectively. Nevertheless, the term remains popular and commonly used in clinical practice and the literature.

Two states of intermediate hyperglycemia are recognized – impaired fasting glucose and impaired glucose tolerance. In 1979 the National Diabetes Data Group (1979) introduced the category of impaired glucose tolerance to denote a state of increased risk of progressing to diabetes, although it was also noted that many would revert to normal. This term was introduced to remove the stigma of diabetes from the other terms in use at the time to denote the range between "normal" and diabetes. This category and definition was included in the 1980 World Health Organization report (World Health Organization 1980). Impaired glucose tolerance is not a clinical entity but is a risk factor for future diabetes and/or adverse outcomes. The universally accepted definition of impaired glucose tolerance includes a fasting plasma glucose <7.0 mmol/L (126 mg/dl) and 2-h post-load plasma glucose of 7.8–11.0 mmol/L (140–199 mg/dl) (Table 2) (World Health Organization & International Diabetes Federation 2006).

In 1997 an expert committee (the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997) introduced impaired fasting glucose to describe a range of fasting plasma glucose equivalent to impaired glucose tolerance, and this was included in the 1999 World Health Organization technical report (World Health Organization 1999). As with impaired glucose tolerance, impaired fasting glucose is a not clinical entity but rather a risk factor for future diabetes and adverse outcomes.

When this category was initially introduced and adopted, the World Health Organization and American Diabetes Association used the same definition, namely,

a fasting plasma glucose of 6.1–6.9 mmol/L (110–125 mg/dl). However the definition of impaired fasting glucose is currently not universally agreed. The World Health Organization continues to recommend diagnosis of impaired fasting glucose based on a fasting plasma glucose 6.1–6.9 mmol/L (110–125 mg/dl) and 2-h postload plasma glucose <7.8 mmol/L (140 mg/dl) (if measured) (Table 2; World Health Organization & International Diabetes Federation 2006). However in 2003 the American Diabetes Association changed its diagnostic criteria and lowered the fasting plasma glucose range to 5.6–6.9 mmol/L (100–125 mg/dl) to define impaired fasting glucose (Table 2; the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003). The World Health Organization decision to continue with the original impaired fasting glucose criteria was based on concerns about the implications of the significant global increase in impaired fasting glucose prevalence with the lower cut point and the impact on individuals and health systems and in particular the lack of evidence of any benefit in terms of reducing adverse outcomes or progression to diabetes with the lower cut point (World Health Organization and International Diabetes Federation 2006).

There is also no universal agreement on HbA1c to diagnose intermediate hyperglycemia. Currently the World Health Organization does not specify HbA1c diagnostic criteria for intermediate hyperglycemia. The American Diabetes Association recommends an HbA1c 5.7–6.4% (39–47 mmol/mol) to diagnose intermittent hyperglycemia, which the American Diabetes Association terms prediabetes (Table 2; American Diabetes Association 2015). An International Expert Committee with members appointed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation considered this issue in 2008. While not defining a specific cut point, the Committee suggested prevention interventions in very-high-risk individuals with HbA1c values close to the 6.5% (48 mmol/mol) HbA1c threshold of diabetes (i.e., ≥6.0%). However interventions would also be appropriate in individuals with lower HbA1c values with other established risk factors (The International Expert Committee 2009).

Hyperglycemia in Pregnancy

Women with hyperglycemia during pregnancy are at increased risk of adverse outcomes for both themselves and their baby, and treatment is effective in reducing this risk. However, there has been considerable controversy on what constitutes glucose intolerance in pregnancy, and consequently there have been a number of procedures and glucose cutoffs proposed.

The original criteria for gestational diabetes mellitus proposed by O'Sullivan and Mahan in the 1960s used a 3-h 100 g OGTT and was based on risk of the mother developing diabetes in the future (O'Sullivan and Mahan 1964), but it was also observed that treatment with a specific diet and insulin significantly reduced the risk of macrosomia compared with untreated women (O'Sullivan et al. 2003). When the 2-h 75 g OGTT was adopted as the standard procedure in 1979/1980 as

the diagnostic test for diabetes and glucose intolerance, the World Health Organization recommended the 75 g glucose load as the testing procedure for pregnant women and also recommended that the criteria for diabetes and impaired glucose tolerance be used to interpret the results of OGTT testing in pregnant women (World Health Organization 1980). This was subsequently modified by the World Health Organization in 1985 with the term gestational diabetes being used for any glucose intolerance first detected during pregnancy (World Health Organization 1985).

Following the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (HAPO Study Cooperative Research Group 2008), revisions to the diagnostic criteria were suggested. This international multicenter study tested 25,505 pregnant women with a 2-h 75 g OGTT and followed them through pregnancy for adverse maternal and fetal outcomes. In 2013 the World Health Organization revised its diagnosis and classification of hyperglycemia first detected during pregnancy and recommended two categories of glucose intolerance based on a 2-h 75 g OGTT (World Health Organization 2013a):

- Diabetes mellitus in pregnancy
- Gestational diabetes mellitus

This move away from classifying pregnant women with either diabetes or impaired glucose tolerance/impaired fasting glucose in the one category of gestational diabetes mellitus represented a return to the 1980 World Health Organization recommendations (World Health Organization 1980).

The diagnosis of diabetes in pregnancy is based on the 2006 World Health Organization criteria for diabetes (World Health Organization 2006) when one or more of the following criteria are met:

- Fasting plasma glucose $\geq$7.0 mmol/L (126 mg/dl)
- 2-h plasma glucose $\geq$11.1 mmol/L (200 mg/dl) following a 75 g oral glucose load
- Random plasma glucose $\geq$11.1 mmol/L (200 mg/ dl) in the presence of diabetes symptoms

The World Health Organization does not recommend use of HbA1c for the diagnosis of diabetes during pregnancy, whereas the American Diabetes Association includes HbA1c as a diagnostic option (American Diabetes Association 2015).

The World Health Organization criteria for the diagnosis of gestational diabetes mellitus at any time in pregnancy include any one or more of the following (World Health Organization 2013a):

- Fasting plasma glucose 5.1–6.9 mmol/L (92–125 mg/dl)
- 1-h plasma glucose $\geq$10.0 mmol/L (180 mg/dl) following a 75 g oral glucose load
- 2-h plasma glucose 8.5–11.0 mmol/L (153–199 mg/dl) following a 75 g oral glucose load

Methods Used to Derive Diagnostic Cut Points

Two main methods have been used to derive diagnostic cut points for diabetes (World Health Organization 2006) – the population distribution of plasma glucose and plasma glucose levels associated with risk of diabetes-specific microvascular complications, particularly retinopathy.

Some studies have reported a bimodal distribution of plasma glucose in which populations can be divided into two separate but overlapping groups. With a bimodal distribution, the point at which the two curves intersect has been used to separate abnormal from normal. A bimodal distribution of 2-h post-load plasma glucose was first described in a 1971 study in Pima Indians (Rushforth et al. 1971). Later studies on populations with high prevalence of diabetes reported a similar bimodal distribution of glucose (Zimmet and Whitehouse 1978; Raper et al. 1984; Rosenthal et al. 1985; Loo et al. 1993; Dowse et al. 1994; Omar et al. 1994; Engelgau et al. 1997; Lim et al. 2002; Fan et al. 2005). Plasma glucose levels in the higher glucose distribution are associated with symptoms of diabetes and diabetes retinal and renal complications. Data on bimodal distributions were used to set the diagnostic 2-h post-OGTT plasma glucose level which remains in current use (National Diabetes Data Group 1979).

However, an international data pooling study by the DETECT-2 collaboration on bimodal distribution of plasma glucose measured during an OGTT, which included 43 studies from 27 countries, questioned the use of bimodal distribution as a suitable method for identifying diagnostic cut points for diabetes (Vistisen et al. 2009). In studies where a bimodal distribution was observed, the cut point for fasting plasma glucose ranged from 5.7 mmol/L 9103 mg/dl) to 8.5 mmol/L (153 mg/dl) (median 7.1 mmol/L (128 mg/dl)) and for 2-h plasma glucose ranged from 9.1 mmol/L (164 mg/dl) to 17.9 mmol/L (323 mg/dl) (median 12.4 mmol/L (223 mg/dl)).

Since 1997, the occurrence of diabetes-specific complications has been used to derive diagnostic cut points for diabetes, particularly using data from epidemiological studies which have examined both prevalent and incident retinopathy across a range of plasma glucose levels. Typically deciles (ten equal sized groups) of the distribution of plasma glucose are plotted against prevalence of retinopathy. The distribution graphs show that the prevalence of retinopathy remains low but then increases substantially and the diagnostic cut point is determined as the level at which the risk of retinopathy increases significantly. Few studies have been ideal for this purpose and most have limited statistical power. Studies have also differed in methodologies to diagnose retinopathy and whether or not people with previously diagnosed diabetes are included in the analysis. Some of these differences are highlighted in the three studies which have been used to set diagnostic levels. In the Egyptian study retinopathy prevalence increased from the eighth decile (fasting plasma glucose 7.2 mmol/L [130 mg/dl]; 2-h post-load plasma glucose 12.1 mmol/L [218 mg/dl]; HbA1c 6.9% [52 mmol/mol]), the ninth decile (fasting plasma glucose 7.5 mmol/L [135 mg/dl]; 2-h post-load plasma glucose 13.5 mmol/L [243 mg/dl]; HbA1c 6.7% [50 mmol/mol]), in the Pima Indian population, and the tenth decile (fasting plasma glucose 6.7 mmol/L [121 mg/dl]; 2-h post-load plasma glucose

10.8 mmol/L [195 mg/dl]; HbA1c 6.2% [44 mmol/mol]) in a US population (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997).

In order to address the limited statistical power of individual studies, the DECTECT-2 collaboration pooled data on over 45,000 participants from 9 studies which enabled a more detailed analysis of this relationship. The distribution of glycemic measures was plotted in vigintiles (20 equally sized groups) and by 0.5 unit intervals of glycemic measures against the occurrence of retinopathy cases which were unequivocally specific to diabetes (Fig. 1) (Colagiuri et al. 2011). The

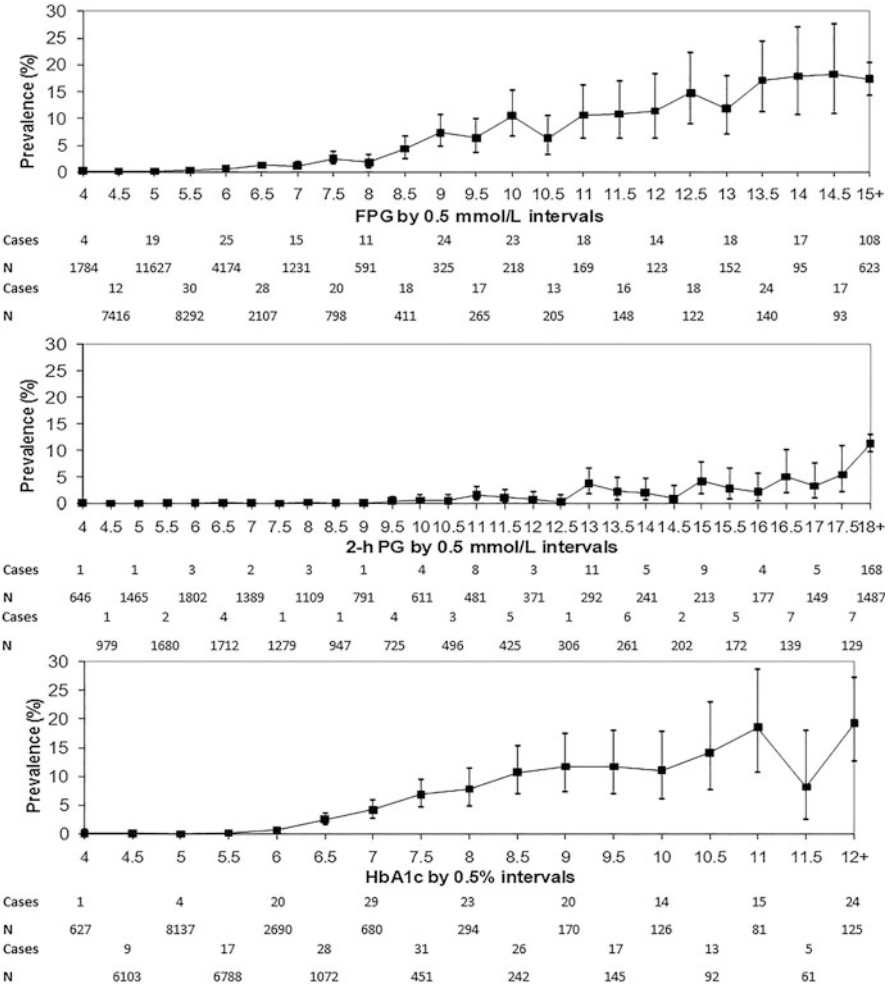


Fig. 1 Prevalence of diabetes-specific retinopathy (moderate or more severe retinopathy) with 95% confidence intervals, number of retinopathy cases, and participants within each interval by 0.5 unit intervals for fasting plasma glucose (FPG), 2-h post-load plasma glucose (2-h PG), and glycated hemoglobin (HbA1c) (Reproduced with permission; Colagiuri et al. 2011)

various analyses performed in that study indicated that HbA1c of 6.5% (48 mmol/mol) was an appropriate alternative diagnostic criterion for diabetes. This study was used by set the HbA1c diagnostic criterion which has now been universally adopted (The International Expert Committee 2009; World Health Organization 2011).

Performance of the Different Criteria on Diabetes Prevalence

Although three measures of glycemia are currently accepted for the diagnosis of diabetes, the results from each of these glycemic biomarkers will not necessarily provide a similar diagnostic result on diabetes status for an individual or for population prevalence. The implications are particularly significant for an individual, but there has been little research on the actual impact, both in terms of societal and health implications. This is one reason why all guidelines recommend repeat confirmatory testing in an asymptomatic individual with an elevated glycemic measure.

Most studies which have compared the various diagnostic criteria have focused on the population impact. The DETECT-2 study on glycemic measures and diabetes-specific retinopathy showed that for the 16,000 participants without known diabetes who had all three glycemic measures, the proportion with newly diagnosed diabetes were 7.7% for fasting plasma glucose $\geq$7.0 mmol/L (126 mg/dl), 13.9% for 2-h post-load plasma glucose $\geq$11.1 mmol/L (200 mg/dl), and 5.7% for HbA1c $\geq$6.5% (48 mmol/mol) (Colagiuri et al. 2011).

A recent study by the NCD Risk Factor Collaboration (NCD-RisC 2015) compared fasting plasma glucose, 2-h plasma glucose in an OGTT, and HbA1c on both the population prevalence of diabetes and previously undiagnosed diabetes. Population prevalence of diabetes based on fasting plasma glucose or 2-h plasma glucose was higher by 2–6% than prevalence based on fasting plasma glucose alone. Overall prevalence based on HbA1c was similar to prevalence based on fasting plasma glucose but was lower than prevalence based on fasting plasma glucose in 42.8% of studies, higher in another 41.6%, and similar in the other 15.6%. Diabetes defined as HbA1c 6.5% (48 mmol/mol) or more had a pooled sensitivity of 52.8% and a pooled specificity of 99.7% compared with fasting plasma glucose 7.0 mmol/L (126 mg/dl) or more for diagnosing previously undiagnosed participants, and sensitivity compared with diabetes defined based on fasting plasma glucose or 2-h plasma glucose was 30.5%. This finding suggests that 47.2% of participants without a previous diagnosis of diabetes who would have diabetes based on their fasting plasma glucose concentration would not have diabetes based on an HbA1c test.

Guideline Recommendations for Procedures for Diagnosing Individual with Diabetes

The American Diabetes Association recommends type 2 diabetes testing be performed on individuals aged $\geq$45 years, and testing should be considered at any age for overweight or obese adults who have at least one risk factor for diabetes and

for children and adolescents who are overweight or obese who have at least two risk factors for diabetes (American Diabetes Association 2015). Repeat testing should be carried out at least every 3 years for those who test normal. In the UK, a two-step approach in identifying type 2 diabetes has been recommended by the National Institute for Health and Care Excellence. The first step is to conduct an assessment with a risk assessment tool or questionnaire on individuals aged ≥40 years or people aged 25–39 years who are of South Asian, Chinese, African-Caribbean, black African, and other black or ethnic minority backgrounds. The second step involves testing with a fasting plasma glucose or HbA1c in those people assessed as high risk according to the risk assessment (National Institute for Health and Care Excellence 2012). Individuals with fasting plasma glucose <5.5 mmol/L (100 mg/dl) or HbA1c <6.0% (42 mmol/mol) should be reassessed at least every 3 years, and those with fasting plasma glucose 5.5–6.9 mmol/L (100–125 mg/dl) or HbA1c 6.0–6.4% (42–47 mmol/mol) should be reassessed at least once a year. In Australia, guideline recommends that risk assessment should be performed on individuals aged ≥40 years or on indigenous people aged ≥18 years. The testing procedure for detecting type 2 diabetes depends on the diagnostic test. A three-step approach is recommended when glucose testing is used and a two-step approach when HbA1c testing is used. The initial step is risk assessment with the AUSDRISK tool (Chen et al. 2010) or risk factors associated with diabetes. If measurement of fasting plasma glucose is used as a second step in high-risk individuals, a third step of an OGTT is recommended for those with fasting plasma glucose 5.5–6.9 mmol/L (100–125 mg/dl) (Colagiuri et al. 2009). Since the introduction of HbA1c as a diagnostic test for diabetes, the Australian Diabetes Society recommends HbA1c for testing as an option in high-risk individuals obviating the need for an OGTT (d'Emden et al. 2015).

Classification of Diabetes

It has long been recognized that diabetes is a heterogeneous group of conditions with many different types, and since the 1965 World Health Organization expert meeting, there have been attempts to develop a standardized classification system. With the advancement of knowledge about the etiology and pathogenesis of diabetes over the past 50 years, classification systems have evolved and further changes are likely.

Having a uniform terminology and functional working classification of diabetes serves a number of purposes including as a basis for research into its causes, treatment, development of complications, and prevention; a framework for the collection of epidemiological data on etiology, natural history, and impact of diabetes and its complications; and an aid to the clinician in selecting appropriate treatment. Ideally classification systems should include classes which are mutually exclusive and homogeneous, require only simple clinical measurement or descriptive observations that are readily obtainable and have biological significance, and be based on knowledge of etiopathology.

History of Classification of Diabetes

The 1965 World Health Organization expert committee recommended classes of diabetes based on age of recognized onset as this was considered the only reliable means of classification (World Health Organization 1965). That committee recommended four classes – "infantile or childhood diabetes" with onset between ages 0 and 14 years, "young diabetes" with age of onset between 15 and 24 years, "adult diabetes" with onset between ages 25 and 64 years, and "elderly diabetes" with onset at age 65 and older. Other clinical types of diabetes were also recognized including "juvenile-onset diabetes" which could occur at any age in which the person required insulin and was ketosis prone, "brittle diabetes" in people with juvenile-onset diabetes which was difficult to control because of episodes of hyperglycemia and ketosis and episodes of hypoglycemia, "insulin-resistant diabetes" in people who required more than 200 units of insulin daily, "gestational diabetes," "pancreatic diabetes," "endocrine diabetes," and "iatrogenic diabetes."

The 1979 National Diabetes Data Group classification moved away from age-based classification and described four classes of diabetes: "insulin-dependent diabetes mellitus (IDDM or type 1 diabetes)"; "non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes)" with two subtypes, obese NIDDM and non-obese NIDDM; "other types of diabetes" including the following subtypes – pancreatic, hormonal, drug, or chemical induced, insulin receptor abnormalities, genetic syndromes, and others; and gestational diabetes. This report also acknowledged that it may be difficult to definitively assign an individual to one specific class because of a lack of all the information required or because there are discrete stages in the natural history of each type of diabetes that may resemble other classes and that it might be necessary to delay a definitive classification until more clinical and diagnostic information becomes available (National Diabetes Data Group 1979). The 1980 World Health Organization expert committee adopted the National Diabetes Data Group classification as an interim measure (World Health Organization 1980). The 1985 World Health Organization report recommended one major change to the classification system for diabetes and added "malnutrition-related diabetes mellitus (MRDM)" as a fifth and separate class of diabetes (World Health Organization 1985).

The 1997 expert committee moved away from a classification system based largely on pharmacological treatment to one based on etiology. Changes made in 1997 included the following:

1. The terms insulin-dependent diabetes mellitus (IDDM) and non–insulin dependent diabetes mellitus (NIDDM) were eliminated because these terms often resulted in classifying individuals on treatment rather than etiology.
2. The terms type 1 and type 2 diabetes were retained but with Arabic rather than Roman numerals.
3. Type 1 diabetes included two subclasses – immune-related and idiopathic. In immune-related type 1 diabetes, there is a recognizable autoimmune process for the pancreatic islet cell destruction, and in the latter the etiology is unknown.

4. Malnutrition-related diabetes mellitus was removed because of lack of evidence that diabetes can be directly caused by protein deficiency.

Therefore, this system which was adopted by the World Health Organization in 1999 (World Health Organization 1999) proposed a return to four basic types of diabetes – type 1 diabetes, type 2 diabetes, other specific types, and gestational diabetes. Because of advances in knowledge, there was a more detailed classification of the other specific classes of diabetes which comprised genetic defects of β-cell function including maturity-onset diabetes of the young (MODY), genetic defects in insulin action, diseases of the exocrine pancreas (including fibrocalculous pancreatopathy), endocrinopathies, drug- or chemical-induced diabetes, infections (e.g., congenital rubella), uncommon forms of immune-mediated diabetes (e.g., anti-insulin receptor antibodies), and other genetic syndromes sometimes associated with diabetes (e.g., Wolfram's syndrome). In addition to types of diabetes, the classification system recognized different stages in the natural history of diabetes including normoglycemia, intermediate hyperglycemia (impaired glucose tolerance and impaired fasting glucose), and three stages of diabetes – not insulin requiring, insulin requiring for control, and insulin required for survival. It was recognized that the stages of hyperglycemia may change over time, and movement between these stages can be bi-directional. Also the underlying disease process may be identifiable at any stage in the development of diabetes, even at the stage of normoglycemia. For example, individuals with islet cell antibodies may be normoglycemic, and in people with type 2 diabetes, the severity of hyperglycemia may regress with weight loss or progress with weight gain (World Health Organization 1999).

Future Directions

While the application of the current classification system to individuals is at times straightforward, this is not always the case, especially with respect to etiology and severity of the defect resulting in hyperglycemia and treatment requirements. There are many examples of the clinical challenge in classifying individuals including obese adolescents where differentiating between type 1 and type 2 diabetes at diagnosis can be very difficult and in the case of latent autoimmune diabetes of adults (LADA) (Botero and Wolfsdorf 2005; Farsani et al. 2013).

All methods currently available to assist with the classification of individuals have limitations including phenotypic characteristics such as age of onset and weight, genotyping since most forms of diabetes are polygenic, humoral or cellular immune biomarkers, and assessment of β-cell function (C-peptide) and insulin resistance.

There have been recent calls to review the classification system (Leslie et al. 2016; Schwartz et al. 2016) in an attempt to better contribute to an understanding of etiology, natural history, pathophysiology, consequences, and treatment (Leslie et al. 2016).

Schwartz et al. (2016) have proposed a β-cell-centric classification system based on an abnormal β-cell being the final common denominator of all diabetes. This proposal suggests that the diabetes spectrum results from interactions between genetically predisposed β-cells with other factors, including insulin resistance, environmental influences, and immune dysregulation. This could lead the way to choice of therapy based on the particular pathway(s) which lead to hyperglycemia that could optimize processes of care and precision medicine in the treatment of diabetes.

While there is a move to align classification systems and precision medicine in the future, a range of challenges will need to be overcome including deficiencies in our current knowledge base and limited access to currently available diagnostic tests to classify individuals with hyperglycemia, especially on a global scale.

Conclusion

The classification and diagnostic criteria of diabetes have changed over time. With the continued advancement in diabetes research, the classification and diagnostic criteria will continue to evolve. Regardless of how diabetes is defined and detected, early detection, prevention, and treatment remain the most important steps in halting the increasing global burden of diabetes.

References

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33:S62–9.

American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2015;38:S8–16.

Botero D, Wolfsdorf JI. Diabetes mellitus in children and adolescents. Arch Med Res. 2005;36:281–90.

Chen L, Magliano DJ, Balkau B, Colagiuri S, Zimmet PZ, Tonkin AM, Mitchell P, Phillips PJ, Shaw JE. AUSDRISK: an Australian type 2 diabetes risk assessment tool based on demographic, lifestyle and simple anthropometric measures. Med J Aust. 2010;192:197–202.

Colagiuri S, Davies D, Girgis S, Colagiuri R. National evidence based guideline for case detection and diagnosis of type 2 diabetes. Canberra: Diabetes Australia and the NHMRC; 2009.

Colagiuri S, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Jonhsen K. The DETECT-2 collaboration writing group. Glycemic thresholds for diabetes-specific retinopathy. Implications for diagnostic criteria for diabetes. Diabetes Care. 2011;34:145–50.

d'Emden MC, Shaw JE, Jones GR, Cheung NW. Guidance concerning the use of glycated haemoglobin (HbA1c) for the diagnosis of diabetes mellitus. A position statement of the Australian Diabetes Society. Med J Aust. 2015;203:89–91.

Dowse GK, Spark RA, Mavo B, Hodge AM, Erasmus RT, Gwalimu M, Knight LT, Koki G, Zimmet PZ. Extraordinary prevalence of non-insulin-dependent diabetes mellitus and bimodal plasma glucose distribution in the Wanigela people of Papua New Guinea. Med J Aust. 1994; 160:767–74.

Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, Nadran A, Sous ES, Ali MA. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diabetes Care. 1997;20:785–91.

Fan J, May SJ, Zhou Y, Barrett-Connor E. Bimodality of 2-h plasma glucose distributions in whites: the Rancho Bernardo study. Diabetes Care. 2005;28:1451–6.
Farsani SF, van der Aa MP, van der Vorst MM, Knibbe CA, de Boer A. Global trends in the incidence and prevalence of type 2 diabetes in children and adolescents: a systematic review and evaluation of methodological approaches. Diabetologia. 2013;56:1471–88.
HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358:1991–2002.
International Diabetes Federation. Diabetes atlas 2000. Brussels: IDF; 2000.
International Diabetes Federation. IDF diabetes atlas. 7th ed. Brussels: IDF; 2015.
Leslie RD, Palmer J, Schloot NC, Lemmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. Diabetologia. 2016;59:13–20.
Lim TO, Bakri R, Morad Z, Hamid MA. Bimodality in blood glucose distribution. Is it universal? Diabetes Care. 2002;25:2212–7.
Loo SG, Gowse GK, Finch C, Zimmet P. Bimodality analysis of frequency distributions of 2-hour plasma glucose concentrations in the urban Micronesian population of Kiribati. J Diabetes Complicat. 1993;7:73–80.
National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes. 1979;28:1039–57.
National Institute for Health and Care Excellence. Type 2 diabetes: prevention in people at high risk. London: National Institute for Health and Care Excellence; 2012.
NCD Risk Factor Collaboration (NCD-RisC). Effects of diabetes definition on global surveillance of diabetes prevalence and diagnosis: a pooled analysis of 96 population-based studies with 331,288 participants. Lancet Diabetes Endocrinol. 2015;3:624–37.
NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet. 2016;387:1513–30.
O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes. 1964;13:278–85.
O'Sullivan JB, Gellis SS, Dandrow RV, Tenney BO. The potential diabetic and her treatment in pregnancy. Obstet Gynecol. 1966;27:683–9. Obstet Gynecol. 2003;102:7
Omar MA, Seedat MA, Dyer RB, Motala AA, Knight LT, Becker PJ. South African Indians show a high prevalence of NIDDM and bimodality in plasma glucose distribution patterns. Diabetes Care. 1994;17:70–3.
Raper LR, Taylor R, Zimmet P, Milne B, Balkau B. Bimodality in glucose tolerance distributions in the urban Polynesian population of Western Samoa. Diabetes Res. 1984;1:1–8.
Report of a World Health Organization Consultation. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Diabetes Res Clin Pract. 2011;93:299–309.
Rosenthal M, McMahan CA, Stern MP, Eifler CW, Haffner SM, Hazuda HP, Franco LJ. Evidence of bimodality of two hour plasma glucose concentrations in Mexican Americans: results from the San Antonio heart study. J Chronic Dis. 1985;38:5–16.
Rushforth NB, Bennett PH, Steinberg AG, Burch TA, Miller M. Diabetes in the Pima Indians. Evidence of bimodality in glucose tolerance distribution. Diabetes. 1971;20:756–65.
Schwartz SS, Epstein S, Corkey BE, Grant SF, Gavin IIIJR, Aguilar RB. The time is right for a new classification system for diabetes: rationale and implications of the β-cell-centric classification schema. Diabetes Care. 2016;39:179–86.
The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 1997;20:1183–97.
The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care. 2003;26:3160–7.
The International Expert Committee. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care. 2009;32:1327–34.

Vistisen D, Colagiuri S, Borch-Johnsen K. The DETECT-2 collaboration. Bimodal distribution of glucose is not universally useful for diagnosing diabetes. Diabetes Care. 2009;32:397–403.
World Health Organization. Diabetes mellitus: report of a WHO expert committee, Technical report series, vol. 310. Geneva: World Health Organization; 1965.
World Health Organization. Expert committee on diabetes mellitus, Technical report series, vol. 646. Geneva: World Health Organization; 1980.
World Health Organization. Diabetes mellitus: report of a WHO study group, Technical report series, vol. 727. Geneva: World Health Organization; 1985.
World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; 1999.
World Health Organization. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: World Health Organization; 2013a.
World Health Organization. Global action plan for the prevention and control of noncommunicable diseases 2013–2020. Geneva: World Health Organization; 2013b.
World Health Organization, International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO/IDF consultation. Geneva: World Health Organization; 2006.
World Health Organization. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Abbreviated report of a WHO Consultation. Geneva: World Health Organization; 2011.
Zimmet P, Whitehouse S. Bimodality of fasting and two-hour glucose tolerance distributions in a Micronesian population. Diabetes. 1978;27:793–800.

Epidemiology and Risk Factors of Type 1 Diabetes

3

Chiara Guglielmi, Richard David Leslie, and Paolo Pozzilli

Contents

C. Guglielmi
Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio Medico, Rome, Italy

R. David Leslie
Centre of Diabetes, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK
e-mail: r.d.g.leslie@qmul.ac.uk

P. Pozzilli (✉)
Department of Endocrinology and Diabetes, University Campus Bio-Medico, Rome, Italy

Centre of Diabetes, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Unit of Endocrinology and Diabetes, Department of Medicine, University Campus Bio Medico, Rome, Italy
e-mail: p.pozzilli@unicampus.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_4

Abstract

Type 1 diabetes (T1D) is one of the most widespread chronic diseases of childhood. T1D results from the autoimmune destruction of insulin-producing beta cells in the pancreas. Genetic, epigenetic, metabolic, and environmental factors act together to precipitate the onset of the disease. Clinical T1D represents the end stage of a process resulting from the progressive beta-cell destruction following an asymptomatic period that may last for years. This knowledge, together with recent advances in the ability to identify individuals at increased risk for clinical disease, has paved the way for trials aimed at preventing or delaying the clinical onset of T1D. Individuals at risk for T1D can be identified by a positive family history or by genetic, immunological, or metabolic markers. These markers can be combined to achieve a higher positive predictive value for T1D and to identify those individuals to be selected for intervention trials.

The purpose of this chapter is to set out the epidemiology and the main risk factors which characterizes T1D.

Keywords

Age · Body mass index (BMI) · Epidemiology · Geography · Gender · Risk factors · Seasonality · Type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an heterogeneous disorder characterized by damage of pancreatic beta cells, terminating in absolute insulin deficiency. Genetic, metabolic, and environmental factors act together to precipitate the onset of the disease. The excess mortality associated with complications of T1D and the increasing incidence of childhood T1D emphasize the importance of therapeutic strategies to prevent this chronic metabolic disorder. T1D is one of the most widespread chronic diseases of childhood, affecting children, adolescents, and young adults (International Diabetes Federation (IDF)).

The global incidence of T1D in children and adolescents is rising with an estimated overall annual increase of approximately 3%. T1D accounts for about 10% of all cases of diabetes, occurs most commonly in people of European descent, and affects two million people in Europe and North America. The lowest incidence has been found in Asia and Oceania and the highest in Europe.

The increase in incidence of T1D has been shown in countries having both high- and low-prevalence figures (see Fig. 1), with an indication of a steeper increase in some of the low-prevalence countries. Several European studies have suggested that, in relative terms, the increase is more pronounced in young children. Although T1D usually accounts for only a minority of the total burden of diabetes in a population, it is the predominant form of the disease in younger age groups in most developed countries.

There are strong indications of geographic differences in trend, but the overall annual increase is estimated around 3%. About 79,100 children under 15 years are

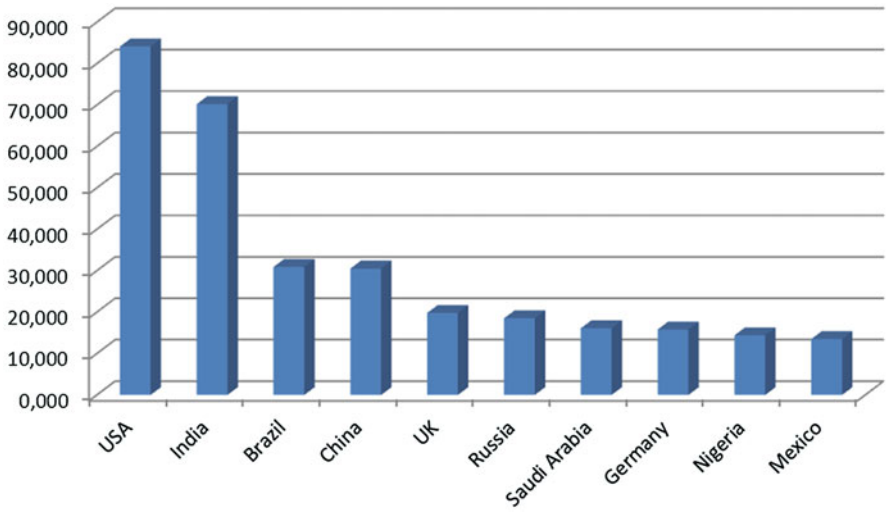


Fig. 1 Top 10 countries for number of children with type 1 diabetes (0–14 years) (Data from Diabetes ATLAS (International Diabetes Federation (IDF)))

estimated to develop T1D annually worldwide. Of the estimated 497,100 children living with T1D, 26% live in Europe Region and 22% in the North America and Caribbean Region (https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf).

Increasingly efforts need to be directed toward early diagnosis of T1D because it is a condition leading to early complications and the potential availability of disease-modifying interventions underscores the need for early diagnosis.

Incidence of Type 1 Diabetes

The incidence of T1D differs based upon geography, ethnicity, age, gender, family history, and BMI. The incidence of T1D begins sharply to rise at about 9 months of age, continues to rise until age 12–14 years, and then declines (Tuomilehto 2013). A similar pattern is seen in many other countries irrespective of whether the overall incidence of T1D is low or high (Patterson et al. 2014).

Geographical Differences

The incidence of childhood T1D varies worldwide (You and Henneberg 2016; Beyerlein et al. 2015; Miller et al. 2011). In Europe and China, the risk appears to rise as the geographical latitude increases (Kalliora et al. 2011; Patterson et al. 2012), but this North-South disparity is not found in the United States, even after adjusting for racial and ethnic variation (Liese et al. 2010).

In Europe, the highest incidence rates are in Finland (Harjutsalo et al. 2013) and in Sardinia (Italy) (Fortunato et al. 2016).

Rates in these countries are almost 400 times that of Venezuela and parts of China, which have the lowest incidence (0.1–0.5 per 100,000 children) (Zhao et al. 2014). The incidence rate of T1D in the white population of the United States is higher than those recorded for countries of Northern Europe but significantly lower than those in Sweden and Finland. In the United States, the incidence of T1D in non-Hispanic white children and adolescents is 23.6 per 100,000 per year, and rates are substantially lower in other racial or ethnic groups (Bell et al. 2009).

Extensive dissimilarities in incidence occur between neighboring areas of similar latitude, suggesting the presence of other contributing risk factors and demonstrating the complexity of the pathogenesis of T1D and more interestingly the observation that when people relocate from a region of low to high incidence, their risk of developing T1D also increases, underlying a causative role for environmental factors.

Seasonality

Seasonal disparity at the time of diagnosis of T1D has been described from many records both in Europe (Moltchanova et al. 2009) and worldwide (Kalliora et al. 2011) with most reports suggesting a winter peak. Seasonal variation in sunshine hours is particularly relevant to vitamin D levels because most of the body's vitamin D is synthesized through the action of sunlight on the skin. The evidence from animal experiments and observational studies in humans of a role for vitamin D in the etiology of T1D has been widely described in literature, and some data suggest a role of vitamin D in the pathogenic process leading to the destruction of the insulin-producing cells (Sørensen et al. 2016; Altieri et al. 2016; Mäkinen et al. 2016).

Two meta-analyses of retrospective studies indicated that the risk of T1D was lower in infants who were supplemented with vitamin D (calcitriol) compared with those who were not supplemented (pooled odds ratio 0.71) (Miettinen et al. 2012; Zipitis and Akobeng 2008). On the other hand, the DAISY study examined 25-hydroxyvitamin D concentrations in infancy and throughout childhood and found no association with islet autoimmunity or progression to T1D (Simpson et al. 2011), and also two studies carried out in new-onset T1D reported no effect of vitamin D supplementation on sustained insulin production (Bizzarri et al. 2010; Walter et al. 2010).

In summary, despite continuing interest in vitamin D supplementation as a potential intervention to prevent islet autoimmunity and T1D, there is surprisingly little supporting evidence from prospective birth cohort studies (Rewers and Ludvigsson 2016).

Age

T1D is a heterogeneous disease, and it is the major type of diabetes in youth, accounting for $\geq$85% of all diabetes cases in youth $<$20 years of age worldwide (Kalliora et al. 2011; Chowdhury 2015). In general, the incidence rate increases from

birth and peaks between the ages of 10 and 14 years during puberty (Kalliora et al. 2011; Chowdhury 2015). Incidence rates decline after puberty and appear to stabilize in young adulthood (15–29 years).

A subgroup of individuals who develop diabetes in later life with clinical features of T2D but test positive for GAD autoantibodies are called LADA (latent autoimmune diabetes in the adults) (Leslie et al. 2006, 2008). The three criteria conventionally used to describe LADA are non-specific, namely, age at diagnosis, autoantibody positivity, and need for insulin treatment. Definitions of adult age range from 15 to 30 years, extending to all ages or up to 70 years. Up to 10% of adults initially thought to have T2D are found to have antibodies associated with T1D, and beta-cell destruction in adults appears to occur at a much slower rate than in young T1D cases, often delaying the need for insulin therapy after diagnosis.

The increasing incidence of T1D throughout the world is especially marked in young children (Patterson et al. 2009). The incidence of T1D in adults is lower than in children, although approximately one-fourth of persons with T1D are diagnosed as adults (Chiang et al. 2014; Lado and Lipman 2016).

Gender

Although most common autoimmune diseases disproportionately affect females, on average girls and boys are equally affected with T1D in young populations (Soltesz et al. 2007). A distinctive pattern has been observed such that regions with a high incidence of T1D (populations of European origin) have a male excess, whereas regions with a low incidence (populations of non-European origin) report a female excess (Svensson et al. 2003; Ostman et al. 2008). In contrast, clear male dominance has emerged from most studies of patients with T1D diagnosed between 15 and 40 years (Gale and Gillespie 2001; Kyvik et al. 2004). Adult T1D appears to differ from other common autoimmune diseases, which typically show a strong female excess, as does diabetes in the nonobese diabetic (NOD) mouse.

BMI

In 2001, Terry Wilkin (2001) presented the "accelerator hypothesis" in which he suggested that T1D and T2D could be defined the same disorder differentiated by the rate of beta-cell loss (Boitard et al. 2005). Since then literature has supported this hypothesis showing that BMI and changes in weight are inversely related to age at diagnosis for T1D. Knerr and colleagues in a large group of T1D children showed that a higher BMI is associated with a younger age at onset of T1D and that an increased weight gain could be considered a risk factor for early manifestation of the disease (Knerr et al. 2005). Moreover, Dabelea et al. (2006), in another study, concluded that an increasing BMI is associated with younger age at diagnosis only in subjects with a reduced beta-cell function and hypothesized that obesity may accelerate the onset of T1D.

Risk Factors for Type 1 Diabetes

Evidence for the role of environmental factors in the development of autoimmune diabetes is provided by population, migration, and twin studies. In North America and Europe, and possibly worldwide, population studies have shown that the incidence of childhood T1D has been increasing over the past 100 years, particularly in younger age groups (Pociot and Lernmark 2016). On the other hand, the proportion of diabetic patients with high diabetes-risk genotypes (DR4-DQ8/DR3-DQ2) has decreased and lower-risk genotypes (DR4-DQ8/X and DR3-DQ2/X) has increased, implying an increasing role in environmental factors (acting in genetically susceptible persons) in promoting diabetes (Rewers and Ludvigsson 2016). Migration studies have shown that Asian children in families who have moved to Britain show an increased incidence in T1D much higher than the incidence in their native countries and approaching that of the indigenous population (Bodansky et al. 1992). Although, it should be noted that in contrast to this, Sardinian migrants to Italy retained their ancestral high incidence of diabetes, suggesting genetics plays a stronger role in determining disease susceptibility (Muntoni et al. 1997). Twin studies have shown that if one of a monozygotic twin pair has diabetes, the risk of their nondiabetic co-twin of developing diabetes after 40 years is estimated to be 50% (Redondo et al. 2001). Interestingly, if the proband is diagnosed before the age of 25 years, the probability of the co-twin developing diabetes is 38%, compared with only 6% for twins of probands diagnosed later (Redondo et al. 2001; Hyttinen et al. 2003). This age of onset-dependent difference in the risk of a monozygotic co-twin developing diabetes cannot be explained by differences in HLA-type distribution (Redondo et al. 2001).

The primary risk factor for beta-cell autoimmunity is genetic, mainly occurring in individuals with either HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes or both, but a trigger from the environment is generally needed (Pociot and Lernmark 2016).

T1D pathogenesis can be divided into three stages, (1) appearance of beta-cell autoimmunity, normoglycemia and no symptoms; (2) beta-cell autoimmunity, dysglycemia and no symptoms; and (3) beta-cell autoimmunity, dysglycemia and symptoms of diabetes (Insel et al. 2015), and the genetic association with each one of the three stages can differ (Pociot and Lernmark 2016).

Portuesi R and colleagues assessed the risk conferred by HLA-DRB1, INS-VNTR, and PTPN22 single genes on the onset of T1D and the joint risk conferred by all these three susceptibility loci using the Bayesian network (BN) approach in a case-control French cohort, consisting of 868 T1D patients and 73 French control subjects, and in a French family data set consisting of 1694 T1D patients and 2340 controls. This is the first study based on both case-control and family data sets, showing the joint effect of HLA, INS, and PTPN22 in a T1D Caucasian population with a wide range of age at T1D onset (Portuesi et al. 2013) (see Fig. 2).

A number of investigations have been made into putative risk factors, other than strictly genetic effects, for the development of autoimmune diabetes, and the major suspects are described below (see Table 1):

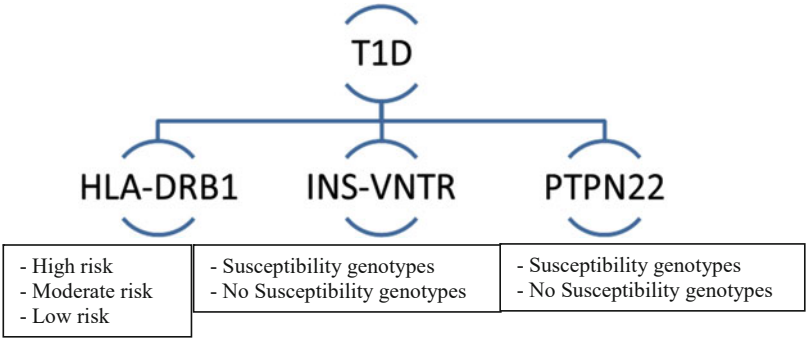
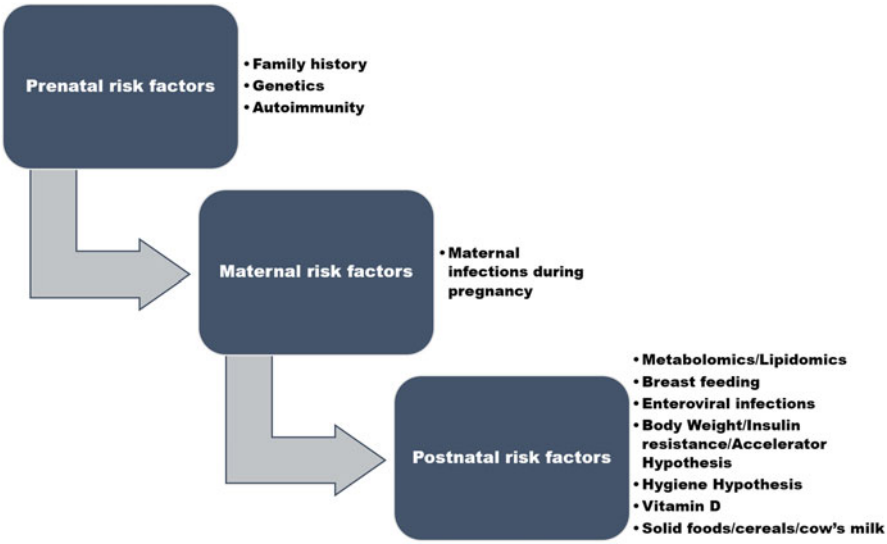


Fig. 2 The Bayesian network implemented to assess risk to develop T1D (Modified from Bodansky et al. (1992))

Table 1 Major risk factors for T1D



- *Family history.* According to a large Swedish study, a family history of diabetes (a first-degree relative diagnosed with diabetes) was associated with a four times increased risk in prevalence of LADA, which may be attributable to an inherited reduction in insulin secretion (Carlsson et al. 2007). Otherwise, the majority of studies into familial risk of autoimmune diabetes have considered only childhood-onset T1D. Siblings of diabetes subjects have a 15 times increased risk of developing diabetes compared to the general population (Insel et al. 2015). Interestingly, familial diabetes risk appears to be transmitted down the paternal line, with the proportion of affected children having a father with T1D exceeding

that of affected children having a mother with T1D (3.4% vs. 1.8%, respectively) (Bingley and Gale 2006). Also, there may be preferential transmission of disease from a father to his daughter than to his son (Gillespie et al. 2002).

- *Infections*. Several ecological reports and case report studies have drawn attention to viral infections as a potential cause of T1D. Bacterial infections are rarely discussed, although bacteria as a cause of pancreatic lesions cannot be excluded. Several viruses have been implicated, with enteroviruses having the strongest evidence from studies in animal models and also in human beings (Rewers and Ludvigsson 2016). Infection with an enterovirus, particularly the coxsackie B virus, has been the subject of a number of investigations looking at potential risk factors for T1D (Sane et al. 2013). Although the results have not always been consistent, a recent systematic review and meta-analysis of case-control studies have shown a strong association between enteroviral infection and T1D-related autoimmunity (odds ratio 3.7) and clinical T1D (odds ratio 9.8). The relationship is most prominent in subjects carrying high-risk HLA-DQB1 genotypes, representing a gene-environment interaction, which precipitates diabetes. Although the causal mechanism connecting enteroviral infection to T1D is not well understood, hypotheses include direct infection of beta cells causing functional impairment and cell lysis (Wen et al. 2008; Jaïdane et al. 2010) and molecular mimicry resulting in autoimmune destruction of beta cells (Stene et al. 2010). The seasonal variation in the first appearance of diabetes-associated antibodies, considered the earliest predictors of T1D and being higher during colder months, also provides some (weak) evidence for an association between viral infections in (also more frequent in winter) T1D. Finally, a fascinating line of evidence proposes that enteroviral infections during pregnancy might result in persistent infection and islet autoimmunity in the mother and offspring (Viskari et al. 2012).
- *Intestinal microbiota*. Some of the candidate environmental factors for T1D (cesarean delivery, early childhood diet, and use of antibiotics) are intertwined with the development and function of the human microbiome (Rewers and Ludvigsson 2016). Gut microbes influence lipid and glucose metabolism, as well as immunity and systemic inflammation outside of the intestine (Wen et al. 2008). Commensal microbiota might modulate the risk of T1D, but studies so far have been underpowered. Some have reported lower microbial diversity in children with islet autoimmunity before progression to diabetes, compared with healthy controls (Rewers and Ludvigsson 2016).
- *Solid food/cereals*. Dietary exposures in infancy have been implicated in the etiology of T1D, and there is evidence in literature that too early or too late introduction of solids might increase baby's risk for T1D. In the DAISY study, the timing of introduction of any type of cereal (gluten and non-gluten containing) was associated with an increased risk of islet autoimmunity with nadir at introduction at 4–6 months of life (Norris et al. 2003), while the BABYDIET study, a primary prevention trial, was designed to investigate whether delay of the introduction of dietary gluten can prevent the development of islet autoimmunity in newborns with a first-degree relative with T1D, who are at genetically high risk of

T1D 14; children who participate in BABYDIET were randomly assigned to one of two dietary intervention groups that introduced cereals that contain gluten either at age 6 months, but no benefit was found in delaying gluten exposure with respect to autoimmunity associated with diabetes or celiac disease (Hummel et al. 2011).

- *Cord blood/metabolomic/lipidomic*. Children developing T1D may have risk markers already in their umbilical cord blood. It is hypothesized that the risk for T1D at an early age may be increased by a pathogenic pregnancy and be reflected in altered cord blood composition. La Torre and colleagues (La Torre et al. 2013) identified a total of 106 lipid metabolites in the cord blood samples of the 152 children, including phospholipids (PLs) and triglycerides (TGs), and they were able to demonstrate that low levels of phosphatidylcholines and phosphatidylethanolamines increased the risk for T1D diagnosed before 4 years of age.
- *Hygiene hypothesis*. Decreasing infections in Europe in the last 50 years correlates with the trend for increasing autoimmune disease. A number of explanations have been proposed including infection-induced upregulation of Treg cells and the subsequent suppression of autoimmunity-promoting Th1 responses. The timing of the infection is also important, and appropriate "protective infections" may delay the onset of diabetes in susceptible populations (Jaïdane et al. 2010).
- *Body mass index (BMI)*. BMI can predict progression to T1D in children, particularly in the context of impaired beta-cell function (low fasting C-peptide) (Dabelea et al. 2006), and in this context, Barker and colleagues investigated whether BMI measured at diagnosis was an independent predictor of C-peptide decline 1-year post-diagnosis (Lauria et al. 2015). A multicentre longitudinal study was carried out at the time of T1D diagnosis and up to 1-year follow-up in more than 3000 subjects. In individuals diagnosed between 0 and 5 years and 5 and 10 years and those diagnosed >18 years, no association was found between BMI and C-peptide declines. In patients aged 10–18 years, higher BMI at baseline was associated with a greater decline in fasting C-peptide over 1 year with a decrease (β 95% CI; P value) of 0.025 (0.010, 0.041) nM/kg per m^2 higher baseline BMI ($P = 0.001$). This association remained significant after adjusting for gender and differences in HbA1c and insulin dose ($\beta = 0.026$, 95% CI = 0.0097, 0.042; $P = 0.002$). This study indicates that increased body weight and increased insulin demand are associated with more rapid disease progression after diagnosis of T1D in an age group 10–18 years (Lauria et al. 2015).
- *Early weight gain*. Weight gain in the first 2 years of life, particularly in the context of HLA-susceptible persons, is associated with increased ICA in first-degree relatives (as children) of T1D patients; relatives of adult-onset diabetes cases have not been studied (Couper et al. 2009).
- *Insulin resistance*. In subjects with ICA, insulin resistance can predict those that are likely to progress to diabetes (Fourlanos et al. 2004). Insulin resistance could be accelerating the development of clinically overt diabetes or could be secondary to the systemic changes which occur in autoimmune disease, i.e., release of insulin resistance-promoting cytokines such as TNFα (Meah et al. 2016).

- *Breast-feeding*. Duration of breast-feeding, particularly short-term exclusive breast-feeding, particularly in the context of HLA-associated diabetes susceptibility genotypes has been found to be associated with childhood-onset type 1 diabetes (Nucci et al. 2015), although this has not been a consistent finding in similar investigations (Ziegler et al. 2003; Virtanen et al. 2006; Norris et al. 2003).
- *Cow's milk*. Most prospective birth cohort studies have not shown any link between early exposure to cows' milk and either islet autoimmunity or T1D. A large worldwide trial called TRIGR aimed to answer the question of whether cow's milk administered in early life is diabetogenic and whether the use of cow's milk hydrolysate can protect from the disease. The rationale behind the use of cow's milk hydrolysate for primary prevention of T1D is based on several epidemiological and in vitro studies indicating that intact cow's milk, if given before 3 months of age, may induce an immune response toward beta cells (Pozzilli et al. 2003). TRIGR is a double-blind randomized clinical trial of 2159 infants with HLA-conferred disease susceptibility and a first-degree relative with T1D recruited from May 2002 to January 2007 in 78 study centers in 15 countries; 1078 were randomized to be weaned to the extensively hydrolyzed casein formula, and 1081 were randomized to be weaned to a conventional cows' milk-based formula (TRIGR Study Group 2007; Knip et al. 2014). Primary outcome was positivity for at least two diabetes-associated autoantibodies out of four analyzed. Autoantibodies to insulin, glutamic acid decarboxylase, and the insulin-associated-2 (IA-2) molecule were analyzed using radiobinding assays and islet cell antibodies with immunofluorescence during a median observation period of 7.0 years (mean, 6.3 years). The absolute risk of positivity for two or more islet autoantibodies was 13.4% among those randomized to the casein hydrolysate formula ($n = 139$) versus 11.4% among those randomized to the conventional formula ($n = 117$). The unadjusted hazard ratio for positivity for two or more autoantibodies among those randomized to be weaned to the casein hydrolysate was 1.21 (95% CI, 0.94–1.54), compared with those randomized to the conventional formula, while the hazard ratio adjusted for HLA risk, duration of breast-feeding, vitamin D use, study formula duration and consumption, and region was 1.23 (95% CI, 0.96–1.58). In conclusion, TRIGR study showed that among infants at risk for T1D, the use of a hydrolyzed formula compared with a conventional formula did not reduce the incidence of diabetes-associated autoantibodies (Knip et al. 2014). The results of the effect of this treatment on diabetes insurgence are expected in 2017.
- *Age of introduction of complex nutrients*. Early introduction of gluten (e.g., via cow's milk) into an infant's diet has been shown to increase the risk of developing diabetes. A pilot intervention trial in which infants with risk-associated HLA-DQB1 haplotypes were given either conventional cow's milk or casein hydrolysate demonstrated decreased frequency of seroconversion to ICA in the casein hydrolysate arm (Akerblom et al. 2005), although a larger study will be needed to confirm the results.
- *Vitamin D*. Dietary vitamin D may be protective against T1D. Vitamin D levels may affect the immune response through the modulation of relative pro- and anti-inflammatory cytokine levels (Fronczak et al. 2003).

- *The overload hypothesis*. A number of environmental factors – in particular child growth and weight and fetal priming (where overfeeding or starving the fetus may alter metabolic programming, tending toward increased insulin resistance or increased liability to beta-cell death) – increase beta-cell stress on a background of autoimmunity, which could explain the tendency toward earlier development of T1D in European populations (Dahlquist 2006).
- *The accelerator hypothesis*. Previous reports have predicted greater risk of T1D among people who were heavier as young children. The accelerator hypothesis predicts earlier onset in heavier people. The relationships between fatness and age at diagnosis were examined in context of birth weight, weight change since birth, weight at diagnosis, BMI at diagnosis, and BMI 12 months later in 94 children aged 1–16 years presenting for management of acute-onset T1D by Kibirige and colleagues (2003), and the results of the study were consistent with the hypothesis that the age at presentation of T1D is associated with fatness.

Summary

T1D is one of the most common chronic diseases of childhood, and it accounts for approximately two-thirds of all cases of diabetes in patients younger than 18 years of age. T1D incidence varies up to 100-fold among different countries, and the incidence increases with the age of the children/adolescents.

Research on risk factors for T1D is an active area of research that will help to classify more precisely genetic and environmental triggers that could theoretically be targeted for intervention. While significant advances have been made in the clinical care of T1D with resultant improvements in quality of life and clinical outcomes, much more needs to be done to improve care of and ultimately to find a cure for T1D (Gale 2002).

Future research should focus on knowing environmental and genetic risk factors of T1D and its complications, preventive strategies, and causal treatment options. The prevalence, which doubled worldwide over the last decades, will increase further, and T1D will shift more and more into the focus of general practitioners. It becomes conclusive that T1D will be a burden for more and more patients and for the majority of health-care systems.

References

Akerblom HK, Virtanen SM, Ilonen J, et al. Dietary manipulation of beta cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study. Diabetologia. 2005;48:829–37.

Altieri B, Grant WB, Casa SD, et al. Vitamin D and pancreas: the role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. Crit Rev Food Sci Nutr. 2017;57(16):3472–3488.

Bell RA, Mayer-Davis EJ, Beyer JW, SEARCH for Diabetes in Youth Study Group, et al. Diabetes in non-Hispanic white youth: prevalence, incidence, and clinical characteristics: the SEARCH for Diabetes in Youth Study. Diabetes Care. 2009;32(Suppl 2):S102.

Beyerlein A, Krasmann M, Thiering E, et al. Ambient air pollution and early manifestation of type 1 diabetes. Epidemiology. 2015;26:e31–2.
Bingley PJ, Gale EA, European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. Progression to type 1 diabetes in islet cell antibody-positive relatives in the European Nicotinamide Diabetes Intervention Trial: the role of additional immune, genetic and metabolic markers of risk. Diabetologia. 2006;49:881–90.
Bizzarri C, Pitocco D, Napoli N, for the IMDIAB Group, et al. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. Diabetes Care. 2010;33:1962–3.
Bodansky HJ, Stephenson C, Haigh D, et al. Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population. BMJ. 1992;304:1020–2.
Boitard C, Efendic S, Ferrannini E, et al. A tale of two cousins: type 1 and type 2 diabetes. Diabetes. 2005;54:S1–3.
Carlsson S, Midthjell K, Grill V. Influence of family history of diabetes on incidence and prevalence of latent autoimmune diabetes of the adult: results from the Nord-Trøndelag Health Study. Diabetes Care. 2007;30:3040–5.
Chiang JL, Kirkman MS, Laffel LMB, et al. Type 1 diabetes through the life span: a position statement of the American Diabetes Association. Diabetes Care. 2014;37:2034–54.
Chowdhury S. Puberty and type 1 diabetes. Indian J Endocrinol Metab. 2015;19:S51–4.
Couper JJ, Beresford S, Hirte C, et al. Weight gain in early life predicts risk of islet autoimmunity in children with a first-degree relative with type 1 diabetes. Diabetes Care. 2009;32:94–9.
Dabelea D, D'Agostino RB Jr, Mayer-Davis EJ, et al. Testing the accelerator hypothesis: body size, β-cell function, and age at onset of type 1 (autoimmune) diabetes. Diabetes Care. 2006;29:290–4.
Dahlquist G. Can we slow the rising incidence of childhood-onset autoimmune diabetes? The overload hypothesis. Diabetologia. 2006;49:20–4.
Fortunato F, Cappelli MG, Vece MM, et al. Incidence of type 1 diabetes among children and adolescents in Italy between 2009 and 2013: the role of a regional childhood diabetes registry. J Diabetes Res. 2016;2016:7239692.
Fourlanos S, Narendran P, Byrnes GB, et al. Insulin resistance is a risk factor for progression to type 1 diabetes. Diabetologia. 2004;47:1661–7.
Fronczak CM, Barón AE, Chase HP, et al. In utero dietary exposures and risk of islet autoimmunity in children. Diabetes Care. 2003;26:3237–42.
Gale EA. The rise of childhood type 1 diabetes in the 20th century. Diabetes. 2002;51:3353–61.
Gale EA, Gillespie KM. Diabetes and gender. Diabetologia. 2001;44:3–15.
Gillespie KM, Gale E, Bingley PJ. High familial risk and genetic susceptibility in early onset childhood diabetes. Diabetes. 2002;51:210–4.
Harjutsalo V, Sund R, Knip M, et al. Incidence of type 1 diabetes in Finland. JAMA. 2013;310:427–8.
Hummel S, Pflüger M, Hummel M, et al. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care. 2011;34:1301–5.
Hyttinen V, Kaprio J, Kinnunen L, et al. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs. Diabetes. 2003;52:1052–5.
Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015;38:1964–74.
International Diabetes Federation (IDF). Atlas data. http://www.diabetesatlas.org/.
Jaïdane H, Sauter P, Sane F, et al. Enterovirus and type 1 diabetes: towards a better understanding of the relationship. Rev Med Virol. 2010;20:265–80.
Kalliora MI, Vazeou A, Delis D, Bozas E, Thymelli I, Bartsocas CS. Seasonal variation of type 1 diabetes mellitus diagnosis in Greek children. Hormones (Athens). 2011;10:67–71.
Kibirige M, Metcalf B, Renuka R, et al. Testing the accelerator hypothesis: the relationship between body mass and age at diagnosis of type 1 diabetes. Diabetes Care. 2003;26:2865–70.

Knerr I, Wolf J, Reinehr T, et al. The 'accelerator hypothesis': relationship between weight, height, body mass index and age at diagnosis in a large cohort of 9,248 German and Austrian children with type 1 diabetes mellitus. Diabetologia. 2005;48:2501–4.
Knip M, Åkerblom HK, Becker D, Dosch HM, Dupre J, Fraser W, Howard N, Ilonen J, Krischer JP, Kordonouri O, Lawson ML, Palmer JP, Savilahti E, Vaarala O, Virtanen SM, TRIGR Study Group. Hydrolyzed infant formula and early β-cell autoimmunity: a randomized clinical trial. JAMA. 2014;311:2279–87.
Kyvik KO, Nystrom L, Gorus F, et al. The epidemiology of type 1 diabetes mellitus is not the same in young adults as in children. Diabetologia. 2004;47:377–84.
La Torre D, Seppänen-Laakso T, Larsson HE, et al. Decreased cord-blood phospholipids in young age–at–onset type 1 diabetes. Diabetes. 2013;62:3951–6.
Lado JJ, Lipman TH. Racial and Ethnic disparities in the incidence, treatment, and outcomes of youth with type 1 diabetes. Endocrinol Metab Clin N Am. 2016;45:453–61.
Lauria A, Barker A, Schloot N, et al. BMI is an important driver of b-cell loss in type 1 diabetes upon diagnosis in 10 to 18-year-old children. Eur J Endocrinol. 2015;172:107–13.
Leslie RD, Williams R, Pozzilli P. Clinical review: type 1 diabetes and latent autoimmune diabetes in adults: one end of the rainbow. J Clin Endocrinol Metab. 2006;91:1654–9.
Leslie RD, Kolb H, Schloot NC, et al. Diabetes classification: grey zones, sound and smoke: action LADA 1. Diabetes Metab Res Rev. 2008;24:511–9.
Liese AD, Lawson A, Song HR, et al. Evaluating geographic variation in type 1 and type 2 diabetes mellitus incidence in youth in four US regions. Health Place. 2010;16(3):547–56.
Mäkinen M, Mykkänen J, Koskinen M, et al. Serum 25-Hydroxyvitamin D concentrations in children progressing to autoimmunity and clinical type 1 diabetes. J Clin Endocrinol Metab. 2016;101:723–9.
Meah FA, DiMeglio LA, Greenbaum CJ, Type 1 Diabetes TrialNet Study Group, et al. The relationship between BMI and insulin resistance and progression from single to multiple autoantibody positivity and type 1 diabetes among TrialNet Pathway to Prevention participants. Diabetologia. 2016;59:1186–95.
Miettinen ME, Reinert L, Kinnunen L, et al. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the off spring. Diabetologia. 2012;55:1291–4.
Miller LJ, Willis JA, Pearce J, et al. Urban-rural variation in childhood type 1 diabetes incidence in Canterbury, New Zealand, 1980–2004. Health Place. 2011;17:248–56.
Moltchanova EV, Schreier N, Lammi N, et al. Seasonal variation of diagnosis of type 1 diabetes mellitus in children. Diabet Med. 2009;26:673–8.
Muntoni S, Fonte MT, Stoduto S, et al. Incidence of insulin-dependent diabetes mellitus among Sardinian-heritage children born in Lazio region, Italy. Lancet. 1997;349:160–2.
Norris JM, Barriga K, Klingensmith G, et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA. 2003;290:1713–20.
Nucci AM, Virtanen SM, Becker DJ. Infant feeding and timing of complementary foods in the development of type 1 diabetes. Curr Diab Rep. 2015;15:62.
Ostman J, Lönnberg G, Arnqvist HJ, et al. Gender differences and temporal variation in the incidence of type 1 diabetes: results of 8012 cases in the nationwide Diabetes Incidence Study in Sweden 1983–2002. J Intern Med. 2008;263:386–94.
Patterson CC, Dahlquist GG, Gyurus E, et al. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. Lancet. 2009;373:2027–33.
Patterson CC, Gyürüs E, Rosenbauer J, et al. Trends in childhood type 1 diabetes incidence in Europe during 1989–2008: evidence of non-uniformity over time in rates of increase. Diabetologia. 2012;55:2142–7.
Patterson C, Guariguata L, Dahlquist G, Soltész G, Ogle G, Silink M. Diabetes in the young – a global view and worldwide estimates of numbers of children with type 1 diabetes. Diabetes Res Clin Pract. 2014;103:161–75.
Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet. 2016;387:2331–9.

Portuesi R, Pozzilli P, Boehm B, Buzzetti R, Filippi S. Assessment of type 1 diabetes risk conferred by HLA-DRB1, INS-VNTR and PTPN22 genes using the Bayesian network approach. PLoS One. 2013;8:e79506.
Pozzilli P, Manfrini S, Picardi A. Cow's milk and trials for prevention of type 1 diabetes. Diabet Med. 2003;20:871–2.
Redondo MJ, Yu L, Hawa M, et al. Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. Diabetologia. 2001;44:354–62.
Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet. 2016;387:2340–8.
Sane F, Caloone D, Gmyr V, et al. Coxsackievirus B4 can infect human pancreas ductal cells and persist in ductal-like cell cultures which results in inhibition of Pdx1 expression and disturbed formation of islet-like cell aggregates. Cell Mol Life Sci. 2013;70:4169–80.
Simpson M, Brady H, Yin X, et al. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia. 2011;54:2779–88.
Soltesz G, Patterson CC, Dahlquist G. Worldwide childhood type 1 diabetes incidence – what can we learn from epidemiology? Pediatr Diabetes. 2007;8:6–14.
Sørensen IM, Joner G, Jenum PA, et al. Vitamin D-binding protein and 25-hydroxyvitamin D during pregnancy in mothers whose children later developed type 1 diabetes. Diabetes Metab Res Rev. 2016;32:883.
Stene LC, Oikarinen S, Hyöty H, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes. 2010;59:3174–80.
Svensson J, Carstensen B, Mortensen HB, et al. Gender-associated differences in type 1 diabetes risk factors? Diabetologia. 2003;46:442–3.
TRIGR Study Group. Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR). Pediatr Diabetes. 2007;8:117–37.
Tuomilehto J. The emerging global epidemic of type 1 diabetes. Curr Diab Rep. 2013;13:795–804.
Virtanen SM, Kenward MG, Erkkola M, et al. Age at introduction of new foods and advanced beta cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes. Diabetologia. 2006;49:1512–21.
Viskari H, Knip M, Tauriainen S, et al. Maternal enterovirus infection as a risk factor for type 1 diabetes in the exposed off spring. Diabetes Care. 2012;35:1328–32.
Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1alpha, 25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. Diabetes Care. 2010;33:1443–8.
Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature. 2008;455:1109–13.
Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. Diabetologia. 2001;44:914–22.
You WP, Henneberg M. Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. BMJ Open Diabetes Res Care. 2016;4:e000161.
Zhao Z, Sun C, Wang C, et al. Rapidly rising incidence of childhood type 1 diabetes in Chinese population: epidemiology in Shanghai during 1997–2011. Acta Diabetol. 2014;51:947–53.
Ziegler AG, Schmid S, Huber D, et al. Early infant feeding and risk of developing type 1 diabetes–associated autoantibodies. JAMA. 2003;290:1721–8.
Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child. 2008;93:512–7.

Epidemiology and Risk Factors of Type 2 Diabetes

4

Sylvia H. Ley and James B. Meigs

Contents

S. H. Ley
Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
e-mail: sylvia.ley@channing.harvard.edu

J. B. Meigs (✉)
Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
e-mail: JMEIGS@mgh.harvard.edu

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_5

Abstract

Type 2 diabetes has become a serious public health concern. It has multiple behavioral, metabolic, and genetic risk factors. Excess body fat, especially central obesity, is the strongest risk factor for type 2 diabetes. Diets favoring higher intake of whole grains, green leafy vegetables, and coffee and lower intake of refined grains, red and processed meat, and sugar-sweetened beverages have been associated with a lower risk of type 2 diabetes. Regular physical activity, ranging from brisk walking to higher-intensity endurance or resistance training, has been associated with a lower risk of type 2 diabetes. Novel biomarkers, such as adipokines and inflammatory cytokines, and intermediate conditions, such as metabolic syndrome, have offered the potential to improve diabetes prediction. Multiple diabetes genetic variants have been identified, and the collaborative efforts are made to investigate gene-environment interactions. Continued work to prevent diabetes is warranted through development of precision health interventions and public health strategies targeting these risk factors.

Keywords

Type 2 diabetes · Epidemiology · Risk factors

Introduction

Type 2 diabetes has become a major public health concern globally and in the United States (US) (International Diabetes Federation 2015). Type 2 diabetes is a heterogeneous disease involving multiple risk factors. Large prospective studies have improved the understanding of modifiable risk factors for type 2 diabetes (Ley et al. 2014). However, individual responses to these environmental risk factors vary, potentially explained by individual differences in intervention adherence and complex gene-environment interactions (Cornelis and Hu 2012). Research on novel biomarkers and intermediate conditions associated with diabetes risk has improved understanding on risk factors for type 2 diabetes and the disease progress and etiology (Meigs 2010).

Epidemiology

The global estimate of adults living with diabetes is 415 million in 2015 and is projected to rise to 642 million by 2040 (International Diabetes Federation 2015) (Fig. 1). Approximately 12% of global health expenditure is spent on diabetes-related treatments, and 46.5% of adults with diabetes is undiagnosed (International Diabetes Federation 2015). Based on the 2011–2012 National Health and Nutrition Examination Survey (NHANES), the unadjusted prevalence in US population was 14.3% for total diabetes, 9.1% for diagnosed diabetes, 5.2% for undiagnosed diabetes, and 38% for prediabetes (Menke et al. 2015). Type 2 diabetes has become an important global health priority in recent decades, and considerable efforts have been made to identify effective preventive strategies (Hu 2011).

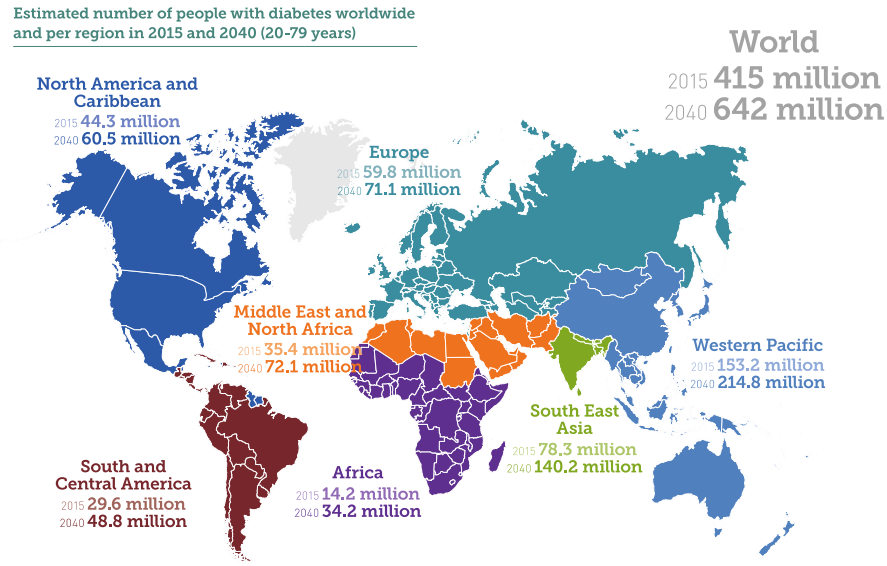


Fig. 1 Estimated number of adults aged 20–79 years with diabetes globally and per region in 2015 and 2040 (Adapted from International Diabetes Federation (2015))

Demographic Risk Factors

Based on NHANES data, the prevalence of diabetes increases with age (Centers for Disease Control and Prevention 2011). In most populations, the incidence of type 2 diabetes is low before age 30 years but increases rapidly and continuously with aging (Geiss et al. 2006; González et al. 2009). This is a particular concern at a time when life expectancy is increasing. In the European countries, higher risk of diabetes in men compared with women was observed (The InterAct Consortium 2011a). However, this was not as consistently evident in the US population; the incidence of diabetes among men compared to women was higher in 2010 but lower in 2013 based on the National Health Interview Survey (NHIS) data (Centers for Disease Control and Prevention 2016). The age-standardized prevalence of diabetes was higher among non-Hispanic black (21.8%), non-Hispanic Asian (20.6%), and Hispanic (22.6%) compared with non-Hispanic white (11.3%) in the NHANES 2011–2012 population (Menke et al. 2015). Ethnic differences can be explained only in part by differences in the prevalence of obesity, behavioral risk factors, and socioeconomic status (SES). Asian, Hispanic, and black ethnicity were each associated with higher diabetes risk compared to white participants after adjustment for differences in age, body mass index (BMI), family history of diabetes, and lifestyle risk factors (i.e., alcohol consumption, smoking, physical activity, and diet) in the Nurses' Health Study (NHS) (Shai et al. 2006). In the Multiethnic Cohort Study of

volunteers living in Hawaii and California, diabetes risk for Japanese Americans and Pacific Islanders remained higher compared to white participants after adjustment for BMI and education (Maskarinec et al. 2009).

Genetic Risk Factors

Early efforts to identify genetic variants for type 2 diabetes heritability in epidemiologic studies involved genome-wide linkage and candidate gene approaches. With the introduction of studies incorporating high-throughput, parallel genotyping technologies including genome-wide association studies (GWAS), the field has advanced rapidly. Further, global collaborative efforts have been made to detect small effects of common variants. For example, a collaboration of 23 studies from populations of European ancestry comprising 27,206 type 2 diabetes cases and 57,574 controls led to the identification and fine mapping of numerous new loci for type 2 diabetes (Gaulton et al. 2015). Over 250 genetic loci have been identified for monogenic, syndromic, or common forms of type 2 diabetes and obesity (Qi et al. 2011).

Gene-Environment Interactions

These genetic variations may influence modifiable risk factors for type 2 diabetes evidenced by variations in individual responses to environmental risk factors. Therefore, understanding gene-environment interactions has the potential to benefit strategies for the prevention of type 2 diabetes. However, gene-environment interaction studies have experienced methodological challenges when investigating small effects of common gene variants (Franks 2011). Collaborative efforts have been made to address these challenges, including the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. The consortium conducted a meta-analysis of 14 cohort studies comprising about 48,000 participants of European descent and reported a nominal interaction between *GCKR* (rs780094) variant and whole grain intake on fasting insulin (Nettleton et al. 2010). Constructing a risk score is another approach that has been used to investigate gene-environment interactions. In a nested case-control study within the Health Professionals Follow-up Study (HPFS) and the NHS, a genetic risk score was calculated on the basis of ten polymorphisms in nine loci (Cornelis et al. 2009). The association between genetic score and type 2 diabetes was strengthened with the BMI increase in this study of individuals of European descent (Cornelis et al. 2009). In the Diabetes Prevention Program (Hivert et al. 2011a), a high genetic risk score was associated with increased risk of developing type 2 diabetes, but a lifestyle intervention attenuated this risk. Although information on common genetic variations may not improve prediction of diabetes (Meigs et al. 2008) (Fig. 2), better understanding of genetic susceptibility to the disease and its influence on environmental risk factors may assist in developing future prevention strategies for type 2 diabetes.

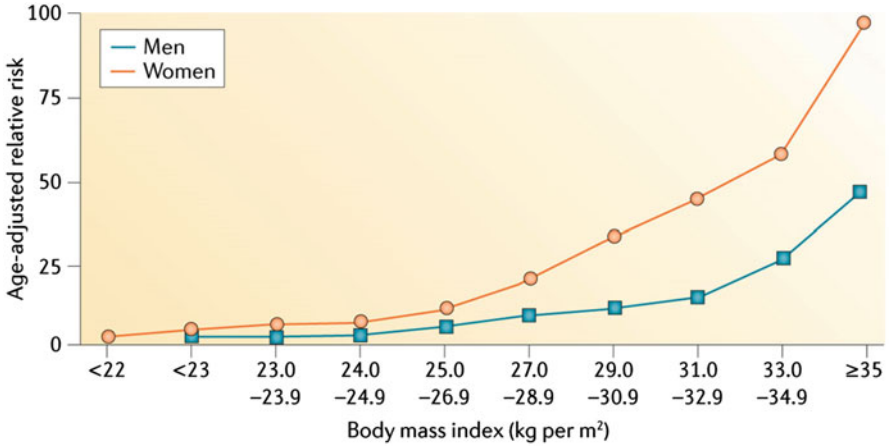


Fig. 2 Association between body mass index and type 2 diabetes (Adapted from DeFronzo et al. (2015))

Behavioral and Lifestyle Risk Factors

Obesity

Excessive body fat is the single largest risk factor for type 2 diabetes (Hu et al. 2001a). Excessive body fat is frequently assessed by BMI (the ratio of body weight in kilograms to squared height in meters) or anthropometric indicators such as a waist circumference or skinfold thickness in epidemiologic studies. Clinical risk categories for obesity measured using BMI (normal BMI 18.5–24.9 kg/m^2, overweight 25–29.9 kg/m^2, and obesity $\geq$30 kg/m^2) are associated with a stepwise increase in diabetes risk (DeFronzo et al. 2015) (Fig. 3). Duration of overweight/obesity is also an important risk factor because each 2 extra years of being obese increased type 2 diabetes risk by 14% (Hu et al. 2014). Further, weight gain during early adulthood aged 25–40 years was more strongly associated with diabetes risk compared with weight gain during later adulthood aged 40–55 years in the European Prospective Investigation into Cancer and Nutrtion (EPIC)-Potsdam Study (Schienkiewitz et al. 2006).

Central obesity captured as a higher waist circumference or waist-to-hip ratio was associated with type 2 diabetes risk in the NHS (Carey et al. 1997). In a meta-analysis of prospective observational studies, the risk associated with a higher waist circumference was slightly stronger than that associated with higher BMI (Vazquez et al. 2007), indicating that central obesity was more important. However, the association of waist-to-hip ratio with type 2 diabetes was slightly weaker compared to BMI. In the prospective EPIC Study, individuals in the overweight BMI and abdominal obesity (waist circumference $\geq$102 cm among men, $\geq$88 cm among women) had a similar risk compared to obese individuals (BMI $\geq$30 kg/m^2) (The

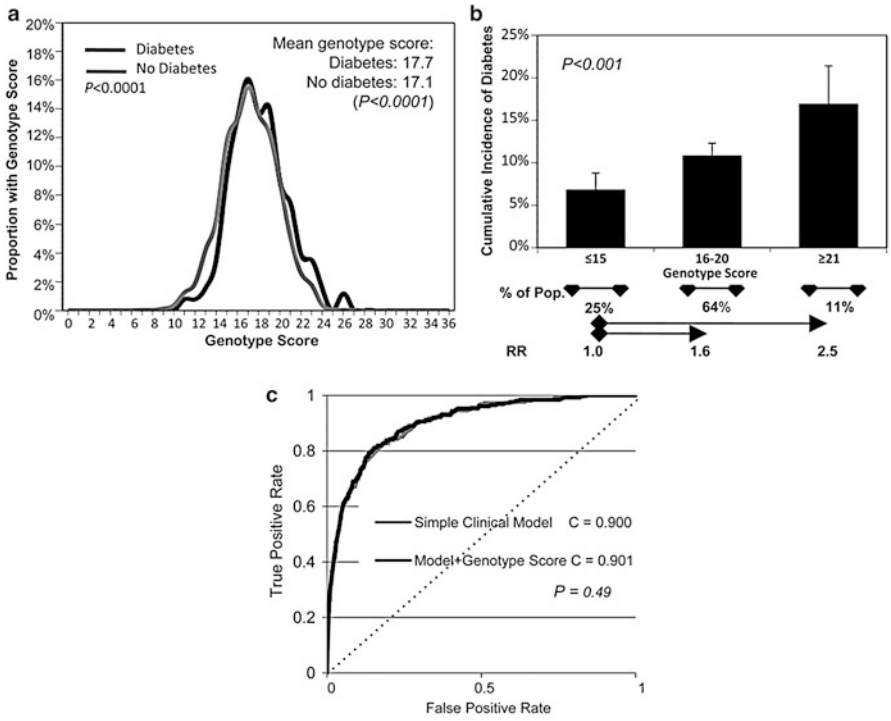


Fig. 3 A genetic risk score of 18 SNPs predicts type 2 diabetes. (**a**) Panel shows the distribution of an 18-SNP genetic risk score, where Framingham Heart Study individuals were scored with a zero if they had no risk alleles at a given SNP, one if they were heterozygous, and two if they were homozygous for the risk allele. Individuals who developed type 2 diabetes over 28 years of follow-up have about 0.6 more risk alleles than those who remain free of diabetes. (**b**) Panel shows that risk for type 2 diabetes increases with increasing genetic risk burden. Those with 16–20 risk alleles have a risk of diabetes of 1.6, and those with ≥21 risk alleles have a risk of 2.5 relative to those with ≤15 risk alleles. However, the genetic risk score does not discriminate those who will develop type 2 diabetes from those who will not after accounting for common clinical risk factors (age, sex, family history of diabetes, and metabolic syndrome traits). (**c**) Panel shows that the area under the receiver operating characteristic curve (the C statistic) was 0.900 for a simple clinical model including clinical risk factors (gray line) and 0.901 for a model including clinical risk factors and the genetic risk score (black line) (Adapted from Meigs (2010))

InterAct Consortium 2012). Therefore, measuring waist circumference in addition to BMI may allow for additional stratification for diabetes risk among overweight individuals.

Diet

Dietary intake has been suspected as a major risk factor for type 2 diabetes for a while, but evidence from prospective studies evaluating diet in relation to the

incidence of diabetes was vastly accumulated in the past couple of decades (Ley et al. 2014).

Dietary Fat and Carbohydrate

Prospective cohort studies demonstrated that total fat intake is not associated with diabetes risk (Hu et al. 2001b). In the Women's Health Initiative (WHI), the incidence of treated diabetes was not different among women who consumed a low-fat diet (24% energy from fat) compared to women who consumed a standard US diet (35% energy from fat) (Tinker et al. 2008). Therefore, the specific type of fat may be more important than the total intake. Diets that favor plant fats over animal fats are advantageous (Hu et al. 2001b; Melanson et al. 2009). A higher intake of polyunsaturated fatty acids (PUFA) was associated with a lower diabetes risk (Meyer et al. 2001; Salmerón et al. 2001). However, the association between the quantity of long-chain n-3 PUFA intake and diabetes risk has been inconsistent, and a meta-analysis including 16 prospective cohorts with 440,873 participants and 21,512 cases of incident diabetes reported nonsignificant association (Wu et al. 2012). Exchanging saturated fatty acids with PUFA was associated with a lower risk (Hu et al. 2001b).

Similarly, the quality of carbohydrate is likely more important than the quantity for diabetes prevention. The relative carbohydrate proportion of the diet did not influence diabetes risk (Hauner et al. 2012). Prospective cohort studies investigating carbohydrate substitutions with other macronutrients reported heterogeneous results (Schulze et al. 2004, 2008). A meta-analysis of eight prospective cohort studies, including five from the US and one each from Finland, Australia, and Germany, demonstrated an inverse association of dietary fiber intake from cereal products with risk of type 2 diabetes (Schulze et al. 2007). However, total fiber or fiber from fruits or vegetables was not associated with diabetes risk in this study (Schulze et al. 2007). The protective impact of cereal fiber was evidenced in several other studies (Hopping et al. 2010; Krishnan et al. 2007), but a few studies did not detect such beneficial impact (Barclay et al. 2007; Wannamethee et al. 2009). Carbohydrate quality can be determined by glycemic index and glycemic load by evaluating the physiologic response to carbohydrate-rich foods. High-quality carbohydrate diets low in average glycemic index and glycemic load are associated with a lower risk for diabetes (Bhupathiraju et al. 2014; Dong et al. 2011b; Liu and Chou 2010), independent of the amount of dietary fiber in the diet.

Vitamins and Minerals

In a meta-analysis of four prospective studies, greater heme-iron intake was associated with a higher risk of type 2 diabetes (Zhao et al. 2012). Further, higher iron stores, reflected by elevated ferritin concentrations, were associated with increased risk of developing type 2 diabetes. A meta-analysis of five prospective studies provided evidence that magnesium intake was inversely associated with type 2 diabetes risk (Dong et al. 2011a). This association was more pronounced among overweight and obese participants (BMI $\geq$25 kg/m^2) but was not significant among those with BMI <25 kg/m^2 (Dong et al. 2011a). In the Framingham Offspring Study,

higher levels of 25-OH vitamin D were associated with lower incidence of type 2 diabetes after accounting for potential confounders (Liu et al. 2010). This potentially protective effect was also reported in the NHS but mainly in the upper levels of circulating 25-OH vitamin D and with a stronger effect in overweight/obese women (Pittas et al. 2010). However, 25-OH vitamin D levels were not associated with type 2 diabetes incidence in the WHI (Robinson et al. 2011). Further, intervention trials investigating the impact of vitamin D supplementation have been mainly inconclusive (Mitri et al. 2011). Therefore, the role of vitamin D in diabetes prevention is currently inconclusive. Further, specific nutrient-based associations with type 2 diabetes may have been confounded by other unaccounted nutrients in food since these nutrients are consumed in combination as food items. For example, dairy products are not only rich in vitamin D but rich in macronutrients and other micronutrients such as magnesium (Dong et al. 2011a).

Food and Beverages

Specific food and beverages have been investigated as risk factors for type 2 diabetes (Ley et al. 2014) (Fig. 4). Whole grain intake was associated with a lower risk of diabetes (Aune et al. 2013; de Munter et al. 2007), even after adjustment for potential confounders including obesity. Conversely, greater intake of white rice which is a form of processed grain was associated with a higher risk for type 2 diabetes (Hu et al. 2012), especially among Asian populations with markedly higher amounts of white rice consumption. Frequent consumption of total red meat and processed meats was associated with higher diabetes risk (Pan et al. 2011b). In a meta-analysis of 13 prospective studies, fish and/or seafood consumption was not significantly associated with a higher risk of type 2 diabetes (Wu et al. 2012). Greater fish/seafood consumption was associated with a higher risk of type 2 diabetes in North America and Europe, while it was associated with a lower risk in Asia (Wallin et al. 2012; Wu et al. 2012). Variations in types of fish consumed and cooking preparation methods used within different geographical locations may explain the differences in results. Total dairy consumption was not associated with type 2 diabetes risk, but greater intake of yogurt was associated with a lower risk of type 2 diabetes (Chen et al. 2014). Although total consumption of fruits and vegetable quantity was not associated with type 2 diabetes, greater green leafy vegetable intake was associated with a lower risk (Carter et al. 2010; Cooper et al. 2012). In NHS cohorts, greater intake of whole fruits rich in anthocyanin such as blueberries, grapes, and apples/pears was also associated with a lower risk of type 2 diabetes (Muraki et al. 2013; Wedick et al. 2012). Nut consumption was associated with a lower risk of diabetes (Jiang et al. 2002; Kendall et al. 2010). In the Prevención con Dieta Mediterránea (PREDIMED) trial, nut supplementation in addition to Mediterranean diet reduced the incidence of type 2 diabetes (Salas-Salvadó et al. 2011).

Based on meta-analyses (Ding et al. 2014; Huxley et al. 2009), total coffee, caffeinated, and decaffeinated coffee consumption was associated with a lower risk of type 2 diabetes. Greater sugar-sweetened beverage consumption was associated with a higher risk of type 2 diabetes (Malik et al. 2010; The InterAct Consortium 2013). Since higher sugar-sweetened beverage intake was associated with more

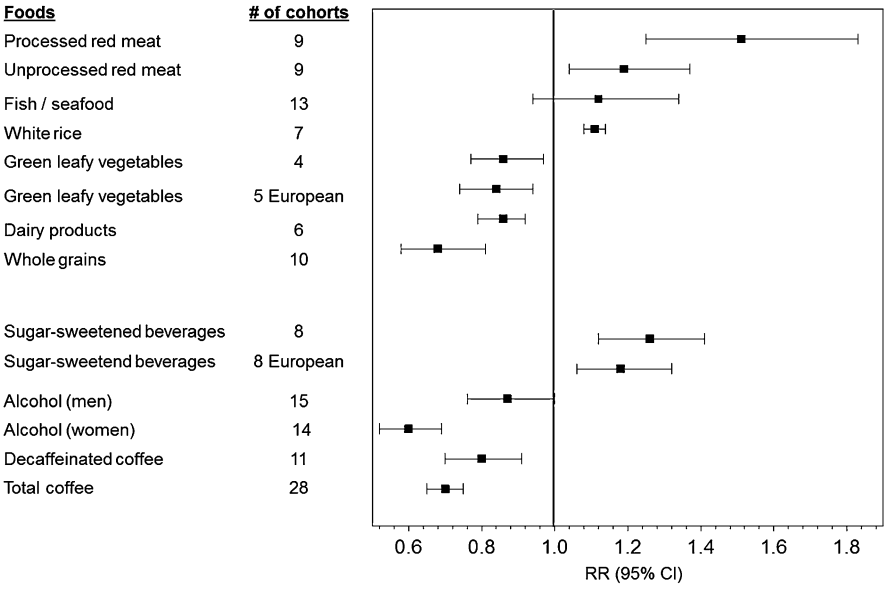


Fig. 4 Summary of meta-analyses of prospective cohort studies on food and beverage intake and type 2 diabetes. Relative risks (RR) are comparison of extreme categories, except for processed meat (per 50 g/day increase), unprocessed red meat and fish/sea food (per 100 g/day), white rice (per each serving/day), whole grains (per three servings/day), sugar-sweetened beverages in European cohorts (per 336 g/day), and alcohol (abstainers with 22 g/day for men and with 24 g/day for women) (Adapted from Ley et al. (2014))

pronounced genetic predisposition to increased BMI and risk for obesity (Qi et al. 2012), this association is likely mediated through weight gain and obesity.

A U-shaped relationship between alcohol consumption and type 2 diabetes was observed with the lowest risk of type 2 diabetes in the moderate range of consumption of about one and half US standard drinks per day (Baliunas et al. 2009; Wannamethee et al. 2003). However, alcohol became harmful at a consumption level above four US standard drinks per day (50 g/day in women and 60 g/day in men) (Baliunas et al. 2009).

Dietary Patterns

Several healthful dietary patterns have been associated with a lower risk of type 2 diabetes (Ley et al. 2014). Mediterranean-style diets were associated with a lower risk of type 2 diabetes (Esposito et al. 2010; Salas-Salvadó et al. 2011, 2014; The InterAct Consortium 2011b). Alternative Healthy Eating Index (AHEI) (Chiuve et al. 2012) and the Dietary Approaches to Stop Hypertension (DASH) diets were also associated with lower diabetes risk (de Koning et al. 2011; Liese et al. 2009a). Vegetarian diets were associated with a lower diabetes risk (Tonstad et al. 2013). Further, prospective studies using exploratory methods to define dietary patterns supported dietary patterns favoring fruits, vegetables, whole grains, and legumes at

the expense of red meats, refined grains, and sugar-sweetened beverages for type 2 diabetes prevention (Fung et al. 2004; Heidemann et al. 2005; Imamura et al. 2009; Liese et al. 2009b; McNaughton et al. 2008; Schulze et al. 2005). Several other characteristics of eating patterns such as skipping breakfast (Mekary et al. 2012) and frequent fried food consumption (Cahill et al. 2014) were associated with a higher risk of type 2 diabetes.

Physical Inactivity

Sedentary behaviors such as higher television viewing time are a risk factor for type 2 diabetes (Grøntved and Hu 2011). Physical inactivity defined as insufficient physical activity to meet present global recommendations by the World Health Organization is responsible for 7% of the global burden of type 2 diabetes (Lee et al. 2012). Physical activity of moderate intensity can lower the risk of type 2 diabetes based on a meta-analysis of ten prospective cohort studies (Jeon et al. 2007). Regular walking defined as $\geq$2.5 h/week of brisk walking was associated with a lower risk for type 2 diabetes compared to almost no walking (Jeon et al. 2007). Moderate- to high-intensity exercise is well known to be beneficial for type 2 diabetes prevention (Manson et al. 1991; Meisinger et al. 2005). In addition to aerobic exercise (e.g., brisk walking, jogging, running, bicycling, swimming, tennis, squash, and rowing), weight training was associated with a lower risk of type 2 diabetes (Grøntved et al. 2012). Engaging in weight training or aerobic exercise for $\geq$150 min/week was associated with 34–52% reduced risk of developing type 2 diabetes in men (Grøntved et al. 2012). In women, engaging in both aerobic moderate to vigorous physical activity and muscle-strengthening activity including toning, yoga, and resistance training was associated with a lower risk of type 2 diabetes (Grøntved et al. 2014).

Early-Life Environment

Children who experienced intrauterine exposure to maternal diabetes are more likely to have large for gestational age birth weight (Reece et al. 2009), childhood overweight (Lawlor et al. 2011), and impaired glucose tolerance (IGT) in early adulthood (Silverman et al. 1995). Among individuals born around the time of famine in the Netherlands during 1944–1945, prenatal exposure to famine especially during late gestation was associated with compromised glucose tolerance in adulthood (Ravelli et al. 1998). Fetal exposure to the severe Chinese famine during 1959–1961 was also associated with a higher risk of hyperglycemia in adulthood (Li et al. 2010). The association is exacerbated by a nutritionally rich environment in later life (Li et al. 2010). Birth weight is associated with a risk of type 2 diabetes later in life in a U-shaped fashion (Harder et al. 2007; Whincup et al. 2008). However, evidence suggests that most type 2 diabetes cases can be prevented by the adaptation of a healthier lifestyle later in life although birth weight may influence diabetes risk (Li et al. 2015). Further, early postnatal behavioral exposures such as breastfeeding may have a long-term protective effect against obesity and type 2 diabetes later in life

(Arenz et al. 2004; Owen et al. 2005, 2006). For example, breastfeeding during early life has a protective effect on obesity (Arenz et al. 2004; Owen et al. 2005) and type 2 diabetes later in life (Owen et al. 2006). However, the multiple potential confounding factors including demographic, socioeconomic, educational, ethnic, cultural, and psychological factors for these associations remain to be clarified (Kramer et al. 2009).

Socioeconomic Status (SES)

In a meta-analysis of 23 prospective case-control and cohort studies (Agardh et al. 2011), the overall risk of developing type 2 diabetes was increased among those in a lower socioeconomic position including lower levels of education (relative risk [RR] 1.41), occupation (RR 1.31), and income (RR 1.40) (Agardh et al. 2011). In the Black Women's Health Study (Krishnan et al. 2010), lower education, household income, and neighborhood SES were associated with a higher risk of developing type 2 diabetes. However, these associations were attenuated after adjustment for BMI indicating that BMI might be a key intermediate factor in the pathway between SES and diabetes. SES may also contribute to the development of type 2 diabetes through processes involving lack of access to health-care services, healthy foods, places to exercise, and occupational opportunities, leading to unhealthy lifestyle practices (Brown et al. 2004).

Migration and Acculturation

Urbanization and Westernization associated with inter- and intra-country migration is a contributing risk factor for type 2 diabetes (Zimmet 2000; Zimmet et al. 2001). Stepwise increases across the sociocultural gradient were reported on the prevalence of obesity (5% in Nigeria, 23% in Jamaica, and 39% in the USA) (Luke et al. 2001) and type 2 diabetes (1%, 12%, and 13%, respectively) (Rotimi et al. 1999) among African descents. However, acculturation is a complex and multidirectional process. The prevalence of diabetes varies by country of origin based on NHIS 2000–2005 data within the Hispanic ethnic group (Pabon-Nau et al. 2010). Acculturation within a migrant population can vary in degrees of retaining their cultural roots and integrating the local mainstream culture (Pérez-Escamilla and Putnik 2007). Further, the study participant selection process may introduce bias and may not reflect general representation of the source population.

Sleep

Habitual sleep disturbances are associated with risk of developing type 2 diabetes (Cappuccio et al. 2010). Obstructive sleep apnea is highly prevalent among obese adults (Young et al. 2005). In a meta-analysis of six prospective cohort studies, moderate to severe obstructive sleep apnea was associated with a higher risk for type 2 diabetes (Wang et al. 2013). In another meta-analysis of ten prospective cohorts, shorter duration of sleep ($\leq$5–6 h/night) was associated with a higher risk of type 2

diabetes (RR 1.28), while longer duration of sleep (>8–9 h/night) was also associated with the risk (RR 1.48) (Cappuccio et al. 2010). Type 2 diabetes risk was also increased among those with difficulty in initiating or maintaining sleep (Cappuccio et al. 2010). Lower melatonin secretion measured from first-morning urine samples as an indicator of sleep disruption was also associated with a higher risk of type 2 diabetes (McMullan et al. 2013). Performing night shift work for an extended period was associated with a higher risk of type 2 diabetes (Pan et al. 2011a). Other sleep quality measures such as regular snoring (Al-Delaimy et al. 2002) and difficulty falling or staying asleep were associated with type 2 diabetes risk (Li et al. 2016).

Depression and Antidepressant Medications

The relation between depression and type 2 diabetes is bidirectional (Pan et al. 2010). In a meta-analysis of 13 studies, baseline depression was associated with incident diabetes (RR 1.60), while baseline diabetes was also associated with incident depression (RR 1.15) (Mezuk et al. 2008). In addition, the use of antidepressant medication was associated with a higher risk of type 2 diabetes (Pan et al. 2012).

Smoking

In a meta-analysis of 25 prospective cohort studies, active smokers were at a higher risk for developing type 2 diabetes compared with nonsmokers (RR 1.44) (Willi et al. 2007). Further, heavier active smokers had higher risk for type 2 diabetes (RR 1.61), while the associations were weaker for lighter active smokers (RR 1.29) and former smokers (RR 1.23). Smoking cessation was associated with a short-term increased risk of diabetes likely mediated through weight gain (Yeh et al. 2010). Exposure to passive smoking at work or home was also associated with a higher risk of diabetes (Zhang et al. 2011).

To summarize lifestyle risk factors for type 2 diabetes, more than 90% of type 2 diabetes cases could have been prevented by following a healthy diet, having a healthy body weight, exercising for at least 30 min a day, avoiding smoking, and consuming alcohol in moderation (Hu et al. 2001a). Therefore, combinations of these behavioral factors could be utilized to develop optimal strategies to prevent diabetes.

Metabolic Factors Associated with Risk of Type 2 Diabetes

Biomarkers

Prediabetes and insulin resistance are increasingly characterized by a subclinical pro-inflammatory condition derived from adipose tissue dysregulation. With accumulation of excess weight, macrophages infiltrate adipose tissue leading to secretion of pro-inflammatory cytokines and impaired secretion of adipokines secreted by the

adipose tissue. The liver is involved in the process through secretion of C-reactive protein (CRP) and liver enzymes. The inflammatory process is also linked with endothelial dysfunction markers.

Adiponectin

Adiponectin is an adipokine mainly produced by adipocytes (Scherer et al. 1995) and has anti-inflammatory and insulin-sensitizing effects (Berg et al. 2001; Kadowaki et al. 2006; Ouchi et al. 2000). In a meta-analysis reviewing 13 prospective studies, lower adiponectin concentrations were consistently associated with a higher risk of type 2 diabetes in populations from various ethnic backgrounds and wide ranges of age, sex, and baseline glucose tolerance (Li et al. 2009). Low adiponectin concentrations were associated with a higher risk of type 2 diabetes among individuals who were insulin resistant at baseline (upper quartile of homeostatic model assessment-insulin resistance [HOMA-IR]) in the Framingham Offspring Study and in the Cooperative Health Research in the Region of Augsburg Study (Hivert et al. 2011b), while the association between lower adiponectin concentrations and a higher risk of type 2 diabetes was stronger in individuals with lower HOMA-IR in the Cardiovascular Health Study (Kizer et al. 2012). Using more refined measures of insulin sensitivity based on intravenous glucose tolerance test, the association between adiponectin levels and the type 2 diabetes incidence was no longer significant after adjusting for S_i in the Insulin Resistance Atherosclerosis Study (IRAS) suggesting that insulin sensitivity mediated the association (Hanley et al. 2011).

Pro-inflammatory Cytokines

Pro-inflammatory markers such as tumor necrosis factor-alpha (TNFα), interleukin (IL)-6, and CRP were associated with a higher risk of type 2. When these three biomarkers were mutually adjusted for each other, only CRP remained significantly associated with type 2 diabetes incidence in the NHS (Hu et al. 2004) and WHI Observational Study (Liu et al. 2007). In the EPIC cohort, the association between higher CRP and type 2 diabetes risk was attenuated and became no longer significant after multiple adjustment for waist-to-hip ratio, serum gamma-glutamyltransferase (GGT), and serum adiponectin (Lee et al. 2009). In the Multi-Ethnic Study of Atherosclerosis (MESA), IL-6 and CRP were associated with a risk of type 2 diabetes in white, black, and Hispanic individuals but not in individuals of Chinese origin (Bertoni et al. 2010). In a meta-analysis of 16 prospective studies from various regions and populations from Europe, Asia, and America, higher CRP concentrations were associated with a higher risk of type 2 diabetes (Lee et al. 2009). IL-18 is another cytokine likely involved in pro-inflammatory and insulin resistance pathways. Higher IL-18 concentrations were associated with higher risk of type 2 diabetes in the NHS (Hivert et al. 2009). In the Atherosclerosis Risk in Communities (ARIC) Study, higher IL-18 concentrations were associated with a higher risk of type 2 diabetes in whites, but not in African American descent participants (Negi et al. 2012), suggesting a potential difference between these ethnic backgrounds.

Coagulation and Endothelial Dysfunction Markers

Plasminogen activator inhibitor-1 (PAI-1) is primarily produced by endothelial cells but also secreted by adipose tissues. In the Framingham Offspring Study, higher concentrations of PAI-1 and von Willebrand factor were associated with a higher risk of type 2 diabetes in multivariable models including major diabetes clinical risk factors in addition to CRP levels (Meigs et al. 2006). PAI-1 concentrations were also strongly associated with type 2 diabetes risk in the Health, Aging, and Body Composition Study of black and white older adults (Kanaya et al. 2006) and in the IRAS cohort (Festa et al. 2002).

Endothelial dysfunction can be detected by measurement of elevated plasma concentrations of cellular adhesion molecules, including E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1. These markers were associated with type 2 diabetes risk in the NHS (Meigs et al. 2004) and the multiethnic WHI Observational Study (Song et al. 2007).

Liver Markers

Higher liver enzyme concentrations were associated with type 2 diabetes risk in a meta-analysis of prospective cohorts from various countries in Europe and Asia in addition to the USA (Fraser et al. 2009). Higher baseline GGT or alanine aminotransferase (ALT) concentrations were associated with diabetes status at follow-up in the Bogalusa Heart Study (Nguyen et al. 2011) and in the Coronary Artery Risk Development in Young Adults Study higher GGT concentrations at baseline (Lee et al. 2003). In a cross-sectional analysis of NHANES III, an interaction between BMI and GGT concentrations was reported demonstrating the association between GGT and diabetes prevalence among participants with higher BMI only (Lim et al. 2007). Higher concentrations of fetuin-A, a glycoprotein secreted by the liver, were associated with a higher risk of type 2 diabetes in a meta-analysis of four prospective studies (Sun et al. 2013). In the NHS, the positive association between fetuin-A and diabetes remained after adjustment for liver enzymes.

Insulin-Like Growth Factor Axis

Insulin-like growth factor (IGF)-1 shares structural homology with insulin (Rajpathak et al. 2009). However, total IGF-1 concentrations were not significantly associated with type 2 diabetes risk in the NHS (Rajpathak et al. 2012). A statistically significant interaction was observed. Free IGF-1 was inversely associated with type 2 diabetes risk among women with higher (above median, 4.6 μU/mL) insulin concentrations, while it was positively associated with type 2 diabetes risk among those with lower insulin concentrations (Rajpathak et al. 2012). Further, lower IGF binding protein (IGFBP)-1 and IGFBP-2 and higher IGFBP-3 were associated with a higher risk for diabetes (Rajpathak et al. 2012).

Sex Hormones

In a meta-analysis, low testosterone in men was associated with risk of type 2 diabetes (Ding et al. 2006). In the NHANES III, men in the lowest tertile of

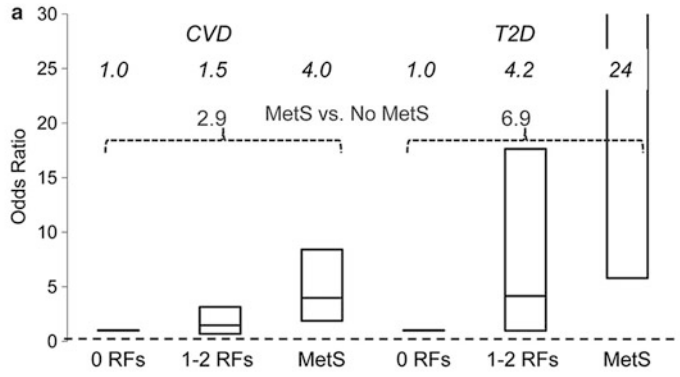
bioavailable testosterone were about four times more likely to have type 2 diabetes compared with men in the upper tertile (Selvin et al. 2007). In the Rancho Bernardo Study, higher bioavailable testosterone concentrations in postmenopausal women were associated with higher risk of type 2 diabetes (Oh et al. 2002). Higher concentrations of bioavailable estradiol were associated with higher risk of type 2 diabetes in women (Oh et al. 2002) but not in men (Haffner et al. 1996; Oh et al. 2002). Further, lower concentrations of sex hormone-binding globulin were associated with higher risk of type 2 diabetes (Ding et al. 2006).

Metabolic Syndrome

Metabolic traits that cluster in metabolic syndrome include central obesity, high fasting glucose, high blood pressure, high triglyceride, and/or low HDL cholesterol levels (Alberti et al. 2006; Grundy et al. 2005). Metabolic syndrome is a strong risk factor of type 2 diabetes (Ford et al. 2008). The presence versus the absence of National Cholesterol Education Program's Adult Treatment Panel III (ATP III) metabolic syndrome among individuals without diabetes at baseline was associated with a 5.3-fold higher risk of developing type 2 diabetes. In community studies, the metabolic syndrome was associated with a higher risk for type 2 diabetes in white participants (Wilson et al. 2005), black participants (Schmidt et al. 2005), Mexican Americans (Lorenzo et al. 2007), Native Americans (Hanson et al. 2002; Russell et al. 2007), and Native Canadians (Ley et al. 2009). Further, metabolic syndrome demonstrates a positive dose-response gradient between the number of metabolic syndrome traits and diabetes risk (Wilson et al. 2005). Women with one or two traits had a sixfold higher risk of type 2 diabetes, and those with three or more traits had a 30-fold higher risk of diabetes in the Framingham Offspring Study. When combined in prediction models for type 2 diabetes risk, metabolic syndrome traits have excellent discriminatory capacity (Wilson et al. 2005, 2007) (Fig. 5).

Summary

Large prospective cohort studies have improved our understanding on environmental risk factors for type 2 diabetes. However, variations between individual responses to risk factor interventions are likely explained by genetic and individual physiologic differences. Therefore, advancement in the knowledge of gene-environment interactions, biomarkers, and intermediate conditions would contribute to the progress of targeted prevention strategies for type 2 diabetes. Continued efforts are warranted to improve the understanding of type 2 diabetes risk to develop optimal strategies for type 2 diabetes prevention with a long-term goal of addressing this major public health concern.



b

Metabolic Syndrome Clusters	Prevalence	RR CVD	RR T2D
high TG + low HDL + high BP	10.3%	1.9	3.5
high waist + high BP + low HDL	9.9%		
high waist + high BP + high TG	7.6%	2.1	5.0
high waist + high TG + low HDL	7.3%		
high FG+ low HDL + high BP	5.4%		
high FG+ high waist + high BP	5.2%		
high FG+ high TG + high BP	4.5%	2.5	11
high FG+ low HDL + high waist	3.9%		
high FG+ high TG + low HDL	3.6%		
high FG+ high TG + high waist	2.9%		

Fig. 5 Metabolic syndrome is a risk factor for both type 2 diabetes (T2D) and cardiovascular disease (CVD). (**a**) Panel shows that among men in the Framingham Heart Study, the 7- to 11-year risk for CVD increases from 1.5 for those with one or two metabolic syndrome risk factors to 4.0 for those with three or more relative to those with no metabolic syndrome risk factors, even after accounting for other CVD-specific risk factors. The bars in the figure represent the odds ratio and its 95% confidence bounds. The relative risk for CVD is 2.9 comparing metabolic syndrome versus no metabolic syndrome. Risk for type 2 diabetes increases from 4.2 for men with one or two metabolic syndrome risk factors to 24 for those with metabolic syndrome relative to those with no metabolic syndrome risk factors, even after accounting for other type 2 diabetes-specific risk factors. The relative risk for type 2 diabetes is 6.9 comparing metabolic syndrome versus no metabolic syndrome. Patterns are similar for Framingham Heart Study women. Risk rises steadily in a dose-response relationship as the number of component traits increases and is increased regardless of which of the various heterogeneous combinations of specific traits are present and even in the absence of impaired glycemia (**b**) (Adapted from Meigs (2010)). *BP* blood pressure, *FG* fasting glucose, *TG* triglycerides

References

Agardh E, Allebeck P, Hallqvist J, Moradi T, Sidorchuk A. Type 2 diabetes incidence and socio-economic position: a systematic review and meta-analysis. Int J Epidemiol. 2011;40:804–18.

Alberti KG, Zimmet P, Shaw J. Metabolic syndrome – a new world-wide definition. A consensus statement from the International Diabetes Federation. Diabet Med. 2006;23:469–80.

Al-Delaimy WK, Manson JE, Willett WC, Stampfer MJ, Hu FB. Snoring as a risk factor for type II diabetes mellitus: a prospective study. Am J Epidemiol. 2002;155:387–93.

Arenz S, Ruckerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity – a systematic review. Int J Obes Relat Metab Disord. 2004;28:1247–56.

Aune D, Norat T, Romundstad P, Vatten LJ. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. Eur J Epidemiol. 2013;28:845–58.

Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, Rehm J. Alcohol as a risk factor for type 2 diabetes. Diabetes Care. 2009;32:2123–32.

Barclay AW, Flood VM, Rochtchina E, Mitchell P, Brand-Miller JC. Glycemic index, dietary fiber, and risk of type 2 diabetes in a cohort of older Australians. Diabetes Care. 2007;30:2811–3.

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med. 2001;7:947–53.

Bertoni AG, Burke GL, Owusu JA, Carnethon MR, Vaidya D, Barr RG, Jenny NS, Ouyang P, Rotter JI. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care. 2010;33:804–10.

Bhupathiraju SN, Tobias DK, Malik VS, Pan A, Hruby A, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and risk of type 2 diabetes: results from 3 large US cohorts and an updated meta-analysis. Am J Clin Nutr. 2014;100(1):218–32.

Brown AF, Ettner SL, Piette J, Weinberger M, Gregg E, Shapiro MF, Karter AJ, Safford M, Waitzfelder B, Prata PA, Beckles GL. Socioeconomic position and health among persons with diabetes mellitus: a conceptual framework and review of the literature. Epidemiol Rev. 2004;26:63–77.

Cahill LE, Pan A, Chiuve SE, Sun Q, Willett WC, Hu FB, Rimm EB. Fried-food consumption and risk of type 2 diabetes and coronary artery disease: a prospective study in 2 cohorts of US women and men. Am J Clin Nutr. 2014;100:667–75.

Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care. 2010;33:414–20.

Carey VJ, Walters EE, Colditz GA, Solomon CG, Willet WC, Rosner BA, Speizer FE, Manson JE. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. Am J Epidemiol. 1997;145:614–9.

Carter P, Gray LJ, Troughton J, Khunti K, Davies MJ. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. BMJ. 2010;341:c4229.

Centers for Disease Control and Prevention. National Diabetes Fact Sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2011.

Centers for Disease Control and Prevention. Diabetes Home, National Diabetes Surveillance System. http://www.cdc.gov/diabetes [accessed Jan 15, 2016].

Chen M, Sun Q, Giovannucci E, Mozaffarian D, Manson JE, Willett WC, Hu FB. Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. BMC Med. 2014;12:1–14.

Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr. 2012;142:1009–18.

Cooper AJ, Forouhi NG, Ye Z, Buijsse B, Arriola L, Balkau B, Barricarte A, Beulens JWJ, Boeing H, Buchner FL, Dahm CC, de Lauzon-Guillain B, Fagherazzi G, Franks PW, Gonzalez C,

Grioni S, Kaaks R, Key TJ, Masala G, Navarro C, Nilsson P, Overvad K, Panico S, Ramon Quiros J, Rolandsson O, Roswall N, Sacerdote C, Sanchez MJ, Slimani N, Sluijs I, Spijkerman AMW, Teucher B, Tjonneland A, Tumino R, Sharp SJ, Langenberg C, Feskens EJM, Riboli E, Wareham NJ. Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. Eur J Clin Nutr. 2012;66:1082–92.

Cornelis MC, Hu FB. Gene–environment interactions in the development of type 2 diabetes: recent progress and continuing challenges. Annu Rev Nutr. 2012;32:245–59.

Cornelis MC, Qi L, Zhang C, Kraft P, Manson J, Cai T, Hunter DJ, Hu FB. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. Ann Intern Med. 2009;150:541–50.

de Koning L, Chiuve SE, Fung TT, Willett WC, Rimm EB, Hu FB. Diet-quality scores and the risk of type 2 diabetes in men. Diabetes Care. 2011;34:1150–6.

de Munter JSL, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med. 2007;4:e261.

DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC, Testa MA, Weiss R. Type 2 diabetes mellitus. Nat Rev Dis Prim. 2015;1:15019.

Ding El, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006;295:1288–99.

Ding M, Bhupathiraju SN, Chen M, van Dam R, Hu FB. Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: a systematic review and a dose–response meta-analysis. Diabetes Care. 2014;37:569–86.

Dong J-Y, Xun P, He K, Qin L-Q. Magnesium intake and risk of type 2 diabetes: meta-analysis of prospective cohort studies. Diabetes Care. 2011a;34:2116–22.

Dong JY, Zhang L, Zhang YH, Qin LQ. Dietary glycaemic index and glycaemic load in relation to the risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Br J Nutr. 2011b;106:1649–54.

Esposito K, Maiorino MI, Ceriello A, Giugliano D. Prevention and control of type 2 diabetes by Mediterranean diet: a systematic review. Diabetes Res Clin Pract. 2010;89:97–102.

Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetes. 2002;51:1131–7.

Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes. Diabetes Care. 2008;31:1898–904.

Franks P. Gene–environment interactions in type 2 diabetes. Curr Diab Rep. 2011;11:552–61.

Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care. 2009;32:741–50.

Fung TT, Schulze M, Manson JE, Willett WC, Hu FB. Dietary patterns, meat intake, and the risk of type 2 diabetes in women. Arch Intern Med. 2004;164:2235–40.

Gaulton KJ, Ferreira T, Lee Y, Raimondo A, Magi R, Reschen ME, Mahajan A, Locke A, William Rayner N, Robertson N, Scott RA, Prokopenko I, Scott LJ, Green T, Sparso T, Thuillier D, Yengo L, Grallert H, Wahl S, Franberg M, Strawbridge RJ, Kestler H, Chheda H, Eisele L, Gustafsson S, Steinthorsdottir V, Thorleifsson G, Qi L, Karssen LC, van Leeuwen EM, Willems SM, Li M, Chen H, Fuchsberger C, Kwan P, Ma C, Linderman M, Lu Y, Thomsen SK, Rundle JK, Beer NL, van de Bunt M, Chalisey A, Kang HM, Voight BF, Abecasis GR, Almgren P, Baldassarre D, Balkau B, Benediktsson R, Bluher M, Boeing H, Bonnycastle LL, Bottinger EP, Burtt NP, Carey J, Charpentier G, Chines PS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Doney ASF, Dorkhan M, Edkins S, Eriksson JG, Esko T, Eury E, Fadista J, Flannick J, Fontanillas P, Fox C, Franks PW, Gertow K, Gieger C, Gigante B, Gottesman O, Grant GB, Grarup N, Groves CJ, Hassinen M, Have CT, Herder C, Holmen OL, Hreidarsson AB, Humphries SE, Hunter DJ, Jackson AU, Jonsson A, Jorgensen ME, Jorgensen T, Kao W-HL,

Kerrison ND, Kinnunen L, Klopp N, Kong A, Kovacs P, Kraft P, Kravic J, Langford C, Leander K, Liang L, Lichtner P, Lindgren CM, Lindholm E, Linneberg A, Liu C-T, Lobbens S, Luan JA, Lyssenko V, Mannisto S, McLeod O, Meyer J, Mihailov E, Mirza G, Muhleisen TW, Muller-Nurasyid M, Navarro C, Nothen MM, Oskolkov NN, Owen KR, Palli D, Pechlivanis S, Peltonen L, Perry JRB, Platou CGP, Roden M, Ruderfer D, Rybin D, van der Schouw YT, Sennblad B, Sigursson G, Stancakova A, Steinbach G, Storm P, Strauch K, Stringham HM, Sun Q, Thorand B, Tikkanen E, Tonjes A, Trakalo J, Tremoli E, Tuomi T, Wennauer R, Wiltshire S, Wood AR, Zeggini E, Dunham I, Birney E, Pasquali L, Ferrer J, Loos RJF, Dupuis J, Florez JC, Boerwinkle E, Pankow JS, van Duijn C, Sijbrands E, Meigs JB, Hu FB, Thorsteinsdottir U, Stefansson K, Lakka TA, Rauramaa R, Stumvoll M, Pedersen NL, Lind L, Keinanen-Kiukaanniemi SM, Korpi-Hyovalti E, Saaristo TE, Saltevo J, Kuusisto J, Laakso M, Metspalu A, Erbel R, Jocke K-H, Moebus S, Ripatti S, Salomaa V, Ingelsson E, Boehm BO, Bergman RN, Collins FS, Mohlke KL, Koistinen H, Tuomilehto J, Hveem K, Njolstad I, Deloukas P, Donnelly PJ, Frayling TM, Hattersley AT, de Faire U, Hamsten A, Illig T, Peters A, Cauchi S, Sladek R, Froguel P, Hansen T, Pedersen O, Morris AD, Palmer CNA, Kathiresan S, Melander O, Nilsson PM, Groop LC, Barroso I, Langenberg C, Wareham NJ, O'Callaghan CA, Gloyn AL, Altshuler D, Boehnke M, Teslovich TM, McCarthy MI, Morris AP, DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nat Genet. 2015;47:1415–25.

Geiss LS, Pan L, Cadwell B, Gregg EW, Benjamin SM, Engelgau MM. Changes in incidence of diabetes in U.S. adults, 1997–2003. Am J Prev Med. 2006;30:371–7.

González ELM, Johansson S, Wallander M-A, Rodríguez LAG. Trends in the prevalence and incidence of diabetes in the UK: 1996–2005. J Epidemiol Community Health. 2009;63:332–6.

Grøntved A, Hu FB. Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. JAMA. 2011;305:2448–55.

Grøntved A, Rimm EB, Willett WC, Andersen LB, Hu FB. A prospective study of weight training and risk of type 2 diabetes mellitus in men. Arch Intern Med. 2012;172:1306–12.

Grøntved A, Pan A, Mekary RA, Stampfer M, Willett WC, Manson JE, Hu FB. Muscle-strengthening and conditioning activities and risk of type 2 diabetes: a prospective study in two cohorts of US women. PLoS Med. 2014;11:e1001587.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome. Circulation. 2005;112:2735–52.

Haffner SM, Shaten J, Stem MP, Smith GD, Kuller L. Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes mellitus in men. MRFIT Research Group. Multiple Risk Factor Intervention Trial. Am J Epidemiol. 1996;143:889–97.

Hanley AJG, Wagenknecht LE, Norris JM, Bergman R, Anderson A, Chen YI, Lorenzo C, Haffner SM. Adiponectin and the incidence of type 2 diabetes in Hispanics and African Americans: the IRAS Family Study. Diabetes Care. 2011;34:2231–6.

Hanson RL, Imperatore G, Bennett PH, Knowler WC. Components of the "metabolic syndrome" and incidence of type 2 diabetes. Diabetes. 2002;51:3120–7.

Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol. 2007;165:849–57.

Hauner H, Bechthold A, Boeing H, Brönstrup A, Buyken A, Leschik-Bonnet E, Linseisen J, Schulze M, Strohm D, Wolfram G. Evidence-based guideline of the German Nutrition Society: carbohydrate intake and prevention of nutrition-related diseases. Ann Nutr Metab. 2012;60:1–58.

Heidemann C, Hoffmann K, Spranger J, Klipstein-Grobusch K, Möhlig M, Pfeiffer A, Boeing H. A dietary pattern protective against type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study cohort. Diabetologia. 2005;48:1126–34.

Hivert M, Sun Q, Shrader P, Mantzoros C, Meigs J, Hu F. Circulating IL-18 and the risk of type 2 diabetes in women. Diabetologia. 2009;52:2101–8.

Hivert M-F, Jablonski KA, Perreault L, Saxena R, McAteer JB, Franks PW, Hamman RF, Kahn SE, Haffner S, the DIAGRAM Consortium, Meigs JB, Altshuler D, Knowler WC, Florez JC, for the Diabetes Prevention Program Research Group. Updated genetic score based on 34 confirmed type 2 diabetes loci is associated with diabetes incidence and regression to normoglycemia in the Diabetes Prevention Program. Diabetes. 2011a;60:1340–8.

Hivert MF, Sullivan L, Shrader P, Fox C, Nathan D, D'Agostino R, Wilson P, Kowall B, Herder C, Meisinger C, Thorand B, Rathmann W, Meigs J. Insulin resistance influences the association of adiponectin levels with diabetes incidence in two population-based cohorts: the Cooperative Health Research in the Region of Augsburg (KORA) S4/F4 study and the Framingham Offspring Study. Diabetologia. 2011b;54:1019–24.

Hopping BN, Erber E, Grandinetti A, Verheus M, Kolonel LN, Maskarinec G. Dietary fiber, magnesium, and glycemic load alter risk of type 2 diabetes in a multiethnic cohort in Hawaii. J Nutr. 2010;140:68–74.

Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care. 2011;34:1249–57.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med. 2001a;345:790–7.

Hu FB, van Dam RM, Liu S. Diet and risk of type II diabetes: the role of types of fat and carbohydrate. Diabetologia. 2001b;44:805–17.

Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004;53:693–700.

Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. BMJ. 2012;344:e1454.

Hu Y, Bhupathiraju SN, de Koning L, Hu FB. Duration of obesity and overweight and risk of type 2 diabetes among US women. Obesity. 2014;22:2267–73.

Huxley R, Lee CM, Barzi F, Timmermeister L, Czernichow S, Perkovic V, Grobbee DE, Batty D, Woodward M. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis. Arch Intern Med. 2009;169:2053–63.

Imamura F, Lichtenstein AH, Dallal GE, Meigs JB, Jacques PF. Generalizability of dietary patterns associated with incidence of type 2 diabetes mellitus. Am J Clin Nutr. 2009;90:1075–83.

International Diabetes Federation. IDF diabetes atlas. 7th ed. Brussels: International Diabetes Federation; 2015.

Jeon CY, Lokken RP, Hu FB, van Dam RM. Physical activity of moderate intensity and risk of type 2 diabetes: a systematic review. Diabetes Care. 2007;30:744–52.

Jiang R, Manson JE, Stampfer MJ, Liu S, Willett WC, Hu FB. Nut and peanut butter consumption and risk of type 2 diabetes in women. JAMA. 2002;288:2554–60.

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116:1784–92.

Kanaya AM, Wassel Fyr C, Vittinghoff E, Harris TB, Park SW, Goodpaster BH, Tylavsky F, Cummings SR. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. Arch Intern Med. 2006;166:350–6.

Kendall CW, Josse AR, Esfahani A, Jenkins DJ. Nuts, metabolic syndrome and diabetes. Br J Nutr. 2010;104:465–73.

Kizer JR, Arnold AM, Benkeser D, Ix JH, Djousse L, Zieman SJ, Barzilay JI, Tracy RP, Mantzoros CS, Siscovick DS, Mukamal KJ. Total and high-molecular-weight adiponectin and risk of incident diabetes in older people. Diabetes Care. 2012;35:415–23.

Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet J-P, Martin RM, Smith GD, Gillman MW, Chalmers B, Hodnett E, Shapiro S. A randomized breast-feeding promotion intervention did not reduce child obesity in Belarus. J Nutr. 2009;139:S417–21.

Krishnan S, Rosenberg L, Singer M, Hu FB, Djousse L, Cupples LA, Palmer JR. Glycemic index, glycemic load, and cereal fiber Intake and risk of type 2 diabetes in US black women. Arch Intern Med. 2007;167:2304–9.

Krishnan S, Cozier YC, Rosenberg L, Palmer JR. Socioeconomic status and incidence of type 2 diabetes: results from the Black Women's Health Study. Am J Epidemiol. 2010;171:564–70.

Lawlor DA, Lichtenstein P, Långström N. Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. Circulation. 2011;123:258–65.

Lee D-H, Jacobs DR, Gross M, Kiefe CI, Roseman J, Lewis CE, Steffes M. Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin Chem. 2003;49:1358–66.

Lee CC, Adler AI, Sandhu MS, Sharp SJ, Forouhi NG, Erqou S, Luben R, Bingham S, Khaw KT, Wareham NJ. Association of C-reactive protein with type 2 diabetes: prospective analysis and meta-analysis. Diabetologia. 2009;52:1040–7.

Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380:219–29.

Ley SH, Harris SB, Mamakeesick M, Noon T, Fiddler E, Gittelsohn J, Wolever TM, Connelly PW, Hegele RA, Zinman B. Metabolic syndrome and its components as predictors of incident type 2 diabetes mellitus in an Aboriginal community. CMAJ. 2009;180:617–24.

Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet. 2014;383:1999–2007.

Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009;302:179–88.

Li Y, He Y, Qi L, Jaddoe VW, Feskens EJM, Yang X, Ma G, Hu FB. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. Diabetes. 2010;59:2400–6.

Li Y, Ley SH, Tobias DK, Chiuve SE, VanderWeele TJ, Rich-Edwards JW, Curhan GC, Willett WC, Manson JE, Hu FB, Qi L. Birth weight and later life adherence to unhealthy lifestyles in predicting type 2 diabetes: prospective cohort study. BMJ. 2015;351:h3672.

Li Y, Gao X, Winkelman JW, Cespedes EM, Jackson CL, Walters AS, Schernhammer E, Redline S, Hu FB. Association between sleeping difficulty and type 2 diabetes in women. Diabetologia. 2016;59:719–27.

Liese AD, Nichols M, Sun X, D'Agostino RB, Haffner SM. Adherence to the DASH Diet is inversely associated with incidence of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes Care. 2009a;32:1434–6.

Liese AD, Weis KE, Schulz M, Tooze JA. Food intake patterns associated with incident type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetes Care. 2009b;32:263–8.

Lim J-S, Lee D-H, Park J-Y, Jin S-H, Jacobs DR. A strong interaction between serum gamma-glutamyltransferase and obesity on the risk of prevalent type 2 diabetes: results from the Third National Health and Nutrition Examination Survey. Clin Chem. 2007;53:1092–8.

Liu S, Chou EL. Dietary glycemic load and type 2 diabetes: modeling the glucose-raising potential of carbohydrates for prevention. Am J Clin Nutr. 2010;92:675–7.

Liu S, Tinker L, Song Y, Rifai N, Bonds DE, Cook NR, Heiss G, Howard BV, Hotamisligil GS, Hu FB, Kuller LH, Manson JE. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. Arch Intern Med. 2007;167:1676–85.

Liu E, Meigs JB, Pittas AG, Economos CD, McKeown NM, Booth SL, Jacques PF. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. Am J Clin Nutr. 2010;91:1627–33.

Lorenzo C, Williams K, Hunt KJ, Haffner SM. The National Cholesterol Education Program – Adult Treatment Panel III, International Diabetes Federation, and World Health Organization

definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. Diabetes Care. 2007;30:8–13.
Luke A, Guo X, Adeyemo AA, Wilks R, Forrester T, Lowe WJ, Comuzzie AG, Martin LJ, Zhu X, Rotimi CN, Cooper RS. Heritability of obesity-related traits among Nigerians, Jamaicans and US black people. Int J Obes Relat Metab Disord. 2001;25:1034–41.
Malik VS, Popkin BM, Bray GA, Després J-P, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care. 2010;33:2477–83.
Manson JE, Stampfer MJ, Colditz GA, Willett WC, Rosner B, Hennekens CH, Speizer FE, Rimm EB, Krolewski AS. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet. 1991;338:774–8.
Maskarinec G, Grandinetti A, Matsuura G, Sharma S, Mau M, Henderson BE, Kolonel LN. Diabetes prevalence and body mass index differ by ethnicity: the Multiethnic Cohort. Ethn Dis. 2009;19:49–55.
McMullan CJ, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. JAMA. 2013;309:1388–96.
McNaughton SA, Mishra GD, Brunner EJ. Dietary patterns, insulin resistance, and incidence of type 2 diabetes in the Whitehall II Study. Diabetes Care. 2008;31:1343–8.
Meigs JB. Epidemiology of type 2 diabetes and cardiovascular disease: translation from population to prevention: the Kelly West award lecture 2009. Diabetes Care. 2010;33:1865–71.
Meigs JB, Hu FB, Rifa IN, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA. 2004;291:1978–86.
Meigs JB, O'Donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PWF. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. Diabetes. 2006;55:530–7.
Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, Manning AK, Florez JC, Wilson PWF, D'Agostino RBS, Cupples LA. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359:2208–19.
Meisinger C, Löwel H, Thorand B, Döring A. Leisure time physical activity and the risk of type 2 diabetes in men and women from the general population. Diabetologia. 2005;48:27–34.
Mekary RA, Giovannucci E, Willett WC, van Dam RM, Hu FB. Eating patterns and type 2 diabetes risk in men: breakfast omission, eating frequency, and snacking. Am J Clin Nutr. 2012;95:1182–9.
Melanson EL, Astrup A, Donahoo WT. The relationship between dietary fat and fatty acid intake and body weight, diabetes, and the metabolic syndrome. Ann Nutr Metab. 2009;55:229–43.
Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and trends in diabetes among adults in the united states, 1988–2012. JAMA. 2015;314:1021–9.
Meyer KA, Kushi LH, Jacobs DR, Folsom AR. Dietary fat and incidence of type 2 diabetes in older Iowa women. Diabetes Care. 2001;24:1528–35.
Mezuk B, Eaton WW, Albrecht S, Golden SH. Depression and type 2 diabetes over the lifespan: a meta-analysis. Diabetes Care. 2008;31:2383–90.
Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. Eur J Clin Nutr. 2011;65:1005–15.
Muraki I, Imamura F, Manson JE, Hu FB, Willett WC, Dam RMv, Sun Q. Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies. BMJ. 2013;347:f5001.
Negi SI, Pankow JS, Fernstrom K, Hoogeveen RC, Zhu N, Couper D, Schmidt MI, Duncan BB, Ballantyne CM. Racial differences in association of elevated interleukin-18 levels with type 2 diabetes: the Atherosclerosis Risk in Communities Study. Diabetes Care. 2012;35:1513–8.
Nettleton JA, McKeown NM, Kanoni S, Lemaitre RN, Hivert M-F, Ngwa J, van Rooij FJA, Sonestedt E, Wojczynski MK, Ye Z, Tanaka T, the CHARGE Whole Grain Foods Study Group. Interactions of dietary whole-grain intake with fasting glucose- and insulin-related

genetic loci in individuals of European descent: a meta-analysis of 14 cohort studies. Diabetes Care. 2010;33:2684–91.
Nguyen QM, Srinivasan SR, Xu J-H, Chen W, Hassig S, Rice J, Berenson GS. Elevated liver function enzymes are related to the development of prediabetes and type 2 diabetes in younger adults: the Bogalusa Heart Study. Diabetes Care. 2011;34:2603–7.
Oh J-Y, Barrett-Connor E, Wedick NM, Wingard DL. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care. 2002;25:55–60.
Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation. 2000;102:1296–301.
Owen C, Martin R, Whincup P, Smith G, Cook D. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics. 2005;115:1367–77.
Owen C, Martin R, Whincup P, Smith G, Cook D. Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. Am J Clin Nutr. 2006;84:1043–54.
Pabon-Nau L, Cohen A, Meigs J, Grant R. Hypertension and diabetes prevalence among U.S. Hispanics by country of origin: the National Health Interview Survey 2000–2005. J Gen Intern Med. 2010;25:847–52.
Pan A, Lucas M, Sun Q, van Dam RM, Franco OH, Manson JE, Willett WC, Ascherio A, Hu FB. Bidirectional association between depression and type 2 diabetes mellitus in women. Arch Intern Med. 2010;170:1884–91.
Pan A, Schernhammer ES, Sun Q, Hu FB. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. PLoS Med. 2011a;8:e1001141.
Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Willett WC, Hu FB. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. Am J Clin Nutr. 2011b;94:1088–96.
Pan A, Sun Q, Okereke OI, Rexrode KM, Rubin RR, Lucas M, Willett WC, Manson JE, Hu FB. Use of antidepressant medication and risk of type 2 diabetes: results from three cohorts of US adults. Diabetologia. 2012;55:63–72.
Pérez-Escamilla R, Putnik P. The role of acculturation in nutrition, lifestyle, and incidence of type 2 diabetes among Latinos. J Nutr. 2007;137:860–70.
Pittas AG, Sun Q, Manson JE, Dawson-Hughes B, Hu FB. Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. Diabetes Care. 2010;33:2021–3.
Qi Q, Bray GA, Smith SR, Hu FB, Sacks FM, Qi L. Insulin receptor substrate 1 gene variation modifies insulin resistance response to weight-loss diets in a 2-year randomized trial: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. Circulation. 2011;124:563–71.
Qi Q, Chu AY, Kang JH, Jensen MK, Curhan GC, Pasquale LR, Ridker PM, Hunter DJ, Willett WC, Rimm EB, Chasman DI, Hu FB, Qi L. Sugar-sweetened beverages and genetic risk of obesity. N Engl J Med. 2012;367:1387–96.
Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GYF, Kaplan RC, Muzumdar R, Rohan TE, Strickler HD. The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. Diabetes Metab Res Rev. 2009;25:3–12.
Rajpathak SN, He M, Sun Q, Kaplan RC, Muzumdar R, Rohan TE, Gunter MJ, Pollak M, Kim M, Pessin JE, Beasley J, Wylie-Rosett J, Hu FB, Strickler HD. Insulin-like growth factor axis and risk of type 2 diabetes in women. Diabetes. 2012;61:2248–54.
Ravelli ACJ, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. Lancet. 1998;351:173–7.
Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. Lancet. 2009;373:1789–97.

Robinson JG, Manson JE, Larson J, Liu S, Song Y, Howard BV, Phillips L, Shikany JM, Allison M, Curb JD, Johnson KC, Watts N. Lack of association between 25(OH)D levels and incident type 2 diabetes in older women. Diabetes Care. 2011;34:628–34.

Rotimi CN, Cooper RS, Okosun IS, Olatunbosun ST, Bella AF, Wilks R, Bennett F, Cruickshank JK, Forrester TE. Prevalence of diabetes and impaired glucose tolerance in Nigerians, Jamaicans and US blacks. Ethn Dis. 1999;9:190–200.

Russell M, de Simone G, Resnick HE, Howard BV. The metabolic syndrome in American Indians: the strong heart study. J Cardiometab Syndr. 2007;2:283–7.

Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Arós F, Ruiz-Gutiérrez V, Ros E, PREDIMED Study Investigators. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes Care. 2011;34:14–9.

Salas-Salvadó J, Bulló M, Estruch R, Ros E, Covas M-I, Ibarrola-Jurado N, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Romaguera D, Lapetra J, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez-González MA. Prevention of diabetes with Mediterranean diets: a subgroup analysis of a randomized trial. Ann Intern Med. 2014;160:1–10.

Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr. 2001;73:1019–26.

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem. 1995;270:26746–9.

Schienkiewitz A, Schulze MB, Hoffmann K, Kroke A, Boeing H. Body mass index history and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study. Am J Clin Nutr. 2006;84:427–33.

Schmidt MI, Duncan BB, Bang H, Pankow JS, Ballantyne CM, Golden SH, Folsom AR, Chambless LE. Identifying individuals at high risk for diabetes. Diabetes Care. 2005;28:2013–8.

Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr. 2004;80:348–56.

Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, Heidemann C, Colditz GA, Hu FB. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. Am J Clin Nutr. 2005;82:675–84.

Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H. Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis. Arch Intern Med. 2007;167:956–65.

Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H. Carbohydrate intake and incidence of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study. Br J Nutr. 2008;99:1107–16.

Selvin E, Feinleib M, Zhang L, Rohrmann S, Rifai N, Nelson WG, Dobs A, Basaria S, Golden SH, Platz EA. Androgens and diabetes in men: results from the Third National Health and Nutrition Examination Survey (NHANES III). Diabetes Care. 2007;30:234–8.

Shai I, Jiang R, Manson JE, Stampfer MJ, Willett WC, Colditz GA, Hu FB. Ethnicity, obesity, and risk of type 2 diabetes in women. Diabetes Care. 2006;29:1585–90.

Silverman BL, Metzger BE, Cho NH, Loeb CA. Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. Diabetes Care. 1995;18:611–7.

Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, Hotamisligil GS, Ridker PM, Rodriguez BL, Margolis KL, Oberman A, Liu S. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. Diabetes. 2007;56:1898–904.

Sun Q, Cornelis MC, Manson JE, Hu FB. Plasma levels of fetuin-A and hepatic enzymes and risk of type 2 diabetes in women in the U.S. Diabetes. 2013;62:49–55.

The InterAct Consortium. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. Diabetologia. 2011a;54:2272–82.
The InterAct Consortium. Mediterranean diet and type 2 diabetes risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study: the InterAct project. Diabetes Care. 2011b;34:1913–8.
The InterAct Consortium. Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: the EPIC-InterAct case-cohort study. PLoS Med. 2012;9:e1001230.
The InterAct Consortium. Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. Diabetologia. 2013;56:1520–30.
Tinker LF, Bonds DE, Margolis KL, Manson JE, Howard BV, Larson J, Perri MG, Beresford SA, Robinson JG, Rodríguez B, Safford MM, Wenger NK, Stevens VJ, Parker LM. Low-fat dietary pattern and risk of treated diabetes mellitus in postmenopausal women: the Women's Health Initiative randomized controlled dietary modification trial. Arch Intern Med. 2008;168: 1500–11.
Tonstad S, Stewart K, Oda K, Batech M, Herring RP, Fraser GE. Vegetarian diets and incidence of diabetes in the Adventist Health Study-2. Nutr Metab Cardiovasc Dis. 2013;23:292–9.
Vazquez G, Duval S, Jacobs DR Jr, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. Epidemiol Rev. 2007;29:115–28.
Wallin A, Di Giuseppe D, Orsini N, Patel PS, Forouhi NG, Wolk A. Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic review and meta-analysis of prospective studies. Diabetes Care. 2012;35:918–29.
Wang X, Bi Y, Zhang Q, Pan F. Obstructive sleep apnoea and the risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Respirology. 2013;18:140–6.
Wannamethee S, Camargo CA Jr, Manson JE, Willett WC, Rimm EB. Alcohol drinking patterns and risk of type 2 diabetes mellitus among younger women. Arch Intern Med. 2003;163: 1329–36.
Wannamethee SG, Whincup PH, Thomas MC, Sattar N. Associations between dietary fiber and inflammation, hepatic function, and risk of type 2 diabetes in older men: potential mechanisms for the benefits of fiber on diabetes risk. Diabetes Care. 2009;32:1823–5.
Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, Willett W, Hu FB, Sun Q, van Dam RM. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr. 2012;95:925–33.
Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BE, Carlsson S, de Rooij SR, Dyck RF, Eriksson JG, Falkner B, Fall C, Forsén T, Grill V, Gudnason V, Hulman S, Hyppönen E, Jeffreys M, Lawlor DA, Leon DA, Minami J, Mishra G, Osmond C, Power C, Rich-Edwards JW, Roseboom TJ, Sachdev HS, Syddall H, Thorsdottir I, Vanhala M, Wadsworth M, Yarbrough DE. Birth weight and risk of type 2 diabetes: a systematic review. JAMA. 2008;300:2886–97.
Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2007;298:2654–64.
Wilson PWF, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005;112:3066–72.
Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med. 2007;167:1068–74.
Wu JH, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O, Djousse L, Hu FB, Mozaffarian D. Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. Br J Nutr. 2012;107:S214–27.
Yeh H-C, Duncan BB, Schmidt MI, Wang N-Y, Brancati FL. Smoking, smoking cessation, and risk for type 2 diabetes mellitus: a cohort study. Ann Intern Med. 2010;152:10–7.

Young T, Peppard PE, Taheri S. Excess weight and sleep-disordered breathing. J Appl Physiol. 2005;99:1592–9.

Zhang L, Curhan GC, Hu FB, Rimm EB, Forman JP. Association between passive and active smoking and incident type 2 diabetes in women. Diabetes Care. 2011;34:892–7.

Zhao Z, Li S, Liu G, Yan F, Ma X, Huang Z, Tian H. Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2012;7: e41641.

Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted? J Intern Med. 2000;247:301–10.

Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001;414:782–7.

Genetics of Diabetes and Diabetic Complications

5

Rashmi B. Prasad, Emma Ahlqvist, and Leif Groop

Contents

Rashmi B. Prasad and Emma Ahlqvist have contributed equally with all other contributors.

R. B. Prasad · E. Ahlqvist
Department of Clinical Sciences, Diabetes and Endocrinology Unit, Lund University Diabetes Centre, Malmo, Sweden
e-mail: Rashmi.Prasad@med.lu.se; Emma.Ahlqvist@med.lu.se

L. Groop (✉)
Department of Clinical Sciences, Diabetes and Endocrinology Unit, Lund University Diabetes Centre, Malmo, Sweden

Finnish Institute of Molecular Medicine, Helsinki University, Helsinki, Finland
e-mail: Leif.Groop@med.lu.se

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_6

Abstract

Diabetes is a collection of diseases characterized by defective glucose homeostasis. Different diabetes types have different etiologies and their genetic architecture ranges from highly penetrant monogenetic diseases, such as MODY and neonatal diabetes, to polygenic diseases, such as type 1 and type 2 diabetes that are caused by numerous genetic variants adding up to the individual risk. While both diabetes and diabetic complications have been known to be partly heritable for a long time, identification of risk variants was originally limited to a few variants with relatively modest effect sizes. This changed with the advent of genome-wide association studies (GWAS), which has led to the identification of hundreds of common risk variants for diabetes. Still, these variants only explain part of the heritability of complex diabetes types. Further technical development in the field, such as next-generation sequencing, has recently enabled identification of rare variants. Epigenetics, epistasis, gene-environment interactions, parent-of-origin effects, and noncoding RNAs are current research areas that provide additional layers to the genetic architecture and might reveal some of the missing heritability. In this chapter, we review the genetic basis of different diabetes types and diabetic complications and the major methodological milestones that have enabled the many success stories of the last decade.

Keywords

Type 2 diabetes (T2D) · Heterogeneity of T2D · Heritability · Genetic association · Linkage studies · Candidate studies · Genome-wide association studies (GWAS) · Next generation sequencing (NGS) · Whole genome sequencing (WGS) · Whole exome sequencing (WES) · Rare variants · Protective variants · Parent-origin · Epigenetics · Gene expression · Gene-gene interactions · Epistasis · Non-coding RNA · Gene-environment interactions · Complications

The Genetic Architecture of Diabetes

Diabetes is considered to result from a collision between genetic predisposition and environment but their respective roles and interactions differ between different types of diabetes and are still relatively poorly understood, especially in the case of type 2 diabetes (T2D).

The disease clusters in certain families supporting a clear heritable component. The "genetic architecture" of diabetes describes the genetic basis for differences between individuals and is defined by the number, frequencies, and effect sizes of causal alleles. The diabetes spectrum includes everything from strongly penetrant monogenic types, like MODY and neonatal diabetes, to the highly complex polygenic and multifactorial T2D, whose architecture is still under debate. One hypothesis suggests that T2D represents the extreme of a normal distribution where a large number of common variants with small additive effects contribute to the disease (the common disease common variant hypothesis – CDCV) (Plomin et al. 2009), whereas an alternative hypothesis proposes that rare alleles cause the effects observed with common variants (synthetic associations) and thereby explain most of the heritability (Lupski et al. 2011). Studies performed thus far suggest that the truth is somewhere in between, with contributions from both common and rare variants (Agarwala et al. 2013).

The genetic architecture can also vary within a diabetic subtype; especially T2D is heterogeneous and could include forms caused by rare variants with high penetrance as well as forms caused by many common risk alleles. A gene locus can also harbor different susceptibility variants in different individuals including both common and rare alleles, as has been observed at the *HNF1A* and *HNF4A* loci. These gene regions contain both rare MODY causing variants and common variants associated with T2D.

Heritability estimates how much variation in a phenotypic trait in a population is due to genetic variation. Heritability in its traditional form estimates phenotypic similarities between family members or, ideally, between monozygotic and dizygotic twins. In genetic terms, heritability can be quantified in two ways; broad-sense heritability (H^2) captures the proportion of phenotypic variation due to genetic effects including dominance (allelic interactions within loci) and epistasis (interactions between loci), whereas narrow-sense heritability (h^2) covers variation due to additive genetic effects only.

It is well known from both family and population studies that both type 1 diabetes (T1D) and T2D are partially heritable but the hitherto identified risk loci explain less than 20% of the heritability of T2D whereas for T1D, this number is >80% (Groop and Pociot 2014). This missing heritability could have multiple explanations, including incorrect estimations of heritability or incorrect definitions of the disease (Visscher et al. 2008). Applying an approach that considered all SNPs on the chip could explain a much larger proportion of the heritability of T2D supporting the existence of numerous yet unidentified loci with even smaller effects than those identified to date (Visscher et al. 2008). Other explanations include gene-gene interactions, also referred to as epistasis, gene-environment interactions, and epigenetics. Parent-of-origin effects, where the same allele can have different effects depending on whether it is inherited from the mother or the father, add another dimension to the genetic architecture and could play a key role in fetal programming of the disease (Kong et al. 2009; Prasad et al. 2016a). Noncoding RNAs and microRNAs add a further layer of complexity in the regulation of gene expression.

The Spectrum of Diabetes Disorders

Traditionally, diabetes has been divided into T2D and T1D. However, this is clearly an obsolete view, and it has become clear that diabetes encompasses a range of heterogeneous metabolic disorders discussed below:

Type 1 diabetes (T1D) is a chronic condition caused by autoimmune destruction of pancreatic beta cells and is characterized by (nearly) complete absence of insulin and presence of several autoantibodies reacting with beta-cell auto antigens leading to dependence on insulin injections. It is most often diagnosed in children, adolescents, or young adults less than 35 years old.

LADA (latent autoimmune diabetes in adults) is a subgroup of diabetes defined by presence of autoantibodies to glutamic acid decarboxylase (GADA) with onset after age 35. These patients may be controlled by oral antidiabetic agents during the first 6 months after diagnosis (Tuomi et al. 1993a; Groop et al. 2006), but become more T1D-like with time.

MODY (maturity-onset diabetes of the young) refers to monogenic forms of diabetes with unique mutations in more than ten different genes, a number which is still increasing. The disease is characterized by autosomal dominant transmission of early-onset (<25 years) diabetes and varying degrees of beta-cell dysfunction (Tattersall 1974).

Maternally inherited diabetes and deafness (MIDD) is due to the A3242G mutation in mitochondrial DNA (mtDNA) (van den Ouweland et al. 1992), exclusively transmitted from the mother as sperm lacks mitochondria. Symptoms also include hearing loss, and neurological problems particularly in patients with the MELAS syndrome (Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke), which is caused by the same mutation in mtDNA.

Neonatal diabetes is defined as diabetes with onset at birth or during the first 6 months of life. Both transient and permanent forms exist (Murphy et al. 2008a).

Gestational diabetes mellitus (GDM) represents a transitory form of diabetes that manifests as hyperglycemia during pregnancy and usually resolves postpartum.

Secondary diabetes is caused by pancreatic disease (pancreatitis or cancer) or other endocrine disorders.

Type 2 diabetes is the most prevalent form of diabetes comprising today >80% of all reported patients with diabetes. T2D develops when pancreatic beta cells can no longer produce enough insulin to compensate for the insulin resistance imposed by increasing obesity. There is no formal definition of T2D; individuals with diabetes who do not fulfill criteria for any of the diabetes forms mentioned above are considered to have T2D. While T2D is mostly diagnosed in elderly people (Groop and Pociot 2014), it is increasingly reported already in adolescents from India and China (WHO 2014) but also in Hispanics and African Americans. The diabetes subtypes represent a diverse range of genetic etiologies and manifestations and usually require separate therapeutic strategies.

The **ANDIS** (All New Diabetics in Scania) project in Southern Sweden represents a new attempt to reclassify diabetes into subgroups based upon phenotypic indications, genetic markers, and other biomarkers (Fig. 1). The aim of ANDIS is to

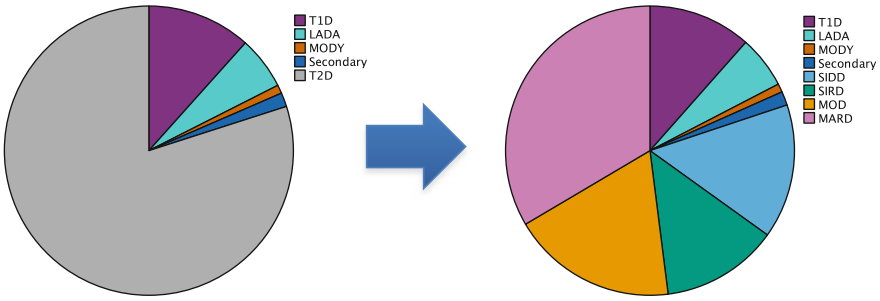


Fig. 1 Distributions of diabetes patient in the Swedish ANDIS cohort where type 2 diabetes patients have been subclassified into four new subgroups: severe insulin-deficient diabetes (SIDD; 17.5%), severe insulin-resistant diabetes (SIRD; 15.3%), moderate obesity-related diabetes (MOD; 21.6%), and moderate age-related diabetes (MARD; 39.1%)

register all new cases of diabetes in Scania and improve diagnosis and treatment strategies. At the time of registration, blood samples are drawn to determine the presence of GAD-antibodies, measure C-peptide, biobanking, and for genetic analysis. The data is used to classify the disease into subtypes and to study genetic causes of diabetes, diabetic complications, and other disorders related to diabetes (http://andis.ludc.med.lu.se/all-new-diabetics-in-scania-andis/). A similar project has been initiated in Uppsala with the same goal (ANDIU – All new diabetics in Uppsala; http://www.andiu.se/english/). Recently these cohorts were used to subgroup patients using unsupervised clustering, based on age at diagnosis, BMI, HbA1c at diagnosis, presence of GAD antibodies and measures of insulin secretion and insulin resistance (c-peptide based HOMA2-B and HOMA2-IR respectively; Fig. 1). Using this strategy patients could be divided into five subtypes, of which one, referred to as severe autoimmune diabetes (SAID) corresponded to T1D and LADA. Of the four type 2 diabetes subgroups, one was characterized by low insulin secretion and poor metabolic control (severe insulin-deficient diabetes; SIDD) and had an increased risk of retinopathy, whereas a subtype defined by strong insulin resistance (severe insulin-resistant diabetes; SIRD) had increased risk of kidney disease (Ahlqvist et al. 2018).

Development of the Field of Complex Genetics

The field of genetics has been revolutionized in the last decade driven by technical advances in sequencing and genotyping techniques (Fig. 2).

Linkage Analysis

Many genetic diseases have been mapped to disease causing genes using data from affected families. Family-based linkage analysis is a method that takes advantage of

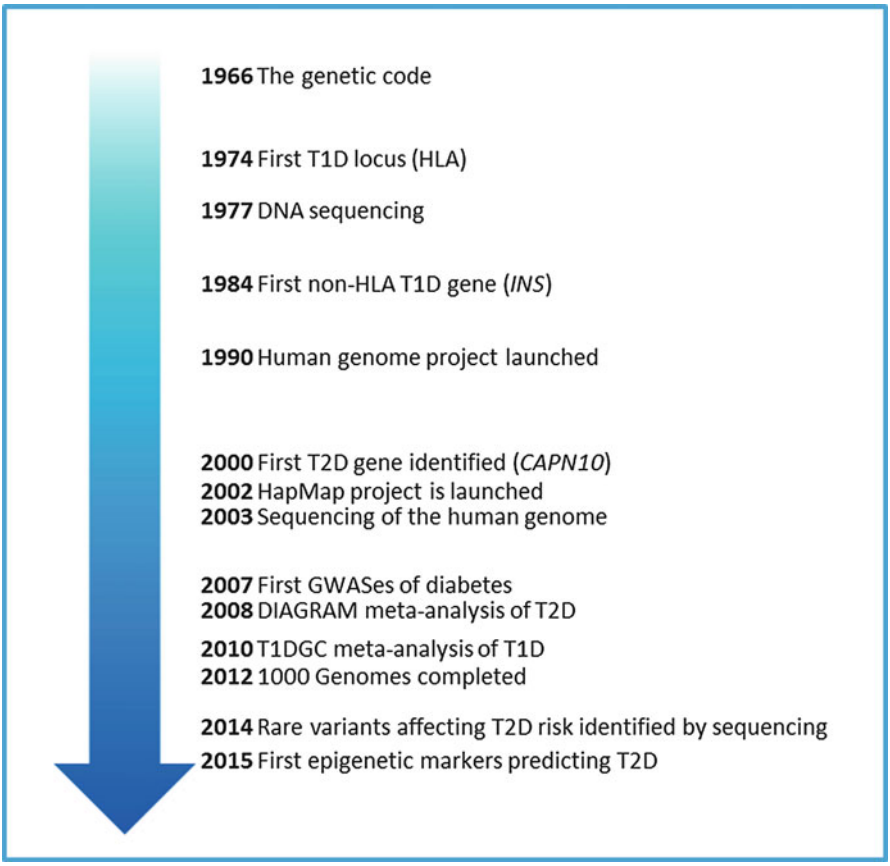


Fig. 2 Major landmarks in the history of genomics and diabetes genetics

the long stretches of chromosomes in linkage within a family that stem from the genetic recombination process during meiosis. Finding that affected family members share a certain genetic region that is identical by decent (i.e., identical because it was inherited from the same parent) more often than expected by chance is evidence that a disease causing variant is in linkage with that marker. Thanks to the long linked regions, disease loci could be mapped on a genome-wide level without any prior hypothesis by genotyping only 400–500 genetic markers (microsatellites). However, the low number of genetic recombinations also results in very low resolution, making it difficult to go from locus to disease causing gene. This strategy is very successful in mapping diseases like MODY that have a strong penetrance and a known mode of inheritance, but much less fruitful for complex diseases such as T1D and T2D.

A modern application of linkage analysis uses a dense GWAS (see below) with about 2.5 M SNPs to identify the shortest, transmitted haplotype followed by sequencing of the most informative individuals to identify the causal variant on

the haplotype. The resolution of this approach is much better than traditional linkage studies, but still it sometimes remains a challenge to identify the functional causal variant.

Candidate Genes, Haplotypes, and Association Studies

The common disease/common variant hypothesis suggests that common disorders are caused by aggregation of common risk alleles with small individual effects (Lupski et al. 2011). This hypothesis stimulated the development of novel tools for genetic association studies. Given the high cost of genotyping, genetic association studies were first restricted to testing a single nucleotide polymorphism (SNP) in a functional candidate gene, e.g., PPARγ (the Pro12Ala variant) for association with a phenotype, insulin resistance, and/or T2D (Deeb et al. 1998)

An important step in the development of association studies was the realization that we inherit short stretches of the chromosomes where variants are in linkage disequilibrium (LD) with another so-called haplotypes. The Human Genome Project (Collins et al. 2003) pioneered and identified >100,000 SNPs spread all over the genome, thereby providing a first catalogue of markers for genetic studies. This allowed studies of larger cohorts with improved statistical power and resolution of observed association signals. A drawback was the need for very large numbers of genetic markers to cover a region, and association studies were therefore, in the early days primarily performed on small regions known to harbor genes that were known or expected to be involved in pathogenesis of the disease.

The next important step came with the the HapMap project which provided a catalogue of haplotypes across the genome and demonstrated that genotyping of 500,000 SNPs was enough to cover about 75% of common variants with minor allele frequency > 5% in the genome (International HapMap Consortium 2003).

Genome-Wide Association Studies (GWAS)

The rapid improvement in high throughput technology for SNP genotyping, allowing simultaneous genotyping of hundreds of thousands of SNPs, as well as the HapMap project, opened new possibilities for performing association studies on the genome-wide level, so-called Genome-Wide Association Studies (GWAS). In 2007, the first GWASes in T2D were published describing a modest list of about ten variants associated with T2D. This list has continuously grown and include today (2017) >140 SNPs showing association with T2D or glycemic traits like glucose and/or insulin. A state-of-the-art GWAS today interrogates over 10 million variants across the genome. This has been made possible not only by development of better genotyping technology but also by the development of reference genomes (usually based on sequencing of thousands of full genomes) that allows inference of SNPs without genotyping, so-called imputation, which takes advantage of the known correlation (LD) between markers in the population.

The huge number of variants that can be tested requires strict correction for multiple testing. A commonly adopted thresholds for genome-wide statistical significance is $p < 5 \times 10^{-8}$, which is equivalent to Bonferroni correction for a million independent tests. The GWAS approach has proven highly effective in identifying robustly associated loci for many complex traits and diseases. In recent years, we have seen many international collaborations joining efforts to combine GWAS studies in ever-larger meta-analyses resulting in the identification of genetic loci with smaller and smaller effects.

Next-Generation Sequencing

Since natural selection usually removes deleterious variants, rare risk alleles are often more recent and likely to have arisen in extended pedigrees in isolated populations. Their term "Clan genomics" has been used to describe the concept of rare variant combinations in families and their role in disease etiology (Lupski et al. 2011).

Techniques for large-scale genomic analysis have continued to evolve thereby making detection of rare variants feasible. One important advancement was the advent of whole exome sequencing (WES) and whole genome-sequencing (WGS) made possible by the continuously improved next-generation sequencing technologies, allowing affordable high - throughput sequencing of entire genomes. While the Human Genome Project, using capillary electrophoresis-based Sanger sequencing, took over 10 years and cost several billion US dollars, the current figures for a full genome is in the range of a few days and 1,000$. WES is a highly effective method to capture more than 90% of the coding DNA of an individual. This is accomplished by applying various "exome capture" techniques that extract the protein-coding portion of the genome using specific DNA probes. The exome represents only 1–1.5% of the genome, but since many disease-causing mutations are located in coding regions, this is a cost-effective approach to identify such rare variants and WES has proven to be very successful in identifying novel genes and disease pathways.

Gene-Gene Interactions

Epistasis is a well-known phenomenon in genetics and refers to interactions between genetic loci resulting in greater effects on a phenotype than expected from the sum of the effects of the involved loci. While epistasis was described more than 100 years ago and has been demonstrated many times in model organisms, there is relatively little evidence for substantial amounts of statistical epistasis in human populations or most natural populations of other organisms, which does not mean that it is not important (Sackton and Hartl 2016). The study of epistasis in complex diseases is severely hampered by the huge sample sizes needed to discover small or medium interaction effect variants with statistical significance. Exhaustively evaluating all of

the possible combinations of SNPs is not computationally feasible. A genome-wide data set including one million SNPs generates 5×10^{11} possible two-SNP models, which requires extensive computing resources and p-values below 10–11 to claim statistical significance after correction for the number of tests. Models including three or more SNPs would of course be even more problematic. Numerous methods have been developed to make whole genome epistasis analysis more computationally tractable (Wei et al. 2014). Still, the power is limited by the sample size. A number of filtering approaches have been suggested to overcome these problems. One is to include only SNPs shown to have an independent effect that is below a certain p-value. While this has been shown to have high power (Evans et al. 2006) and has identified significant interactions for some diseases, SNPs that have effects only through their interactions with other genes would be missed. Another strategy is to use biological knowledge, such as genes belonging to the same pathway or having similar functions, to filter SNPs and then evaluate multi-marker combinations based on biological criteria (Carlson et al. 2004). However, this will bias the analysis in favor of models with an already known biological foundation and miss new potentially more interesting interactions.

Gene-Environment Interactions

It is well known that most forms of diabetes result from a complex interplay between genes and environment. The T2D epidemic is quite recent, dating back ~50 years, and it is evident that during this period, there has been a substantial change in the environment and lifestyle. In contrast, it takes much longer to change our genetic architecture, which determines how we respond to the effects of the environment and which is therefore an important aspect in determining diabetes etiology. For instance, genetic variation affecting metabolic processes could render an individual more susceptible to the effects of a poor diet, while variants affecting personality traits could influence the individual's risk to over-consume food.

A common argument against models that includes genetic variants with strong effects is that if alleles are associated with negative health effects they should have been removed from the population by natural selection (Diamond 2003). However, in the case of diabetes, it is important to remember that the penetrance of the genetic effect depends on interactions with the environment, which has dramatically changed in the recent years.

Epigenetics

The environment can also influence the manifestation of a trait through epigenetic effects on the genome. Epigenetics is defined as a heritable change in gene expression that can be passed on from one cell generation to another through mitotic inheritance or between generations of species (meiotic inheritance) without changing the DNA sequence (Chong and Whitelaw 2004; Chong et al. 2007; Bird 2007). This

can be in the form of epigenetic modifications, such as addition of methyl group to the DNA sequence, or post-translational modification of histones or microRNAs. The addition of a methyl group often occurs at CpG sites, whereby cytosine is converted into a 5-methylcytosine. If occurring at the promoter, this is usually associated with reduced transcriptional activity and silencing of gene expression, imprinting. Methylation can be studied by bisulfate sequencing. Treatment of DNA with bisulfite converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Bisulfate sequencing can be targeted or global, i.e., the entire genome. There is emerging evidence that environmental factors such as diet and exercise can change the degree of DNA methylation and thereby cause changes in gene expression. It has been shown that poor physical fitness and activity and a low VO_{2max} increase risk of developing T2D. Obesity and insulin resistance, mitochondrial dysfunction, and changes in muscle fiber – type composition are potential mechanisms linking poor physical fitness with an increased risk for disease Exercise is also a potential environmental factor which could exert effects on gene expression by methylation (White et al. 2013).

Noncoding RNAs: microRNAs

Noncoding RNAs are important regulators of gene expression and function. MicroRNAs (miRNAs) piRNAs (PIWI-interacting RNAs), snoRNAs (small nucleolar RNAs), lincRNAs (long intergenic noncoding RNAs), and lncRNAs (long noncoding RNAs) represent different forms of noncoding RNAs that can regulate gene expression and eventually contribute to the development of diabetes. For instance, the efficiency of miRNAs binding to target transcripts depends on both the sequence and the intra-molecular structure of the transcript. SNPs can contribute to alterations in the structure of regions flanking them or may alter the target sequence, thereby influencing the accessibility for miRNA binding (http://200.12.130.109/nrdr/) (Fernandez-Valverde et al. 2011; Hariharan et al. 2009). Manipulation of specific miRNAs is now being explored as novel therapeutic modalities (Davidson and McCray Jr. 2011).

Parent-of-Origin Effects

The risk of developing T2D is higher if the mother has T2D than the father, whereas the opposite is seen for T1D. These phenomena conflict with the classical Mendelian inheritance patterns, which assume functional equivalence of maternal and paternal alleles (Groop et al. 1996; Hemminki et al. 2010). Sex specific parental effects have also been reported for glucose stimulated insulin secretion and HDL concentrations (Groop et al. 1996). A potential explanation for this could be preferential parental transmission of causative alleles to offspring, which is often associated with DNA methylation and imprinting. Certain epigenetic modifications have the potential to be

stable and heritable across cell divisions and manifest as parent-of-origin effects (Chong and Whitelaw 2004).

The conflict hypothesis, or the kinship theory of genomic imprinting, suggests that inequality between the parental genomes results from a genomic tug-of-war between mothers and fathers over the use of maternal resources for the fetus. The paternal imprinting maximizes the utilization of intrauterine resources to the offspring to increase his evolutionary fitness whereas the maternal imprinting tries to minimize utilization of these resources to conserve them for her own survival and for her future offspring (Moore and Haig 1991). In contrast, the co-adaptation hypothesis suggests that imprinted genes coevolve to improve fetal development and maternal provisioning of nutrition and care (Wolf and Hager 2006). While there is insufficient evidence to favor either theory over the other, imprinting nevertheless plays a key role in defining paternal and maternal effects on the offspring.

Parent-of-origin effects (POE) can also be caused by intrauterine effects, which could play a role in fetal programming. Poor nutrition can affect fetal growth and produce permanent changes in glucose-insulin metabolism, often associated with low birth weight (Hales and Barker 2001). Low birth weight can induce permanent changes in metabolism and increase susceptibility to chronic diseases as diabetes as proposed by the Developmental Origins of Health and Disease (DoHAD) hypothesis (Barker 2007). If intrauterine programming results in a reduced β-cell mass, it could predispose to diabetes later in life if insufficient to increase insulin secretion to meet increased demands imposed by insulin resistance. Gestational diabetes in the mother can lead to a hyperglycemic environment, which, in turn, is associated with both macrosomia and low birth weight (Young and Ecker 2013; Group HSCR et al. 2008). A "U" shaped curve has been observed for the association of low and high birth weight and risk of T2D and obesity (Harder et al. 2007).

Investigation of parent-of-origin effects requires family-based cohorts with pedigree information. Long-range phasing and imputation methods allow for predicting genotypes, thereby assigning "surrogate" parents despite availability of DNA from only a few family members. Novel POE methods allow detection of imprinting effects from differences in the phenotypic variance of heterozygotes in very large case-control studies (Hoggart et al. 2014). Parent-of-origin effects could explain part of the missing heritability and must be taken into consideration in investigations of etiology of diabetes.

A large family-based study on Iceland showed that variants in the *KCNQ1*, *KLF14,* and *MOB2* genes show higher risk of T2D when the risk allele is transmitted from the mother than from the father (Kong et al. 2009; Small et al. 2011); these findings have subsequently been replicated in our own studies (Hanson et al. 2013). We have also provided evidence for excess maternal transmission of variants in the *THADA* gene to offspring with T2D (Prasad et al. 2015). The *KCNQ1* gene is an example of fetal programming showing monoallelic expression in fetal islets but biallelic expression in adult islets (Travers et al. 2013) Moreover, paternal mutations at this locus show reduced pancreatic beta cell mass (Asahara et al. 2015).

Genetics of Specific Diabetes Types

Type 1 Diabetes

T1D accounts for 5–10% of diabetes cases worldwide. It is a chronic disease characterized by an autoimmune reaction to the pancreatic beta cells and presence of autoantibodies, leading to nearly complete absence of insulin secretion and dependence on insulin injections. T1D is usually diagnosed in children or adults younger than 35 years. Incidence (cases per year) varies depending on geography, age, and family history, with the highest incidence rates observed in Finland and Sardinia and the lowest in China and Venezuela (Karvonen et al. 2000). The first sign of disease is the appearance of beta-cell autoantibodies, which can occur very early in childhood. The first antibodies are usually directed against either insulin or glutamic acid decarboxylase (GAD), but additional antibodies against ZnT8A and islet antigen-2 (IA2) are common; IA2 are especially frequent in young children. The appearance of auto-antibodies can be followed by a period of slight elevation of blood glucose until overt symptomatic diabetes develops (Pociot and Lernmark 2016).

Heritability

The genetic component in T1D is strong. The average prevalence risk is 0.4% for children with no family history of T1D, but ~6% when one parent has T1D, and >30% when both parents are affected. There is also a great difference in concordance rates between dizygotic (7–11%) and monozygotic (40–50%) twins (Kyvik et al. 1995; Hyttinen et al. 2003). Interestingly, as previously mentioned, the risk of inheriting T1D differs depending on which parent is affected with approximately double risk if inherited from the father (5–8%) than from the mother (2–4%) (Kyvik et al. 1995; Pociot et al. 1993). The sibling relative risk of T1D is about 15 (Patterson et al. 2009; Dahlquist et al. 1989) as compared to 3 for T2D.

Genetic Risk Loci

The main susceptibility locus for T1D is the Human Leukocyte Antigen (HLA) gene complex encoding the major histocompatibility complex (MHC) in humans. This locus accounts for up to 50% of genetic T1D risk and was identified already in the 1970s (Singal and Blajchman 1973; Nerup et al. 1974).

HLA molecules are cell-surface proteins that bind and present peptide antigens to T-lymphocytes. HLA is categorized into two classes. Class I molecules (A, B, and C) consist of a polypeptide chain that form a heterodimer with β-2 microglobulin which is not encoded by the HLA complex. Class II molecules (DR, DQ, and DP) consist of a heterodimer created from two polypeptides (α and β). Class I molecules present peptides from inside the cells and activate cytotoxic T-cells, whereas class II molecules present extra-cellular antigens to T-helper cells that stimulate B-cells to produce antibodies. Peptide binding, and thus antigen presentation, is determined by the shape and electrical charges of the peptide binding groove and the ability of the T-cell receptor to bind to the HLA-peptide complex.

The HLA region exhibits strong linkage disequilibrium, so that within a population individual alleles are usually found in only one or a few haplotype combinations. The highest T1D risk is attributable to class II loci HLA-DR3-DQ2 and HLA-DR4-DQ8. Nearly 90% of children diagnosed with T1D in Scandinavia have either HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes (Sanjeevi et al. 1995). The association between HLA and diabetes seems to be related to risk of developing the first auto-antibody, so that children homozygous for HLA-DR3-DQ2 are more likely to have GADA antibodies as their first antibody and children with HLA-DR4-DQ8 haplotype more likely to have insulin autoantibodies first (Ilonen et al. 2013). Other class II haplotypes have also been associated with risk of T1D with smaller effects, e.g., the DPB1 locus is associated with both protection (DPB1*04:02) and susceptibility (DPB1*03:01 and DPB1*02:02) (Noble 2015). HLA risk alleles also differ between populations. The HLA-DR7 haplotype including *DRB1*07:01* is protective in the European population but confers risk in Africans (Erlich et al. 2008). Similarly, an African specific DR3 haplotype (DRB1*03:02-DQA1*04:01-DQB1*04:02) protects from T1D (Erlich et al. 2008).

Multiple non-HLA loci contribute to disease risk with smaller effects. The first, and strongest (OR 2.4), non-HLA locus, in the insulin gene (*INS*), was identified already in 1984 (Bell et al. 1984). The promotor region was found to have a variable number of repeats (VNTR) marking alleles with different expression of the INS gene, which is postulated to affect susceptibility by modulating thymic expression of insulin and affecting T-cell education (Pugliese 2005). Susceptibility loci in the *CTLA4*, *PTPN22,* and *IL2RA* regions were all identified in candidate gene studies. Since the introduction of GWAS more than 50 loci have been identified, explaining ~80% of the narrow sense heritability of T1D (Pociot et al. 2010). One of the largest efforts was the type 1 Diabetes Genetics Consortium (T1DGC), an international collaboration through which >14,000 samples were collected and genotyped. Of the identified loci only *PTPN22* and *IL2RA* have ORs greater than 1.5; most are in the range of 1.1–1.3, underscoring the importance of the HLA region (Pociot et al. 2010).

Recognition of a specific antigen and HLA by the T-cell receptor may result in autoimmune attack, which could be further potentiated by gene variants that impair antigen presentation or T-cell signaling. Functional insights into the role of T1D susceptibility loci has revealed that many candidate genes are involved in functions related to T-cell-mediated adaptive immune response and tolerance mechanisms and also to innate immunity involved in recognition of β-cell antigens (Zhernakova et al. 2009). Many genetic associations are also shared with other autoimmune diseases (Zhernakova et al. 2009). For example, a common loss-of-function allele in the tyrosine phosphatase *PTPN22* locus decreases the risk of Crohn disease but increases the risk of rheumatoid arthritis and T1D. Interestingly, at least 50% of the identified candidate genes, including *CTRB1/2*, *IFIH1*, *GLIS3*, and *PTPN2* are also expressed in beta-cells supporting the concept that genetic susceptibility to T1D influences both the immune system and beta-cell function (Bergholdt et al. 2012). Post-GWAS fine mapping and functional characterization remain to be performed for most loci.

Gene-Gene and Gene-Environment Interactions

A number of gene-gene interactions have been identified for T1D, primarily between HLA and non-HLA loci, e.g., an interaction between the *PTPN22* locus and *DR3/DR4-DQ302* where the effect of *PTPN22* is stronger with low risk HLA (Smyth et al. 2008).

As for gene-environment interactions, T1D is most likely triggered by an environmental factor but the initiating events that lead to the presentation of beta-cell antigens to T cells for their activation are yet to be elucidated.

Epigenetics

Many processes involved in T1D could be influenced by epigenetic mechanisms, including beta-cell development, metabolism, and regeneration. Immune responses, including activation of T cells and induction of regulatory T-cells, rely on epigenetic regulation. The pattern of four CpG sites proximal to the transcription start site of the *INS* gene has been shown to differ between T1D patients and controls, with three sites being less methylated and one more methylated (Fradin et al. 2012). Similarly, CpG sites in the promoter of *IL2* were more densely methylated in T1D patients than in controls (Belot et al. 2013).

Histone modifications may also be relevant for T1D, For example, case-control studies have revealed different levels of acetylated histone H4 or of H3K9 acetylation in T1D patients compared with controls (Miao et al. 2012), and increased levels of H3K9me2 in T1D-related genes, including *CTLA4*, in lymphocytes from T1D patients compared with controls (Miao et al. 2008).

A growing number of observations suggest that miRNAs can also contribute to the development of T1D. Experimental studies in animal models and cultured cells have provided convincing evidence that miRNA can participate in controlling autoimmune damage of β-cells, regulation of insulin synthesis and secretion (Zheng et al. 2017). The expression of specific miRNAs in blood and lymphocytes has also been shown to differ between T1D patients and controls and to be correlated with disease severity (Zullo et al. 2017). Measurement of these miRNAs may therefore be useful for identifying people at risk of developing T1D and for disease prevention.

Type 2 Diabetes

Heritability

Heritability estimates for T2D have varied between 25% and 80% in different studies; the highest estimates seen in those with the longest follow-up period. The lifetime risk of developing T2D is 40% for individuals who have one parent with T2D and almost 70% with two affected parents (Köbberling and Tillil 1982). The concordance rate of T2D in monozygotic twins is ~70%, while the concordance in dizygotic twins is only 20–30% (Kaprio et al. 1992; Newman et al. 1987; Poulsen et al. 1999; Medici et al. 1999).

The relative risk for first-degree relatives is approximately 3 and ~6 if both parents are affected (Meigs et al. 2000). The prevalence of T2D varies from a few

percent among Caucasians in Europe to 50% among Pima Indians in Arizona (Diamond 2003). Thus, there is no doubt that the risk of T2D is partly determined by genetic factors. However, the genetic factors discovered thus far, mostly by GWAS, explain only 10–15% of the heritability of T2D.

Genetic Risk Loci

Linkage studies identified the first T2D gene *CAPN10* on chromosome 10 encoding calpain 10, a cysteine protease with largely unknown functions in glucose metabolism (Horikawa et al. 2000). However, this finding has been difficult to replicate. The *TCF7L2* gene variant, which shows the strongest association with T2D, was originally identified in a region showing modest linkage with T2D on chromosome 10q. Luckily, fine-mapping identified the *TCF7L2* intronic rs7903146 SNP contributing to, but not fully explaining, the original linkage (Duggirala et al. 1999; Reynisdottir et al. 2003; Grant et al. 2006). This association has since been confirmed in various populations world-wide rendering it the most consistent association with T2D to date, conferring a relative risk of ~1.4 (Tong et al. 2009).

Candidate gene studies have robustly associated two loci, *PPARG* and *KCNJ11,* with T2D (Deeb et al. 1998; Hani et al. 1998; Gloyn et al. 2003). The *KCNJ11* E23K and *PPARG* P12A polymorphisms act in an additive manner to increase T2D risk (Hansen et al. 2005). *PPARG* encodes the nuclear receptor PPAR-γ which is a molecular target for thiazolidinediones, a class of insulin sensitizing drugs used to treat T2D. This variant was associated with increased transcriptional activity, increased insulin sensitivity, and protection against T2D (Deeb et al. 1998). *KCNJ11* encodes four out of eight subunits of the ATP-sensitive potassium (K-ATP) channel in pancreatic beta-cells, the other four coded by ABCC8 (SUR1). In pancreatic beta cells, ATP-potassium channels are crucial for the regulation of glucose stimulated insulin secretion and are targets for the antidiabetic drugs sulfonylureas, which act by stimulating insulin secretion. Activating mutations in this gene also cause neonatal diabetes while loss-of-function mutations in *KCNJ11* and *ABCC8* cause hyperinsulinemia associated with hypoglycemia in infancy (Gloyn et al. 2004).

Genome-wide association studies have been successful in identifying numerous loci associated with T2D and related traits. The first four GWASes for T2D were published in 2007, also by the Science magazine coined "Breakthrough of the Year" (Diabetes Genetics Initiative of Broad Institute of H et al. 2007; Scott et al. 2007; Wellcome Trust Case Control C 2007; Sladek et al. 2007). Unforeseen in genetics of T2D, three of the studies reported the same top findings!

Association studies require large study populations for sufficient power. A second wave of GWAS combined existing or new GWAS to meta-analyze >50,000 individuals (Voight et al. 2010). Many research groups worked together in consortia like DIAGRAM (DIAbetes Genetics Replication and Meta-analysis Consortium) and MAGIC (Meta-Analyses of Glucose-and Insulin-related traits Consortium) to facilitate this. Since the most strongly associated SNPs are often only markers for the functional variant responsible for the observed genetic effect, additional fine mapping of the loci is necessary.

Impact of ethnicity: A number of GWAS and meta-analysis studies have also been performed in non-European cohorts, adding several new loci to the list of variants associated with T2D (Cho et al. 2012; Imamura et al. 2012; Kooner et al. 2011; Li et al. 2013; Palmer et al. 2012; Parra et al. 2011; Shu et al. 2010; Replication et al. 2014). Interestingly, most associations found in one ethnic group also show some evidence of association in populations of other ethnicities.

In total, GWAS performed until date have identified ~248 variants for T2D mapping to >150 loci as well as numerous loci for glucose or insulin-related traits and more are likely to come (online table with references – Tables 1 and 2) (Fig. 3).

Rare and Protective Variants

A study applying WGS and imputation in an Icelandic population with follow-up in Danish and Iranian populations identified rare variants in the *PAM* and *PDX1* genes associated with T2D (Steinthorsdottir et al. 2014). WES and GWAS of a small founder population in Greenland identified the p.Arg684Ter variant (allele frequency of 17%) in the *TBC1D4* gene associated with glucose and insulin concentrations and muscle insulin resistance (Moltke et al. 2014).

Very often is the common variant the risk variant. In fact, the average T2D risk variant frequency in the population is ~54% which raises the question whether T2D is the default condition? If so, do rare protective variants make any difference in disease susceptibility? The ideal population to identify protective variants is a population that despite having a clustering of risk factors for T2D have escaped the disease. A rare loss of function mutation (R138X) was detected in the *SLC30A8* gene in the Botnia region from Finland and subsequently replicated applying the Exome chip in>150,000 individuals from other European countries. Another protective loss-of-function frameshift mutation in the same gene was identified on Iceland. The *SLC30A8* gene encodes the islet zinc transporter 8 with a putative effect on insulin secretion. Notably, a common variant in the same gene increases susceptibility to T2D whereas autoantibodies to T1D predispose to T1D.

Collectively, carriers of these protein-truncating mutations have a 65% lower risk of T2D (Flannick et al. 2014). Other studies based on Icelandic, Danish, and Iranian populations identified a low frequency variant in the *CCND2* gene which reduced T2D risk by half (Steinthorsdottir et al. 2014). Moreover, variants in *TCF2* were found to be protective against T2D (Gudmundsson et al. 2007). It is likely that more recent variants could be detected by sequencing large families.

In addition to SNPs, structural variants could also contribute to T2D risk. A common copy number variation (CNV), CNVR5583.1 in the *TSPAN8* gene has been repeatedly shown to be associated with T2D (Wellcome Trust Case Control C et al. 2010; Zeggini et al. 2008).

Gene-Gene and Gene-Environment Interactions

Few studies have reported significant gene-gene interactions on risk of T2D, and all of them have been based on previously established T2D risk loci. Further studies in large populations using unbiased and novel approaches will most likely be necessary to identify such effects.

Table 1 Genetic loci associated with T2D risk and glycemic traits

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
1	rs17106184	*FAF1*	Intron	1	G	1.1	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
2	rs2296172	*MACF1*	Coding – missense	1	G	1.1	2D	(Albrechtsen et al. 2013)
3	rs10923931	*NOTCH2*	Intron	1	T	1.13	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
4	rs340874	*PROX1*	Intergenic	1	C	1.07	Fasting glucose/HOMA B/T2D	(Dupuis et al. 2010)
5	rs243021	*BCL11A*	Intergenic	2	A	1.08	T2D	(Voight et al. 2010)
6	rs243088	*BCL11A*	Intergenic	2	T	1.07	T2D	(Morris et al. 2012a)
7	rs2975760	*CAPN10*	Intron	2	C	1.17	T2D	(Horikawa et al. 2000; Weedon et al. 2003)
8	rs3792267	*CAPN10*	Intron	2	G	1.17	T2D	(Horikawa et al. 2000; Weedon et al. 2003)
9	rs7607980	*COBLL1*	Coding – missense	2	T	1.14	T2D	(Albrechtsen et al. 2013)
10	rs560887	*G6PC2/ABCB11*	Intron	2	T	1.03	Fasting glucose/T2D/ HOMA B	(Dupuis et al. 2010)
11	rs780094	*GCKR*	Intron	2	C	1.06	T2D/fasting glucose/beta cell function/triglycerides/ fasting insulin	(Dupuis et al. 2010)
12	rs3923113	*GRB14*	Intergenic	2	A	1.07	T2D	(Morris et al. 2012a; Kooner et al. 2011)
13	rs13389219	*GRB14*	Intergenic	2	C	1.07	T2D	(Morris et al. 2012a)
14	rs2943641	*IRS1*	Intergenic	2	C	1.19	Fasting glucose/T2D/ HOMAB, HOMA IR/ AUC ins/AUC ratio/ISI	(Rung et al. 2009)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
15	rs7578326	*KIAA1486/IRS1*	Intron of uncharacterized LOC646736	2	A	1.11	T2D	(Voight et al. 2010)
16	rs7593730	*RBMS1/ITGB6*	Intronic	2	C	1.11	T2D	(Qi et al. 2010)
17	rs7560163	*RND3*	Intergenic	2	G	1.33	T2D	(Palmer et al. 2012)
18	rs7578597	*THADA*	Coding – missense	2	T	1.15	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
19	rs10200833	*THADA*	Intron	2	G	1.06	T2D	(Zeggini et al. 2008; Saxena et al. 2012)
20	rs6723108	*TMEM163*	Intergenic	2	T	1.31	Decreased fasting plasma insulin/HOMA-IR/T2D	(Tabassum et al. 2013)
21	rs998451	*TMEM163*	Intron	2	G	1.56	Decreased fasting plasma insulin/HOMA-IR/T2D	(Tabassum et al. 2013)
22	rs4607103	*ADAMTS9-AS2*	Intron	3	C	1.09	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
23	rs6795735	*ADAMTS9-AS2*	Intron	3	C	1.09	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
24	rs11708067	*ADCY5*	Intron	3	A	1.12	T2D/2hr glucose/HOMA B	(Dupuis et al. 2010; Saxena et al. 2010)
25	rs2877716	*ADCY5*	Intron	3	C	1.12	2 hr insulin adjusted for 2 hr glucose/2 hr glucose/ T2D	(Dupuis et al. 2010; Saxena et al. 2010)
26	rs11071657	*FAM148B*	Intergenic	3	A	1.03	Fasting glucose/T2D/ HOMA B	(Dupuis et al. 2010)
27	rs4402960	*IGF2BP2*	Intron	3	T	1.11	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007)

28	rs1470579	*IGF2BP2*	Intron	3	C	1.15	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Scott et al. 2012; Zeggini et al. 2007; Unoki et al. 2008)
29	rs6808574	*LPP*	Intergenic	3	C	1.07	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
30	rs1801282	*PPARG*	Coding – missense	3	C	1.09	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007)
31	rs13081389	*PPARG*	Intergenic	3	A	1.24	T2D	(Voight et al. 2010; Zeggini et al. 2007; Deeb et al. 1998; Saxena et al. 2007)
32	rs17036160	*PPARG*	Intron	3	C	1.11	T2D	(Saxena et al. 2012)
33	rs1797912	*PPARG*	Intron	3	A	1.06	T2D	(Saxena et al. 2012)
34	rs831571	*PSMD6*	Intergenic	3	C	1.09	T2D	(Cho et al. 2012a)
35	rs7647305	*SFRS10*	Intergenic	3	C	1.08	BMI/obesity T2D	(Thorleifsson et al. 2009)
36	rs16861329	*ST6GAL1*	Intron	3	G	1.09	T2D	(Kooner et al. 2011)
37	rs6780569	*UBE2E2*	Intergenic	3	G	1.21	T2D	(Yamauchi et al. 2010)
38	rs6815464	*MAEA*	Intron	4	C	1.13	T2D	(Cho et al. 2012a)
39	rs7656416	*MAEA*	Intron	4	C	1.15	T2D	(Cho et al. 2012a; Imamura et al. 2012)
40	rs6813195	*TMEM154*	Intergenic	4	C	1.08	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
41	rs10010131	*WFS1*	Intron	4	G	1.14	T2D	(Lyssenko et al. 2008; Sandhu et al. 2007)
42	rs4689388	*WFS1*	NearGene-5	4	T	1.16	T2D	(Rung et al. 2009)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
43	rs6446482	*WFS1*	Intron	4	G	1.11	T2D	(Voight et al. 2010; Sandhu et al. 2007; Minton et al. 2002)
44	rs1801214	*WFS1*	Coding – missense	4	T	1.13	T2D	(Voight et al. 2010; Sandhu et al. 2007; Minton et al. 2002)
45	rs459193	*ANKRD55*	Intergenic	5	G	1.08	T2D	(Morris et al. 2012a)
46	rs702634	*ARL15*	Intron	5	A	1.06	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
47	rs4457053	*ZBED3*	Intron of ZBED3-AS1	5	G	1.08	T2D	(Voight et al. 2010)
48	rs1048886	*C6orf57*	Coding – missense	6	G	1.54	T2D	(Sim et al. 2011)
49	rs7754840	*CDKAL1*	Intron	6	C	1.17	T2D	(Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012a)
50	rs7756992	*CDKAL1*	Intron	6	G	1.2	T2D	(Steinthorsdottir et al. 2007)
51	rs2206734	*CDKAL1*	Intron	6	T	1.2	T2D	(Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012a)

52	rs4712523	*CDKAL1*	Intron	6	G	1.27	T2D	(Voight et al. 2010; Rung et al. 2009; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012a)
53	rs10946398	*CDKAL1*	Intron	6	C	1.12	T2D	(Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012a)
54	rs7766070	*CDKAL1*	Intron	6	A	1.23	T2D	(Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012a)
55	rs2244020 (rs9266650)	*HLA-B*	Intergenic	6	G	1.09	T2D	(Ng et al. 2014)
56	rs1535500	*KCNK16*	Coding – missense	6	T	1.08	T2D	(Cho et al. 2012a)
57	rs3130501	*POU5F1-TCF19*	NearGene-5	6	G	1.07	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
58	rs9505118	*SSR1-RREB1*	Intron	6	A	1.06	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
59	rs9470794	*ZFAND3*	Intron	6	C	1.12	T2D	(Cho et al. 2012a)
60	rs17168486	*DGKB*	Intergenic	7	T	1.15	T2D	(Morris et al. 2012b)
61	rs2191349	*DGKB/ TMEM195*	Intergenic	7	T	1.06	Fasting glucose, Homa B/ T2D	(Dupuis et al. 2010)
62	rs6467136	*GCC1-PAX4*	Intergenic	7	G	1.11	T2D	(Cho et al. 2012a)
63	rs4607517	*GCK*	Intergenic	7	A	1.07	Fasting glucose/T2D/ HOMA B	(Dupuis et al. 2010)
64	rs864745	*JAZF1*	Intron	7	T	1.1	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
65	rs849134	*JAZF1*	Intron	7	A	1.13	T2D	(Zeggini et al. 2008; Voight et al. 2010)
66	rs12113122	*JAZF1*	Intron	7	G	1.55	T2D	(Saxena et al. 2012)
67	rs972283	*KLF14*	Intergenic	7	G	1.07	Reduced insulin sensitivity T2D	(Voight et al. 2010)
68	rs516946	*ANK1*	Intron	8	C	1.09	T2D	(Morris et al. 2012b)
69	rs515071	*ANK1*	Intron	8	G	1.18	T2D reduced beta-cell function	(Imamura et al. 2012; Morris et al. 2012b)
70	rs13266634	*SLC30A8*	Coding – missense	8	C	1.19	T2D	(Sladek et al. 2007)
71	rs11558471	*SLC30A8*	UTR-3	8	A	1.15	Fasting glucose, HOMA B T2D	(Dupuis et al. 2010; Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Zeggini et al. 2007; Takeuchi et al. 2009; Perry et al. 2012a; Sladek et al. 2007)

72	rs3802177	*SLC30A8*	UTR-3	8	G	1.26	T2D	(Dupuis et al. 2010; Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Zeggini et al. 2007; Takeuchi et al. 2009; Perry et al. 2012a; Sladek et al. 2007)
73	rs896854	*TP53INP1*	Intron	8	T	1.06	T2D	(Voight et al. 2010)
74	rs10965250	*CDKN2A/2B*	Intergenic	9	G	1.2	T2D	(Voight et al. 2010; Saxena et al. 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012b)
75	rs2383208	*CDKN2A/2B*	Intergenic	9	A	1.19	T2D	(Voight et al. 2010; Saxena et al. 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012b)
76	rs7018475	*CDKN2A/2B*	Intergenic	9	G	1.35	T2D	(Voight et al. 2010; Saxena et al. 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012b)
77	rs564398	*CDKN2A/2B*	Intergenic	9	T	1.12	T2D	(Voight et al. 2010; Saxena et al. 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012b)
78	rs10757282	*CDKN2A/2B*	Intergenic	9	C	1.14	T2D	(Voight et al. 2010; Saxena et al. 2007; Yamauchi et al. 2010; Steinthorsdottir et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Perry et al. 2012b)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
79	rs10811661	*CDKN2B*	Intergenic	9	T	1.2	T2D	(Zeggini et al. 2008; Voight et al. 2010; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Zeggini et al. 2007; Saxena et al. 2007; Scott et al. 2007; Li et al. 2013; Takeuchi et al. 2009; Parra et al. 2011)
80	rs7034200	*GLIS3*	Intron	9	A	1.03	Fasting glucose/T2D/ HOMA B	(Dupuis et al. 2010)
81	rs7041847	*GLIS3*	Intron	9	A	1.1	T2D	(Cho et al. 2012a; Li et al. 2013)
82	rs10814916	*GLIS3*	Intron	9	C	1.11	T2D	(Dupuis et al. 2010; Cho et al. 2012a; Li et al. 2013)
83	rs17584499	*PTPRD*	Intron	9	T	1.57	T2D	(Tsai et al. 2010)
84	rs2796441	*TLE1*	Intergenic	9	G	1.07	T2D	(Morris et al. 2012b)
85	rs13292136	*TLE4 (CHCHD9)*	Intergenic	9	C	1.11	T2D	(Voight et al. 2010)
86	rs553668	*ADRA2A*	UTR-3	10	A	1.42	T2D	(Rosengren et al. 2010)
87	rs10885122	*ADRA2A*	Intergenic	10	G	1.04	Fasting glucose/HOMA B/T2D	(Dupuis et al. 2010)
88	rs12779790	*CDC123, CAMK1D*	Intergenic	10	G	1.11	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
89	rs11257655	*CDC123/ CAMK1D*	Intergenic	10	C	1.15	T2D	(Zeggini et al. 2008; Li et al. 2013; Shu et al. 2010)
90	rs10906115	*CDC123/ CAMK1D*	Intergenic	10	A	1.13	T2D	(Zeggini et al. 2008; Li et al. 2013; Shu et al. 2010)
91	rs10886471	*GRK5*	Intron	10	C	1.12	T2D	(Li et al. 2013)

92	rs5015480	*HHEX*	Intergenic	10	C	1.13	T2D	(Voight et al. 2010; Saxena et al. 2007; Takeuchi et al. 2009; Sladek et al. 2007; Perry et al. 2012b)
93	rs1111875	*HHEX/IDE*	Intergenic	10	C	1.13	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007)
94	rs7903146	*TCF7L2*	Intronic/promoter	10	T	1.35	T2D, fasting glucose, 2 hr glucose	(Grant et al. 2006)
95	rs4506565	*TCF7L2*	Intron	10	T	1.34	Fasting glucose, HOMA B T2D	(Zeggini et al. 2008; Voight et al. 2010; Zeggini et al. 2007; Saxena et al. 2007; Steinthorsdottir et al. 2007; Scott et al. 2007; Takeuchi et al. 2009; Sladek et al. 2007; Perry et al. 2012b; Grant et al. 2006; Saxena et al. 2006; Salonen et al. 2007; Timpson et al. 2009; Wellcome Trust Case Control Consortium 2007)
96	rs7901695	*TCF7L2*	Intron	10	C	1.37	T2D	(Zeggini et al. 2008; Voight et al. 2010; Zeggini et al. 2007; Saxena et al. 2007; Steinthorsdottir et al. 2007; Scott et al. 2007; Takeuchi et al. 2009; Sladek et al. 2007; Perry et al. 2012b; Grant et al. 2006; Saxena et al. 2006; Salonen et al. 2007; Timpson et al. 2009; Wellcome Trust Case Control Consortium 2007)
97	rs1802295	*VPS26A*	UTR-3	10	A	1.08	T2D	(Kooner et al. 2011)
98	rs12571751	*ZMIZ1*	Intron	10	A	1.08	T2D	(Morris et al. 2012b)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
99	rs11603334	*ARAP1*	UTR-5	11	G	1.13	T2D fasting proinsulin levels/fasting glucose/	(Strawbridge et al. 2011)
100	rs1552224	*CENTD2*	Intergenic	11	A	1.14	T2D	(Voight et al. 2010)
101	rs11605924	*CRY2*	Intron	11	A	1.04	Fasting glucose/HOMA B/T2D	(Dupuis et al. 2010)
102	rs174550	*FADS1*	Intron	11	T	1.04	Fasting glucose/T2D/ HOMA B	(Dupuis et al. 2010)
103	rs2334499	*HCCA2*	Intergenic	11	T	1.35	T2D	(Kong et al. 2009)
104	rs3842770	*INS-IGF2*	Intron	11	A	1.18	T2D – African American	(Ng et al. 2014)
105	rs5219	*KCNJ11*	Coding – missense	11	T	1.14	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Zeggini et al. 2007; Scott et al. 2007; Timpson et al. 2009; Hani et al. 1998)
106	rs5215	*KCNJ11*	Coding – missense	11	C	1.14	T2D	(Diabetes Genetics Initiative of Broad Institute of Harvard and MIT 2007; Zeggini et al. 2007; Scott et al. 2007; Timpson et al. 2009; Hani et al. 1998)
107	rs2237895	*KCNQ1*	Intron	11	C	1.45	T2D	(Yasuda et al. 2008)
108	rs231362	*KCNQ1*	Intron	11	G	1.08	T2D	(Voight et al. 2010)
109	rs163184	*KCNQ1*	Intron	11	G	1.22	T2D	(Morris et al. 2012a; Yasuda et al. 2008)
110	rs2237892	*KCNQ1*	Intron	11	C	1.25	Reduced beta-cell function T2D	(Voight et al. 2010; Unoki et al. 2008; Takeuchi et al. 2009; Tsai et al. 2010; Yasuda et al. 2008)

111	rs10501320	*MADD*	Intron	11	G	1.01	T2D fasting proinsulin levels/fasting glucose	(Strawbridge et al. 2011)
112	rs10830963	*MTNR1B*	Intron	11	G	1.09	T2D	(Prokopenko et al. 2009)
113	rs1387153	*MTNR1B*	Intergenic	11	T	1.09	Reduced beta-cell function T2D	(Voight et al. 2010; Tsai et al. 2010; Prokopenko et al. 2009)
114	rs7138803	*BCDIN3D/ FAIM2*	Intergenic	12	A	1.11	BMI/obesity T2D	(Thorleifsson et al. 2009; Willer et al. 2008)
115	rs11063069	*CCND2*	Intergenic	12	G	1.12	T2D	(Morris et al. 2012b)
116	rs1153188	*DCD*	Intergenic	12	A	1.08	T2D	(Zeggini et al. 2008)
117	rs1531343	*HMGA2*	Intron of pseudogene	12	C	1.1	T2D	(Voight et al. 2010)
118	rs9668162	*HMGA2*	Intron	12	G	1.26	T2D	(Saxena et al. 2012)
119	rs7305618	*HNF1A*	Intergenic	12	C	1.14	T2D	(Voight et al. 2010; Parra et al. 2011)
120	rs35767	*IGF1*	NearGene-5	12	G	1.04	Fasting insulin/T2D/ HOMA IR	(Dupuis et al. 2010)
121	rs10842994	*KLHDC5*	Intergenic	12	C	1.1	T2D	(Morris et al. 2012b)
122	rs4275659	*MPHOSPH9*	Intron	12	C	1.06	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
123	rs7957197	*OASL/TCF1/ HNF1A*	Intron of OASL	12	T	1.07	T2D	(Voight et al. 2010)
124	rs7961581	*TSPAN8,LGR5*	Intergenic	12	C	1.09	T2D	(Zeggini et al. 2008; Lyssenko et al. 2008)
125	rs9552911	*SGCG*	Intron	13	G	1.63	T2D	(Saxena et al. 2013)
126	rs1359790	*SPRY2*	Intergenic	13	G	1.15	T2D	(Shu et al. 2010)
127	rs2028299	*AP3S2*	UTR-3	15	C	1.1	T2D	(Kooner et al. 2011)
128	rs7172432	*C2CD4A/B*	Intergenic	15	A	1.14	Reduced beta-cell function, T2D	(Yamauchi et al. 2010)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
129	rs7178572	*HMG20A*	Intergenic	15	A	1.09	Lean T2D	(Kooner et al. 2011; Perry et al. 2012a)
130	rs7177055	*HMG20A*	Intergenic	15	A	1.08	T2D	(Morris et al. 2012b)
131	rs8042680	*PRC1*	Intron	15	A	1.07	T2D	(Voight et al. 2010)
132	rs7403531	*RASGRP1*	Intron	15	T	1.1	T2D	(Li et al. 2013)
133	rs4502156	*VPS13C/ C2CD4A/B*	Intergenic	15	T	1.07	Fasting proinsulin levels T2D	(Strawbridge et al. 2011)
134	rs11634397	*ZFAND6*	Intergenic	15	G	1.06	T2D	(Voight et al. 2010)
135	rs7202877	*BCAR1*	Intergenic	16	T	1.12	T2D	(Morris et al. 2012b)
136	rs8050136	*FTO*	Intron	16	A	1.17	Increased BMI, reduced insulin sensitivity, T2D	(Zeggini et al. 2008; Voight et al. 2010; Zeggini et al. 2007; Scott et al. 2007; Perry et al. 2012a; Timpson et al. 2009; Wellcome Trust Case Control Consortium 2007; Frayling et al. 2007)
137	rs9939609	*FTO*	Intron	16	A	1.25	T2D (obese)	(Zeggini et al. 2008; Voight et al. 2010; Zeggini et al. 2007; Scott et al. 2007; Perry et al. 2012a; Timpson et al. 2009; Wellcome Trust Case Control Consortium 2007; Frayling et al. 2007)
138	rs11642841	*FTO*	Intron	16	A	1.13	T2D	(Zeggini et al. 2008; Voight et al. 2010; Zeggini et al. 2007; Scott et al. 2007; Perry et al. 2012a; Timpson et al. 2009; Wellcome Trust Case Control Consortium 2007; Frayling et al. 2007)

139	rs4430796	*HNF1B*	Intron	17	G	1.19	Reduced beta-cell function T2D	(Li et al. 2013; Gudmundsson et al. 2007; Winckler et al. 2005a; Winckler et al. 2005b)
140	rs7501939	*HNF1B*	Intron	17	T	1.09	T2D	(Gudmundsson et al. 2007)
141	rs391300	*SRR*	Intron	17	G	1.28	T2D	(Tsai et al. 2010)
142	rs4523957	*SRR*	NearGene-5	17	T	1.27	T2D	(Tsai et al. 2010)
143	rs8090011	*LAMA1*	Intron	18	G	1.13	Lean T2D	(Perry et al. 2012a)
144	rs17782313	*MC4R*	Intergenic	18	C	1.06	BMI/T2D	(Thorleifsson et al. 2009; Willer et al. 2008)
145	rs12970134	*MC4R*	Intergenic	18	A	1.08	T2D/BMI/waist circumference/insulin resistance	(Morris et al. 2012a; Chambers et al. 2008)
146	rs3794991	*GATAD2A/ CILP2*	Intron, intergenic	19	T	1.12	T2D	(Morris et al. 2012a; Saxena et al. 2012)
147	rs8108269	*GIPR*	Intergenic	19	G	1.05	T2D	(Morris et al. 2012a)
148	rs3786897	*PEPD*	Intron	19	A	1.1	T2D	(Cho et al. 2012b)
149	rs10401969	*SUGP1/CILP2*	Intron	19	C	1.13	T2D	(Morris et al. 2012a; Saxena et al. 2012)
150	rs6017317	*FITM2-R3HDML-HNF4A*	Intergenic	20	G	1.09	T2D	(Cho et al. 2012a)
151	rs4812829	HNF4A	Intron	20	A	1.09	T2D	(Kooner et al. 2011)
152	rs5945326	DUSP9	Intergenic	X	A	1.27	T2D	(Voight et al. 2010)
153	rs12010175	FAM58A	Intron	X	G	1.21	T2D	(Li et al. 2013)
154	rs8181588	KCNQ1	Intron	11	A	1.3	T2D	(Hanson et al. 2014)
155	rs10229583	FSCN3 – PAX4	Downstream_gene_ variant	7	G	1.14	T2D	(Ma et al. 2013)
156	rs9936385	FTO	Intron	16	C	1.13	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
157	rs9502570	LOC105374905	Regulatory_region_variant	6	A	1.06	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
158	rs849135	JAZF1	Intron_variant	7	G	1.12	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
159	rs4458523	WFS1	Intron_variant	4	G	1.09	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
160	rs3132524	POU5F1	Intron_variant	6	G	1.07	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
161	rs2943640	LOC646736 – LOC105373913	Intergenic_variant	2	C	1.09	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
162	rs7612463	UBE2E2	Intron_variant	3	C	1.1	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
163	rs1727313	MPHOSPH9	3_prime_UTR_variant	12	C	1.06	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
164	rs11717195	ADCY5	Intron_variant	3	T	1.09	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
165	rs17791513	LOC101927450 – CHCHD2P9	Intergenic_variant	9	A	1.21	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)

166	rs2261181	RPSAP52	Intron_variant	12	T	1.16	T2D	(DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium 2014)
167	rs6931514	CDKAL1	Intron_variant	6	G	1.25	T2D	(Zeggini et al. 2008)
168	rs791595	LOC101928423	Regulatory_region_variant	7	A	1.17	T2D	(Hara et al. 2014)
169	rs312457	SLC16A13	Intron_variant	17	G	1.2	T2D	(Hara et al. 2014)
170	rs11787792	GPSM1	Intron_variant	9	A	1.15	T2D	(Hara et al. 2014)
171	rs163182	KCNQ1	Intron_variant	11	C	1.28	T2D	(Cui et al. 2011)
172	rs4712524	CDKAL1	Intron_variant	6	G	1.22	T2D	(Unoki et al. 2008)
173	rs6769511	IGF2BP2	Intron_variant	3	C	1.23	T2D	(Unoki et al. 2008)
174	rs2244020	DHFRP2 – LOC101929072	Upstream_gene_variant	6	G	1.09	T2D	(Ng et al. 2014)
175	rs231356	LOC105376523, KCNQ1, KCNQ1OT1	Non_coding_transcript_exon_variant	11	T	1.09	T2D	(Ng et al. 2014)
176	rs9348440	CDKAL1	Intron_variant	6	A	0.246	T2D	(Go et al. 2013)
177	rs12229654	LOC105369980 – LOC105369981	Intergenic_variant	12	G	0.277	T2D	(Go et al. 2013)
178	rs11066453	OAS1	Intron_variant	12	G	0.242	T2D	(Go et al. 2013)
179	rs75493593	SLC16A11	Missense_variant	17	?	1.25	T2D	(SIGMA Type 2 Diabetes Consortium 2014a)
180	rs1333051	CDKN2B-AS1 – DMRTA1	Regulatory_region_variant	9	A	1.22	T2D	(Parra et al. 2011)
181	rs10440833	CDKAL1	Intron_variant	6	A	1.25	T2D	(Voight et al. 2010)
182	rs60780116/ rs1996546	ACSL1	Coding	4	T/G	1.09	T2D	(Scott et al. 2017)
183	rs9271774/ rs9271775	HLA-DQA1	Coding	6	C/T	1.09	T2D	(Scott et al. 2017)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
184	rs6918311/ rs4407733	SLC35D3	Intergenic	6	A/ A	1.06	T2D	(Scott et al. 2017)
185	rs1182436/ rs1182397	MNX1	Coding	7	C/ G	1.08	T2D	(Scott et al. 2017)
186	rs635634/ rs495828	ABO	Intergenic	9	T/ T	1.08	T2D	(Scott et al. 2017)
187	rs2292626/ rs2421016	PLEKHA1	Coding	10	C/ C	1.07	T2D	(Scott et al. 2017)
188	rs1061810/ rs3736505	HSD17B12	Coding	11	A/ G	1.07	T2D	(Scott et al. 2017)
189	rs111669836/ rs11227234	MAP3K11	Coding	11	A/ T	1.06	T2D	(Scott et al. 2017)
190	rs10146997/ rs17109256	NRXN3	Coding	14	G/ A	1.07	T2D	(Scott et al. 2017)
191	rs2925979/ rs2925979	CMIP	Coding	16	T/ T	1.07	T2D	(Scott et al. 2017)
192	rs7224685/ rs8068804	ZZEF1	Coding	17	T/ A	1.07	T2D	(Scott et al. 2017)
193	rs78761021/ rs17676067	GLP2R	Coding	17	G/ C	1.06	T2D	(Scott et al. 2017)
194	rs79349575/ rs15563	GIP	Coding	17	A/ G	1.06	T2D	(Scott et al. 2017)
195	rs78124264	IRS1	Intergenic	2			T2D	(Fuchsberger et al. 2016)
196	rs79856023	PPARG	Intergenic	3			T2D	(Fuchsberger et al. 2016)
197	rs9727115	SNX7	Intron	1	G	0.0133	Fasting proinsulin levels adjusted for fasting glucose	(Strawbridge et al. 2011)

198	rs2785980	LYPLAL1	Intergenic	1	T	0.017	Fasting insulin	(Manning et al. 2012)
199	rs4675095	IRS1	Intron	2	A	0.003	Fasting glucose/HOMA-IR	(Dupuis et al. 2010)
200	rs2943634	IRS1	Intergenic	2	C	0.025	Fasting insulin, CAD	(Manning et al. 2012)
201	rs1371614	DPYSL5	Intron	2	T	0.022	Fasting glucose	(Manning et al. 2012)
202	rs11920090	SLC2A2	Intron	3	T	0.02	Fasting glucose/HOMA B/HBA1C	(Dupuis et al. 2010)
203	rs17046216	MSMO1	Intron	4	A	0.18; 0.19	Fasting insulin; insulin resistance	(Chen et al. 2012)
204	rs4691380	PDGFC	Intron	4	C	0.021	Fasting insulin	(Manning et al. 2012)
205	rs6235	PCSK1	Coding – missense	5	G	0.0394/ −0.014	Fasting proinsulin levels/ fasting glucose	(Strawbridge et al. 2011)
206	rs13179048	PCSK1	Intergenic	5	C	0.018	Fasting glucose	(Manning et al. 2012)
207	rs4646949	TAF11	NearGene-3	6	T	0.02	Fasting insulin	(Manning et al. 2012)
208	rs6943153	GRB10	Intron	7	C	0.0154	FG, FI	(Scott et al. 2012)
209	rs4841132	PPP1R3B	Intergenic	8	A	0.03	Fasting glucose	(Manning et al. 2012)
210	rs7077836	TCERG1L	Intergenic	10	T	0.28; 0.34	Fasting insulin; insulin resistance	(Chen et al. 2012)
211	rs7944584	MADD	Intron	11	A	0.021	Fasting proinsulin/fasting glucose/Homa B	(Dupuis et al. 2010)
212	rs10838687	MADD	Intron	11	T	0.0253	Fasting proinsulin levels	(Strawbridge et al. 2011)
213	rs1483121	OR4S1	Intergenic	11	G	0.015	Fasting glucose	(Manning et al. 2012)
214	rs2074356	HECTD4/ C12orf51	Intron	12			1 hr plasma glucose	(Go et al. 2013)
215	rs2293941	PDX1 – AS1	Intron	13	A	0.016	Fasting glucose	(Manning et al. 2012)
216	rs17271305	VPS13C	Intron	15	G	0.07	2 hr glucose/2 hr insulin, adjusted for 2 hr glucose	(Saxena et al. 2010)

(*continued*)

Table 1 (continued)

N	T2D risk SNP	*GENE/nearest Gene*	Gene location	Chr	RA	OR	TRAIT	References
217	rs1549318	LARP6	Iintergenic	15	T	0.0192	Fasting proinsulin levels	(Strawbridge et al. 2011)
218	rs4790333	SGSM2	Intron	17	T	0.0154	Fasting proinsulin levels	(Strawbridge et al. 2011)
219	rs10423928	GIPR	Intron	19	A		2 hr glucose/Insulinogenic index/AUCins/gluc/2 hr insulin, adjusted for 2 hr glucose/T2D	(Saxena et al. 2010)
220	rs6048205	FOXA2/ LINC00261	Intergenic/nearGene-5	20	A	0.,029	Fasting glucose	(Manning et al. 2012)

A number of studies have suggested an interaction between Pro12Ala in the *PPARG* gene with intake of dietary fatty acids and exercise for risk of T2D (Luan et al. 2001; Kahara et al. 2003). Physical exercise has been shown to modify the effect of the *FTO* variant rs9939609 on BMI (Andreasen et al. 2008; Franks et al. 2008). A recent study reported interactions between the *FTO* variant and frequency of alcohol consumption, sleep duration, salt intake, and physical activity (Young et al. 2016). The *HNF1B* rs4430796 variant has been shown to interact with self-reported physical activity (Brito et al. 2009). Further, patients with *HNF1A* mutations respond better to sulfonylureas than to metformin (Pearson et al. 2003). The effect of the *GIPR* rs10423928 variant on incident diabetes risk was modified by dietary fat and carbohydrate intake (Sonestedt et al. 2012).

Epigenetics

Epigenome-wide association studies (EWAS) in blood have reported hypomethylation of a CpG site at the *FTO* locus and at least three studies have reported differential methylation at a CpG site in the *TXNIP* gene (Toperoff et al. 2012; Chambers et al. 2015; Florath et al. 2016; Kulkarni et al. 2015). Further, CpG sites in *TXNIP*, *ABCG1*, *PHOSPHO1*, *SOCS3*, and *SREBF1* have been associated with risk of developing future T2D (Chambers et al. 2015). Studies in human pancreatic islets have reported differential methylation of CpGs in *TCF7L2*, *FTO*, and *KCNQ1;* an additional 102 genes showed differential methylation in the EWAS, but the role for pathogenesis of T2D remains unclear (Chambers et al. 2015). EWAS on human pancreatic islets revealed many differentially expressed genes between type 2 diabetic and non-diabetic donors including *CDKN1A* and *SEPT9* (Dayeh et al. 2014). Aging associated with increased DNA methylation in multiple loci including *KLF14* and some of this associated with impaired insulin secretion (Bacos et al. 2016). One of the earliest studies on whole genome bisulfite sequencing of human pancreatic islets showed >25,000 differentially methylated regions (DMRs) in islets from type 2 diabetics including those with known islet function, e.g., *PDX1*, *TCF7L2*, and *ADCY5* (Volkov et al. 2017).

Further, the *CDKN2A/B* region on chromosome 9 is associated with T2D, as well as cardiovascular disease and a number of other disorders. This region harbors an lncRNA, *ANRIL* (non-protein coding *CDKN2B-AS1 CDKN2B* antisense RNA 1), which can potentially modify and explain some of these associations (Broadbent et al. 2008).

Gene Expression in Pancreatic Islets

Elucidating the molecular mechanisms underlying complex diseases requires understanding of gene expression in relevant cell types and tissues. Multiple novel genes with a potential role in glucose metabolism and insulin secretion have been discovered through global expression studies using microarrays (Taneera et al. 2015). Later, rapid technological advances in next generation sequencing facilitated the identification and precise quantification of all transcripts in the cell through RNA sequencing. This allowed investigation of genetic effects on gene expression, e.g., expression quantitative trait loci (eQTLs), splicing (splice QTLs), allelic imbalance,

Table 2 Rare risk and protective loci associated with T2D and glycemic traits

N	SNPs	*GENE/ nearest gene*	Gene location	Chr	References
1	rs35658696	*PAM*	Coding – missense	5	(Huyghe et al. 2013)
2	rs78408340	*PAM*	Coding – missense	5	(Huyghe et al. 2013)
3	rs36046591	*PPIP5K2*	Coding – missense	5	(Huyghe et al. 2013)
4	p.Lys34Serfs*50	*SLC30A8*	Coding – missense	8	(Flannick et al. 2014)
5	p.Arg138*	*SLC30A8*	Coding – missense	8	(Flannick et al. 2014)
6	rs3824420	*KANK1*	Coding – missense	9	(Huyghe et al. 2013)
7	rs505922	*ABO*	Intronic	9	(Huyghe et al. 2013)
8	rs60980157	*GPSM1*	Coding – missense	9	(Huyghe et al. 2013)
9	p.Leu5Val (20)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
10	p.Ile131Val (1)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
11	p.Gln249Pro (3)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
12	p.Arg392Trp (1)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
13	p.Leu427Gln (3)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
14	p.Gly434Arg (488)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
15	p.X406Gly (200)	*ATG13*	Coding – missense	11	(Huyghe et al. 2013)
16	rs35233100	*MADD*	Coding – missense	11	(Huyghe et al. 2013)
17	p.Arg279Cys (324)	*TBC1D30*	Coding – missense	12	(Huyghe et al. 2013)
18	p.Pro746Leu (427)	*TBC1D30*	Coding – missense	12	(Huyghe et al. 2013)
19	c.1522G>A[p. E508K	*HNF1A*	Coding – missense	12	(SIGMA Type 2 Diabetes Consortium 2014b)
20	rs76895963	*CCND2*	Intergenic	12	(Steinthorsdottir et al. 2014)
21	rs75615236	*CCND2*	Intergenic	12	(Steinthorsdottir et al. 2014)
22	rs150781447	*TBC1D30*	Coding – missense	12	(Huyghe et al. 2013)
23	rs2650000	*HNF1A*	Intergenic	12	(Huyghe et al. 2013)
24	Chr. 13: g.27396636delT	*PDX1*	Coding – missense	13	(Flannick et al. 2014)

(*continued*)

Table 2 (continued)

N	SNPs	*GENE/ nearest gene*	Gene location	Chr	References
25	p.Tyr416Cys (78)	*SGSM2*	Coding – missense	17	(Huyghe et al. 2013)
26	p.Thr789Pro (3),	*SGSM2*	Coding – missense	17	(Huyghe et al. 2013)
27	p.Val996Ile (236)	*SGSM2*	Coding – missense	17	(Huyghe et al. 2013)
28	rs61741902	*SGSM2*	Coding – missense	17	(Huyghe et al. 2013)

(Fadista et al. 2014), cis-regulatory networks (Pasquali et al. 2014), and noncoding RNAs (Moran et al. 2012).

In a heterogeneous tissue like pancreatic islet, containing diverse cell types with myriad functions, distinct expression data from each cell type further facilitates dissection of unique cell functions. Single cell sequencing allows investigation of gene expression in individual cellular subsets. RNA sequencing of α, β, γ, δ, and ε cells from adult and fetal pancreas have generated distinct expression profiles for these specific cell types (Blodgett et al. 2015; Wang et al. 2016) and identified genes that were differentially expressed between T2D and nondiabetic donors (Segerstolpe et al. 2016; Xin et al. 2016). Interestingly, some of the key genes reported in previous studies were missing in data from single cells.

LADA

LADA (latent autoimmune diabetes in adults) accounts for 4–14% of diabetic patients in Europe with the highest prevalence in northern Europe (Laugesen et al. 2015). LADA was originally defined by presence of diabetes-associated autoantibodies, especially GADA, age at onset more than 35 years and no requirement of insulin treatment during the first 6 months (Tuomi et al. 1993b), but the exact criteria remain controversial and different thresholds and cutoffs have been used in different studies. Phenotypically, LADA is an intermediary form between T1D and T2D, where LADA with high antibody titers are more similar to T1D, whereas LADA with lower titers are closer to T2D.

Heritability

A family history of any form of diabetes is a risk factor for LADA (Carlsson et al. 2007). A few genetic studies on LADA have focused on candidate genes associated with T1D and T2D and found LADA to be associated with both T1D (HLA) and T2D susceptibility (e.g., *TCF7L2*) (Andersen et al. 2014). Whether this is due to an admixture of T1D and T2D patients within the LADA group or a disease etiology including both autoimmune and metabolic pathways is unclear. GWAS are still missing and therefore we know nothing about the possible existence of LADA specific loci.

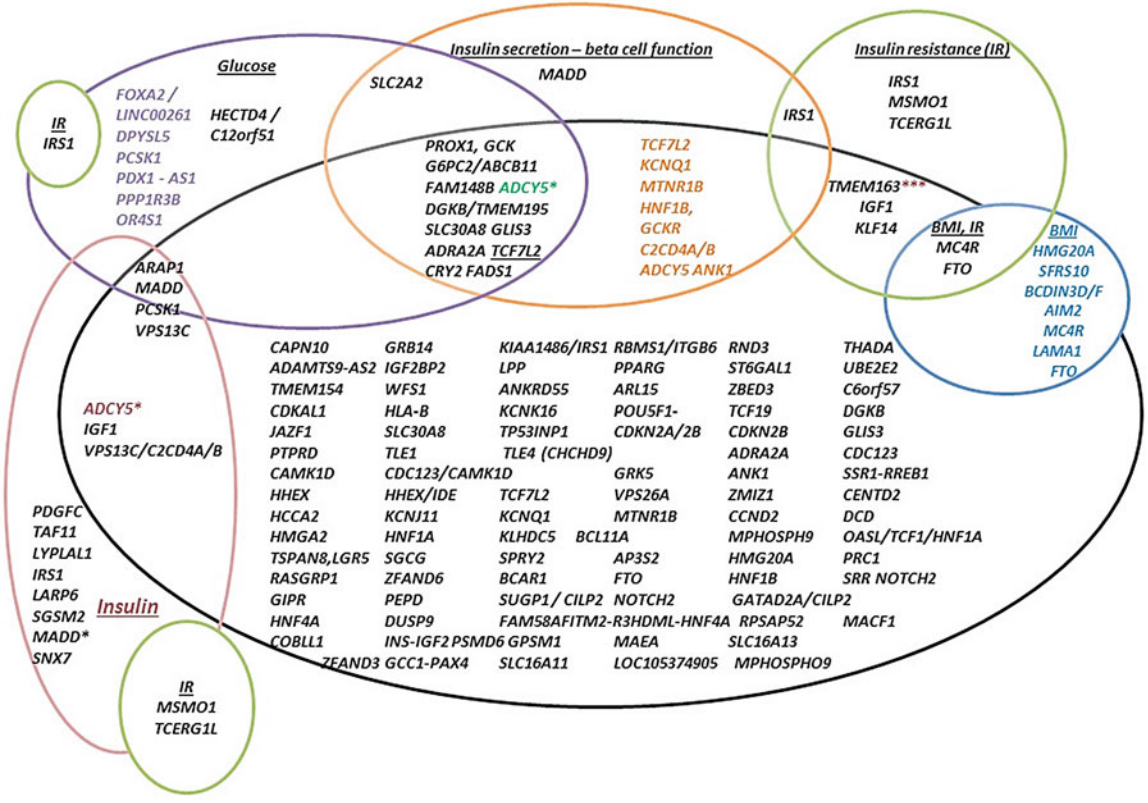


Fig. 3 Loci associated with T2D and glycemic traits. The variants are represented by gene names, which could indicate the location, either in or near the gene. The black circle contains loci associated with T2D. The large number of loci overlapping with insulin secretion traits illustrates the importance of beta cell function in T2D

Genetic Risk Loci

A number of studies have investigated the association between LADA and the HLA locus and found an age-at-diagnosis-dependent association with a higher frequency of *HLA-DRB1*03* (DR3) and *HLA-DRB1*04* (DR4) in younger patients (Horton et al. 1999). DR3 and DR4 were associated with GADA and IA–2A positivity, respectively. The strongest associations outside the HLA region have been found for the T1D loci *INS* and *PTPN22* (Howson et al. 2011). Other T1D loci found to be associated with adult onset autoimmune diabetes in the same direction as for T1D include S*TAT4*, *CTLA4*, *IL2RA*, *ERBB3*, *SH2B3*, and *CLEC16A* (Howson et al. 2011). Common variants in the *TCF7L2* gene help to differentiate autoimmune from non-autoimmune diabetes in young (15–34 years) but not in middle-aged (40–59 years) diabetic patients (Cervin et al. 2008).

MODY

Maturity-onset diabetes of the young (MODY) is a collection of monogenic forms of diabetes often stated to comprise ~1% of diabetes patients; however, the exact prevalence is unclear (Kleinberger and Pollin 2015). MODY is characterized by early-onset, usually before 25 years of age, insulin secretion defects and an autosomal dominant inheritance pattern. However, the penetrance and expression of disease can vary and not all MODY patients have a family history of diabetes. The diagnosis of MODY is a challenge as the phenotype can be quite varying and diagnosis requires sequencing, a tool that has not yet received widespread acceptance in the clinic.

Since the different types of MODY differ from each other and from other diabetes types with regard to severity, course of disease, risk of complications, and response to different therapies, a correct diagnosis is of great importance. Many MODY-patients are treated with insulin even though for some MODY types (MODY 1 and 4) treatment with sulfonylureas is a better alternative.

So far, mutations in at least 14 genes are known to cause MODY. Most of them are encoding transcription factors. The most common MODY types are MODY2 (32% of U.K. MODY cases) caused by mutations in the glucokinase gene (*GCK*) an MODY3 (52% of U.K. cases), caused by mutations in hepatocyte nuclear factor-1alpha (*HNF1A*) (Shields et al. 2010). Within these genes, a large variety of mutations can cause disease (there are more than 200 mutations described in the *GCK* and *HNF1A* genes) and MODY mutations are often unique to a given family (Murphy et al. 2008b). Because of the extreme allelic heterogeneity, the appropriate diagnosis requires sequencing to identify the causal mutation. With the advent of next-generation sequencing, this is becoming much more feasible but still a large proportion of patients with MODY is undiagnosed.

A correct diagnosis of MODY is important for the correct management of the disease. MODY2 is associated with a mild increase in glucose concentrations, the disease does not progress and patients do not develop complications such as diabetic retinopathy or kidney disease and usually does not require pharmacological treatment

(Ajjan and Owen 2014). MODY1 and 3 are often misdiagnosed as T1D and treated with insulin injections but can be better managed with low doses of sulfonylurea (Shepherd et al. 2009). A genetic diagnosis of MODY should prompt genetic screening of other family members to identify undiagnosed cases of MODY.

In addition to the severe MODY causing mutations, many MODY genes also harbor common variants that increase risk of T2D, including *HNF1A*, *HNF4A*, *HNF1B*, *GCK*, and *PDX1* (Voight et al. 2010; Scott et al. 2012; Steinthorsdottir et al. 2007).

Neonatal Diabetes

Neonatal diabetes mellitus (NDM) presents as uncontrolled hyperglycemia within the first 6 months of life with an estimated prevalence of one case per 300,000–500,000 live births (Polak and Cave 2007). NDM often presents with intra - uterine growth retardation (IUGR), failure to thrive, decreased subcutaneous fat, and very low C-peptide levels. NDM is usually subdivided into permanent (PNDM) and relapsing transient (TNDM) forms; the latter form often develops T2D later (von Muhlendahl and Herkenhoff 1995).

The most common form of TNDM is due to imprinting in a locus on chromosome 6q24. Two genes at this locus are exclusively expressed from the paternal copy; *PLAGL1* (Pleiomorphic Adenoma Gene-Like 1), a zinc-finger transcription factor and *HYMAI* (Hydatiform Mole-Associated Imprinted), an untranslated RNA of unknown function (Temple et al. 1995, 1996; Arima et al. 2001). The disease manifests as a consequence of a double dose of either or both genes, which can occur due to (i) paternal isodisomy (both copies of paternal origin), (ii) duplication, which is inherited from the father, and (iii) methylation abnormality, wherein the maternal copy is silenced by methylation (Temple et al. 1995, 1996; Arima et al. 2001).

The etiology of PNMD is much more diverse with most cases being sporadic, but both dominant and recessive autosomal inheritance has also been reported. The most common forms of PNMD are caused by mutations in the *KCNJ11* (K+ Channel inwardly rectifying family J, member 11) or *ABCC8* genes (ATP-Binding Cassette, subfamily C, member8). Some *KCNJ11* mutations can cause both TNDM and PNDM (Gloyn et al. 2004; Babenko et al. 2006). PNDM can also be due to dominant mutations in the insulin gene (Stoy et al. 2007). At least two MODY genes, *GCK* (Glucokinase, MODY2) and *IPF* (insulin promoter factor, necessary for pancreatic development, mutated in MODY4), can cause recessively inherited PNDM when both parents transmit a mutated allele (Njolstad et al. 2003; Gloyn 2003; Stoffers et al. 1997). Other forms of PNMD are associated with mutations in the *FOXP3, EIF2AK3, PDX1, RFX6, and GLIS3* genes (Greeley et al. 2010).

Gestational Diabetes

Gestational diabetes mellitus (GDM) is a transitory form of diabetes that manifests as hyperglycemia during pregnancy and often resolves postpartum. Insulin

resistance begins to develop during mid-pregnancy and escalates until third trimester and requires compensation by increased insulin secretion. If this is not possible, GDM develops (Kuhl 1975). High age, obesity, history of macrosomia, multiparity, and history of polycystic ovary syndrome (PCOS) all increase risk of GDM. The risk is particularly high in women of South Asian, Middle Eastern, or Hispanic (Guariguata et al. 2014; Shaat et al. 2004).

There is emerging evidence that GDM, like T2D, has a genetic basis. GDM is more frequent in women whose mothers had GDM, as well as in women with a maternal family history of T2D (Williams et al. 2003; Martin et al. 1985; Harder et al. 2001). Women with parental history of T2D had a 2.3-fold increased risk of GDM (Williams et al. 2003), and those with a diabetic sibling had an 8.4-fold higher risk of GDM compared to women with no diabetic siblings (Robitaille and Grant 2008). Of note, changes in the diagnostic criteria complicate comparison of studies from different time points.

Many of the GDM-associated genetic risk variants overlap with T2D risk variants (Robitaille and Grant 2008; Cho et al. 2009; Lauenborg et al. 2009; O'Sullivan 1991; Kim et al. 2002). These include variants in the *TCF7L2*, *GCK*, *KCNJ11*, *KCNQ1*, *SLC30A8, HHEX/IDE*, *CDKAL1*, *IGF2BP2*, *FTO*, *PPARG*, *MTNR1B*, and *IRS1* genes (Cho et al. 2009; Lauenborg et al. 2009; Huopio et al. 2013; Kwak et al. 2012). Further, the TCF7L2 rs7903146 and FTO rs8050136 SNPs have been shown to predict diabetes after GDM (Ekelund et al. 2012).

Rare MODY mutations have also been observed in GDM, i.e., mutations in the *GCK* (MODY-2), *HNF1A* (MODY-3) and *PDX1* (MODY-4) genes which account for less than 10% of reported GDM cases (Buchanan and Xiang 2005; Ellard et al. 2000). Defects in β-cell function due to autoimmune destruction of pancreatic β-cells, as in T1D, can also cause GDM characterized by circulating autoantibodies reacting with β-cell antigens (GAD, or insulin autoantibodies, IAA). These patients appear to have evolving T1D, and they rapidly develop overt diabetes after pregnancy. This situation is seen in about 10% of GDM women (Buchanan and Xiang 2005; Catalano et al. 1990).

The role of epigenetics as a trigger of GDM is still unclear but there is some evidence that GDM can result in altered methylation in blood (Enquobahrie et al. 2015; Wu et al. 2018). There are also some epigenetic markers like H3K27 and H3K4 that can predict progression from GDM to overt T2D (Michalczyk et al. 2016).

Women with GDM are at increased risk for adverse pregnancy outcomes including fetal hyperinsulinism and macrosomia (Young and Ecker 2013; Group HSCR et al. 2008). Glucose from the mother passes freely across the placenta to the fetus while insulin cannot cross this barrier. Therefore, the fetal pancreas is stimulated to produce additional insulin which acts as a growth hormone promoting growth and adiposity (Silverman et al. 1991). GDM can also have consequences for the offspring later in life, such as increased predisposition to obesity and T2D (Silverman et al. 1991; Pettitt et al. 1993). Altered placental DNA methylation of CpG sites in the Adiponectin and Leptin genes has been reported in GDM (Bouchard et al. 2012; Lesseur et al. 2014); the expression of Leptin can be mediated by methylation of the

PPARGC1α gene (Cote et al. 2016). Altered methylation patterns in response to maternal metabolic status, e.g., HDL-C levels and glucose have also been reported in placenta and cord blood (Finer et al. 2015) of the *ABCA1* gene (Houde et al. 2013).

Genetics of Diabetic Complications

A major concern in all types of diabetes is the risk of developing complications, including diabetic kidney disease (DKD), diabetic retinopathy (DR), cardiovascular disease (CVD), diabetic neuropathy, and peripheral vascular disease (PVD). DKD and other complications are responsible for most of the morbidity and mortality associated with diabetes (Alberti and Zimmet 2013; American 2008). Diabetes is the leading cause of end-stage renal disease (ESRD) in many countries and a major contributor to blindness, lower limb amputation, and CVD (Centers for Disease Control and Prevention 2011; Gilg et al. 2013).

Diabetic Kidney Disease

Diabetic kidney disease affects as many as ~30% of patients with chronic diabetes (Ritz et al. 2011; Krolewski et al. 1985). It can often be characterized by an early phase of glomerular hyperfiltration, followed by a progressive increase in protein leakage through the glomerular basement membrane. Overt DKD is often preceded by a stage of microalbuminuria (urinary albumin excretion rate [AER] 20–199 μg/min) that will often but not always progress to macroalbuminuria (AER $\geq$ 200 μg/min). In parallel, the glomerular filtration rate (eGFR) decreases, leading first to chronic kidney disease (CKD, eGFR <60 mL/min/1.73 m^2) and subsequently to ESRD (eGFR <15 mL/min/1.73 m^2) when the patient will need dialysis or a kidney transplantation to survive.

The pathologic processes involved in the development of DN are complex and only partly known, but multiple pathways seem to be involved. Hyperglycemia is known to be a major risk factor for all diabetic complications, affecting various kidney structures including podocytes, tubular, mesangial, endothelial, and inflammatory cells but the underlying mechanisms have remained elusive (Forbes and Cooper 2013). Several candidate pathways have been implied, including protein glycation, formation of reactive oxygen species, and increased flux through the polyol pathway. In T2D, insulin resistance seems to be an important driver of DKD.

Heritability

The risk of developing DKD also depends on genetic factors as evidenced by familial aggregation. The estimated heritability of AER is ~20–40% and a sibling of an affected individual has the double risk of developing DKD (Harjutsalo et al. 2004; Langefeld et al. 2004; Krolewski et al. 2006; Forsblom et al. 1999). Prevalence of DKD also differs between ethnic groups with different genetic backgrounds.

Despite this compelling evidence for genetic effects, the search for the specific variants conferring DKD predisposition has been rather unrewarding and only a few robust associations have been found.

Genetic Risk Loci

One of the most plausible and best-supported candidate loci is an insertion/deletion (I/D) variant in the gene encoding ACE. ACE inhibitors confer protection against DKD by decreasing glomerular hypertension and permeability, and the ACE I/D variant is associated with a twofold increase in ACE activity, making this a highly credible biological candidate (Nikzamir et al. 2008; Rigat et al. 1990). This locus has been studied in numerous association studies but a role in DKD susceptibility has still not been proven. A meta-analysis, including over 26,000 individuals from 63 studies, observed some modest evidence for this locus in DKD, but the association was mainly observed in Asian populations with T2D and did not reach genome-wide significance (Wang et al. 2012).

Compared to diabetes and many other traits, there have been relatively few GWAS studies performed on DKD. The GENIE consortium performed the first study to yield a genome-wide significant finding, identifying one locus in the *AFF3* gene and another between the *RGMA* and *MCTP2* gene loci associated with risk of ESRD in patients with T1D (Sandholm et al. 2012). A follow-up study identified a third locus, near *SP3*, that was significantly associated with ESRD only in women (Sandholm et al. 2013). However, these loci still need to be replicated in independent cohorts before they can be universally accepted as DKD risk loci.

The situation for genetics of DKD in T2D is even less rewarding. While a few studies, including family-based linkage analysis and GWAS have been performed and produced interesting candidates; none of the loci have reached genome-wide significance (Ahlqvist et al. 2015).

The limited success of genetic studies in DKD compared to many other diseases is probably due to a combination of factors, including inadequate sample sizes and phenotypic imprecision. DKD typically develops after more than 15 years, which reduces the number of available patients with duration of diabetes sufficient to identify control patients who will escape DKD or at least have a clearly later onset than the case group. Another problem is the high prevalence of other types of kidney disease in T2D patients. These can only be clearly distinguished from DKD by kidney biopsies, which are not routinely taken in most countries (Ruggenenti and Remuzzi 2000).

In order to increase sample sizes, many studies have included patients with early signs of DKD, such as microalbuminuria, as well as cases with macroalbuminuria or ESRD. However, albuminuria is a relatively poor predictor of DKD, especially in the early stages, adding further uncertainty to the classification (Perkins et al. 2010; Boger and Sedor 2012). Reduced kidney function, as revealed by eGFR and ESRD, and dysfunction of the glomerular filtration barrier, reflected by albuminuria, can develop independently suggesting that these processes result from partly different disease mechanisms, with distinct genetic determinants (Perkins et al. 2010; Steinke et al. 2005; Ellis et al. 2012). In spite of these obstacles, ongoing efforts combining

large cohorts (IMI studies SUMMIT and Beat-DKD, the JDRF-funded GENIE consortium, etc.) in meta-analyses will hopefully reach adequate sample sizes to identify robust associations also for DKD in T2D.

Diabetic Retinopathy

Diabetic retinopathy is characterized by microvascular changes in the retina, increasing vascular permeability and capillary degeneration with resulting microaneurysms, exudates, and neovascularization (Forbes and Cooper 2013). The main clinical risk factors for DR are duration of diabetes, chronic hyperglycemia, hypertension, and lipids (Yau et al. 2012).

The prevalence of DR is double for patients with microalbuminuria and sixfold increase in patients with macroalbuminuria compared to patients with no signs of renal dysfunction suggesting both common and unique mechanisms (Rani et al. 2011; Drury et al. 2011; Groop et al. 2009). Clinical data support at least two, potentially distinct, pathological processes for DR, resulting in proliferative retinopathy and macular edema, respectively (Viswanath and McGavin 2003).

The heritability of DR has been estimated to 18–57%, which is consistent with a substantial genetic component but might also reflect challenges in defining the phenotype consistently (Arar et al. 2008; Hietala et al. 2008; Looker et al. 2007).

As for DKD, robust genetic findings are very sparse. Candidate gene studies have produced a plethora of suggestive associations but none has reached genome-wide significance (Cho and Sobrin 2014). One of the best-studied candidate genes is the gene encoding vascular endothelial growth factor A (*VEGFA*). VEGF inhibition is used clinically to treat DR making this a highly credible biological candidate. In spite of this, variants that influence VEGF expression show only marginal association to DR (Qiu et al. 2013; Zhao and Zhao 2010; Abhary et al. 2009). A second biologically credible candidate gene is aldose reductase (*AKR1B1*). Aldose reductase is the rate-limiting enzyme in the polyol pathway, which has been widely implicated in glucose-related tissue damage. However, association data for *AKR1B1* also remains unconvincing (Abhary et al. 2009, 2010). A third locus suggested to contribute to both DKD and DR is variants in the promoter of the gene encoding *EPO* (Tong et al. 2008), but again these have not been consistently replicated (Williams et al. 2012).

Only a few GWAS studies of DR have been performed so far and none of them have yielded significant reproducible associations (Sheu et al. 2013; Huang et al. 2011; Grassi et al. 2011). This is not so surprising given the small sample sizes. The largest reanalysis of the GoKIND and EDIC data sets included only 973 cases and 1856 controls (Cho and Sobrin 2014). Much larger sample sizes will likely be needed given the heterogeneity of the phenotype and progressive nature of the disease. Such studies are currently being assembled by large international consortia such as SUMMIT (Cho and Sobrin 2014).

Diabetic Neuropathy

Diabetic neuropathy is also a highly heterogeneous complication affecting ~10% of individuals with chronic diabetes. The mechanisms leading to nerve damage are poorly understood but likely include both vascular ischemic mechanisms and damage caused by advanced glycation end products that stimulate proinflammatory pathways and matrix-metalloproteinases (Said 2007).

There have been few genetic studies on diabetic neuropathy and no GWAS. A study in T1D suggested that an AKR1B1 polymorphism was involved in decline of nerve function but unfortunately even this study was severely restrained by small sample sizes (Thamotharampillai et al. 2006).

Cardiovascular Complications

Diabetes accelerates the process of atherosclerosis, leading to coronary artery disease (CAD), ischemic stroke (IS) and peripheral arterial disease (PAD). For example, hyperglycemia promotes vascular dysfunction by inhibiting nitric oxide production in endothelial cells and platelets, impairing endothelium-dependent vasodilation and increasing production of reactive oxygen species (ROS). Thereby atherosclerotic plaques are destabilized and rendered more vulnerable to rupture while a hypercoagulable state increases the formation and persistence of thrombi (Beckman et al. 2002). The heritability for CAD in the general population is estimated to be 40–50% and more than 60 variants, many of which are involved in cholesterol and lipid metabolism, have been robustly associated with disease risk (Khera and Kathiresan 2017). These loci are likely to play a role also in diabetic individuals, but additional loci might influence risk of macrovascular disease as a consequence of hyperglycemia. A locus near the glutamate-ammonia ligase (*GLUL*) gene has been associated with coronary heart disease specifically in T2D patients (Qi et al. 2013), but overall, diabetes specific association have not yet been sufficiently studied.

Epigenetics and Diabetic Complications

Epigenetic changes affecting the development of complications have been suggested to explain the observation of "metabolic memory"; a concept coined to describe the observation that early poor metabolic control can be memorized by target tissues and promote diabetic complications despite intensified treatment later in the disease. For example, the UKPDS and DCCT studies showed that an initial good metabolic control was associated with reduced frequency of diabetic complications decades later (DCCT 1993; Writing Team for the Diabetes C et al. 2002, 2003; Holman et al. 2008). The thioredoxin-interacting protein (*TXNIP*) gene is extremely sensitive to increases in glucose and has been ascribed proinflammatory roles in many tissues as well as promoting insulin resistance and glucose increased TXNIP expression histone acetylation in mice could promote diabetic kidney disease (De Marinis et al. 2016).

Summary

While the HLA plays a central role in the genetics of T1D, the role of genetics in T2D and diabetic complications has been more difficult to dissect. There can be several explanations for these shortcomings as discussed in this review, not at least the poorly defined phenotypes. Work is though in progress to refine phenotypes and subgroups of T2D and diabetic complications. Also the rapid improvement of genetic tools will hopefully accelerate the search for these missing genes. Even with the limitations of only partly explained heritability, and small effect loci, genetic studies still provide valuable information about disease mechanisms and identifies new potential therapeutic target. In monogenic diabetes, identification of the underlying variants has already enabled personalized treatment. With refined phenotypes and improved patient stratification this will hopefully be in our near future also for complex diabetes types.

References

Abhary S, Hewitt AW, Burdon KP, Craig JE. A systematic meta-analysis of genetic association studies for diabetic retinopathy. Diabetes. 2009;58(9):2137–47. Epub 2009/07/10

Abhary S, Burdon KP, Laurie KJ, Thorpe S, Landers J, Goold L, et al. Aldose reductase gene polymorphisms and diabetic retinopathy susceptibility. Diabetes Care. 2010;33(8):1834–6. Epub 2010/04/29

Agarwala V, Flannick J, Sunyaev S, Go TDC, Altshuler D. Evaluating empirical bounds on complex disease genetic architecture. Nat Genet. 2013;45(12):1418–27. Epub 2013/10/22

Ahlqvist E, van Zuydam NR, Groop LC, McCarthy MI. The genetics of diabetic complications. Nat Rev Nephrol. 2015;11(5):277–87. Epub 2015/04/01

Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spégel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark Å, Lahti K, Forsén T, Tuomi T, Rosengren AH, Groop L (2018) Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. The Lancet Diabetes & Endocrinology.

Ajjan RA, Owen KR. Glucokinase MODY and implications for treatment goals of common forms of diabetes. Curr Diab Rep. 2014;14(12):559. Epub 2014/10/27

Alberti KG, Zimmet P. Global burden of disease – where does diabetes mellitus fit in? Nat Rev Endocrinol. 2013;9(5):258–60. Epub 2013/03/13

Albrechtsen A, et al. Exome sequencing-driven discovery of coding polymorphisms associated with common metabolic phenotypes. Diabetologia. 2013;56(2):298–310.

American DA. Economic costs of diabetes in the U.S. in 2007. Diabetes Care. 2008;31(3):596–615. Epub 2008/03/01

Andersen MK, Sterner M, Forsen T, Karajamaki A, Rolandsson O, Forsblom C, et al. Type 2 diabetes susceptibility gene variants predispose to adult-onset autoimmune diabetes. Diabetologia. 2014;57(9):1859–68. Epub 2014/06/08

Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes. 2008;57(1):95–101.

Arar NH, Freedman BI, Adler SG, Iyengar SK, Chew EY, Davis MD, et al. Heritability of the severity of diabetic retinopathy: the FIND-Eye study. Investig Ophthalmol Vis Sci. 2008;49(9):3839–45. Epub 2008/09/04

Arima T, Drewell RA, Arney KL, Inoue J, Makita Y, Hata A, et al. A conserved imprinting control region at the HYMAI/ZAC domain is implicated in transient neonatal diabetes mellitus. Hum Mol Genet. 2001;10(14):1475–83.

Asahara S, Etoh H, Inoue H, Teruyama K, Shibutani Y, Ihara Y, et al. Paternal allelic mutation at the Kcnq1 locus reduces pancreatic beta-cell mass by epigenetic modification of Cdkn1c. Proc Natl Acad Sci U S A. 2015;112(27):8332–7.

Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, et al. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med. 2006;355(5):456–66.

Bacos K, Gillberg L, Volkov P, Olsson AH, Hansen T, Pedersen O, et al. Blood-based biomarkers of age-associated epigenetic changes in human islets associate with insulin secretion and diabetes. Nat Commun. 2016;7:11089.

Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007;261(5):412–7.

Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287(19):2570–81.

Bell GI, Horita S, Karam JH. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes. 1984;33(2):176–83. Epub 1984/02/01

Belot MP, Fradin D, Mai N, Le Fur S, Zelenika D, Kerr-Conte J, et al. CpG methylation changes within the IL2RA promoter in type 1 diabetes of childhood onset. PLoS One. 2013;8(7):e68093.

Bergholdt R, Brorsson C, Palleja A, Berchtold LA, Floyel T, Bang-Berthelsen CH, et al. Identification of novel type 1 diabetes candidate genes by integrating genome-wide association data, protein-protein interactions, and human pancreatic islet gene expression. Diabetes. 2012;61(4):954–62. Epub 2012/02/22

Bird A. Perceptions of epigenetics. Nature. 2007;447(7143):396–8. Epub 2007/05/25

Blodgett DM, Nowosielska A, Afik S, Pechhold S, Cura AJ, Kennedy NJ, et al. Novel observations from next-generation RNA sequencing of highly purified human adult and fetal islet cell subsets. Diabetes. 2015;64(9):3172–81.

Boger CA, Sedor JR. GWAS of diabetic nephropathy: is the GENIE out of the bottle? PLoS Genet. 2012;8(9):e1002989. Epub 2012/10/03

Bouchard L, Hivert MF, Guay SP, St-Pierre J, Perron P, Brisson D. Placental adiponectin gene DNA methylation levels are associated with mothers' blood glucose concentration. Diabetes. 2012;61(5):1272–80.

Brito EC, Lyssenko V, Renstrom F, Berglund G, Nilsson PM, Groop L, et al. Previously associated type 2 diabetes variants may interact with physical activity to modify the risk of impaired glucose regulation and type 2 diabetes: a study of 16,003 Swedish adults. Diabetes. 2009;58(6):1411–8.

Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, et al. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet. 2008;17(6):806–14. Epub 2007/12/01

Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest. 2005;115(3):485–91.

Carlson CS, Eberle MA, Kruglyak L, Nickerson DA. Mapping complex disease loci in whole-genome association studies. Nature. 2004;429(6990):446–52. Epub 2004/05/28

Carlsson S, Midthjell K, Grill V. Influence of family history of diabetes on incidence and prevalence of latent autoimmune diabetes of the adult: results from the Nord-Trondelag Health Study. Diabetes Care. 2007;30(12):3040–5. Epub 2007/09/20

Catalano PM, Tyzbir ED, Sims EA. Incidence and significance of islet cell antibodies in women with previous gestational diabetes. Diabetes Care. 1990;13(5):478–82.

Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta: U.S. Department of Health and Human; 2011.

Cervin C, Lyssenko V, Bakhtadze E, Lindholm E, Nilsson P, Tuomi T, et al. Genetic similarities between latent autoimmune diabetes in adults, type 1 diabetes, and type 2 diabetes. Diabetes. 2008;57(5):1433–7. Epub 2008/03/04

Chambers JC, et al. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. Nat Genet. 2008;40(6):716–8.

Chambers JC, Loh M, Lehne B, Drong A, Kriebel J, Motta V, et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. Lancet Diabetes Endocrinol. 2015;3(7):526–34. Epub 2015/06/23

Chen G, et al. Genome-wide association study identifies novel loci association with fasting insulin and insulin resistance in African Americans. Hum Mol Genet. 2012;21(20):4530–6.

Cho YS, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2012a;44(1):67–72.

Cho YS, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2012b;44(1):67–72.

Cho H, Sobrin L. Genetics of diabetic retinopathy. Curr Diab Rep. 2014;14(8):515.

Cho YM, Kim TH, Lim S, Choi SH, Shin HD, Lee HK, et al. Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. Diabetologia. 2009;52(2):253–61.

Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2012;44(1):67–72. Epub 2011/12/14

Chong S, Whitelaw E. Epigenetic germline inheritance. Curr Opin Genet Dev. 2004;14(6):692–6.

Chong S, Youngson NA, Whitelaw E. Heritable germline epimutation is not the same as trans-generational epigenetic inheritance. Nat Genet. 2007;39(5):574–5; author reply 5–6

Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomics research. Nature. 2003;422(6934):835–47. Epub 2003/04/16

Cote S, Gagne-Ouellet V, Guay SP, Allard C, Houde AA, Perron P, et al. PPARGC1alpha gene DNA methylation variations in human placenta mediate the link between maternal hyperglycemia and leptin levels in newborns. Clin Epigenetics. 2016;8:72.

Cui B, et al. A genome-wide association study confirms previously reported loci for type 2 diabetes in Han Chinese. PLoS One. 2011;6(7):e22353.

Dahlquist G, Blom L, Tuvemo T, Nystrom L, Sandstrom A, Wall S. The Swedish childhood diabetes study–results from a nine year case register and a one year case-referent study indicating that type 1 (insulin-dependent) diabetes mellitus is associated with both type 2 (non-insulin-dependent) diabetes mellitus and autoimmune disorders. Diabetologia. 1989;32 (1):2–6. Epub 1989/01/01

Davidson BL, McCray PB Jr. Current prospects for RNA interference-based therapies. Nat Rev Genet. 2011;12(5):329–40. Epub 2011/04/19

Dayeh T, Volkov P, Salo S, Hall E, Nilsson E, Olsson AH, et al. Genome-wide DNA methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. PLoS Genet. 2014;10(3):e1004160.

DCCT. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med. 1993;329(14):977–86.

De Marinis Y, Cai M, Bompada P, Atac D, Kotova O, Johansson ME, et al. Epigenetic regulation of the thioredoxin-interacting protein (TXNIP) gene by hyperglycemia in kidney. Kidney Int. 2016;89(2):342–53.

Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998;20(3):284–7.

Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316(5829): 1331–6.

DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014;46(3):234–44.

Diamond J. The double puzzle of diabetes. Nature. 2003;423(6940):599–602. Epub 2003/06/06

Drury PL, Ting R, Zannino D, Ehnholm C, Flack J, Whiting M, et al. Estimated glomerular filtration rate and albuminuria are independent predictors of cardiovascular events and death in type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetologia. 2011;54(1):32–43. Epub 2010/07/30

Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, et al. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. Am J Hum Genet. 1999;64(4):1127–40. Epub 1999/03/26

Dupuis J, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42(2):105–16.

Ekelund M, Shaat N, Almgren P, Anderberg E, Landin-Olsson M, Lyssenko V, et al. Genetic prediction of postpartum diabetes in women with gestational diabetes mellitus. Diabetes Res Clin Pract. 2012;97(3):394–8.

Ellard S, Beards F, Allen LI, Shepherd M, Ballantyne E, Harvey R, et al. A high prevalence of glucokinase mutations in gestational diabetic subjects selected by clinical criteria. Diabetologia. 2000;43(2):250–3.

Ellis JW, Chen MH, Foster MC, Liu CT, Larson MG, de Boer I, et al. Validated SNPs for eGFR and their associations with albuminuria. Hum Mol Genet. 2012;21(14):3293–8. Epub 2012/04/12

Enquobahrie DA, Moore A, Muhie S, Tadesse MG, Lin S, Williams MA. Early pregnancy maternal blood DNA methylation in repeat pregnancies and change in gestational diabetes mellitus status – a pilot study. Reprod Sci. 2015;22(7):904–10.

Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk. Analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008;57(4):1084–92.

Evans DM, Marchini J, Morris AP, Cardon LR. Two-stage two-locus models in genome-wide association. PLoS Genet. 2006;2(9):e157. Epub 2006/09/28

Fadista J, Vikman P, Laakso EO, Mollet IG, Esguerra JL, Taneera J, et al. Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism. Proc Natl Acad Sci U S A. 2014;111(38):13924–9.

Fernandez-Valverde SL, Taft RJ, Mattick JS. MicroRNAs in beta-cell biology, insulin resistance, diabetes and its complications. Diabetes. 2011;60(7):1825–31. Epub 2011/06/29

Finer S, Mathews C, Lowe R, Smart M, Hillman S, Foo L, et al. Maternal gestational diabetes is associated with genome-wide DNA methylation variation in placenta and cord blood of exposed offspring. Hum Mol Genet. 2015;24(11):3021–9.

Flannick J, Thorleifsson G, Beer NL, Jacobs SB, Grarup N, Burtt NP, et al. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nat Genet. 2014;46(4):357–63.

Florath I, Butterbach K, Heiss J, Bewerunge-Hudler M, Zhang Y, Schottker B, et al. Type 2 diabetes and leucocyte DNA methylation: an epigenome-wide association study in over 1,500 older adults. Diabetologia. 2016;59(1):130–8. Epub 2015/10/05

Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013;93(1):137–88. Epub 2013/01/11

Forsblom CM, Kanninen T, Lehtovirta M, Saloranta C, Groop LC. Heritability of albumin excretion rate in families of patients with type II diabetes. Diabetologia. 1999;42(11):1359–66. Epub 1999/11/07

Fradin D, Le Fur S, Mille C, Naoui N, Groves C, Zelenika D, et al. Association of the CpG methylation pattern of the proximal insulin gene promoter with type 1 diabetes. PLoS One. 2012;7(5):e36278.

Franks PW, Jablonski KA, Delahanty LM, McAteer JB, Kahn SE, Knowler WC, et al. Assessing gene-treatment interactions at the FTO and INSIG2 loci on obesity-related traits in the Diabetes Prevention Program. Diabetologia. 2008;51(12):2214–23.

Frayling TM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316(5826):889–94.

Fuchsberger C, et al. The genetic architecture of type 2 diabetes. Nature. 2016;536(7614):41–7.

Gilg J, Rao A, Fogarty D. UK Renal Registry 16th annual report: chapter 1 UK renal replacement therapy incidence in 2012: national and centre-specific analyses. Nephron Clin Pract. 2013;125 (1–4):1–27. Epub 2013/01/01

Gloyn AL. Glucokinase (GCK) mutations in hyper- and hypoglycemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. Hum Mutat. 2003;22(5):353–62.

Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, et al. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes. 2003;52(2):568–72. Epub 2003/01/24

Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med. 2004;350(18):1838–49. Epub 2004/04/30

Go MJ, et al. New susceptibility loci in MYL2, C12orf51 and OAS1 associated with 1-h plasma glucose as predisposing risk factors for type 2 diabetes in the Korean population. J Hum Genet. 2013;58(6):362–5.

Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006;38(3):320–3.

Grassi MA, Tikhomirov A, Ramalingam S, Below JE, Cox NJ, Nicolae DL. Genome-wide meta-analysis for severe diabetic retinopathy. Hum Mol Genet. 2011;20(12):2472–81. Epub 2011/03/29

Greeley SA, Tucker SE, Naylor RN, Bell GI, Philipson LH. Neonatal diabetes mellitus: a model for personalized medicine. Trends Endocrinol Metab. 2010;21(8):464–72.

Groop L, Pociot F. Genetics of diabetes–are we missing the genes or the disease? Mol Cell Endocrinol. 2014;382(1):726–39. Epub 2013/04/17

Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M, et al. Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. Diabetes. 1996;45(11):1585–93. Epub 1996/11/01

Groop L, Tuomi T, Rowley M, Zimmet P, Mackay IR. Latent autoimmune diabetes in adults (LADA)–more than a name. Diabetologia. 2006;49(9):1996–8. Epub 2006/07/05

Groop PH, Thomas MC, Moran JL, Waden J, Thorn LM, Makinen VP, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes. 2009;58(7):1651–8. Epub 2009/04/30

Group HSCR, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991–2002.

Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. Diabetes Res Clin Pract. 2014;103(2):176–85.

Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat Genet. 2007;39(8):977–83.

Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5–20.

Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, et al. Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. Diabetologia. 1998;41(12):1511–5.

Hansen SK, Nielsen EM, Ek J, Andersen G, Glumer C, Carstensen B, et al. Analysis of separate and combined effects of common variation in KCNJ11 and PPARG on risk of type 2 diabetes. J Clin Endocrinol Metab. 2005;90(6):3629–37.

Hanson RL, Guo T, Muller YL, Fleming J, Knowler WC, Kobes S, et al. Strong parent-of-origin effects in the association of KCNQ1 variants with type 2 diabetes in American Indians. Diabetes. 2013;62(8):2984–91.

Hanson RL, et al. A genome-wide association study in American Indians implicates DNER as a susceptibility locus for type 2 diabetes. Diabetes. 2014;63(1):369–76.

Hara K, et al. Genome-wide association study identifies three novel loci for type 2 diabetes. Hum Mol Genet. 2014;23(1):239–46.

Harder T, Franke K, Kohlhoff R, Plagemann A. Maternal and paternal family history of diabetes in women with gestational diabetes or insulin-dependent diabetes mellitus type I. Gynecol Obstet Investig. 2001;51(3):160–4.
Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol. 2007;165(8):849–57.
Hariharan M, Scaria V, Brahmachari SK. dbSMR: a novel resource of genome-wide SNPs affecting microRNA mediated regulation. BMC Bioinf. 2009;10:108. Epub 2009/04/18
Harjutsalo V, Katoh S, Sarti C, Tajima N, Tuomilehto J. Population-based assessment of familial clustering of diabetic nephropathy in type 1 diabetes. Diabetes. 2004;53(9):2449–54. Epub 2004/08/28
Hemminki K, Li X, Sundquist K, Sundquist J. Familial risks for type 2 diabetes in Sweden. Diabetes Care. 2010;33(2):293–7. Epub 2009/11/12
Hietala K, Forsblom C, Summanen P, Groop PH, FinnDiane Study G. Heritability of proliferative diabetic retinopathy. Diabetes. 2008;57(8):2176–80. Epub 2008/04/30
Hoggart CJ, Venturini G, Mangino M, Gomez F, Ascari G, Zhao JH, et al. Novel approach identifies SNPs in SLC2A10 and KCNK9 with evidence for parent-of-origin effect on body mass index. PLoS Genet. 2014;10(7):e1004508.
Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359(15):1577–89. Epub 2008/09/12
Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet. 2000;26(2):163–75.
Horton V, Stratton I, Bottazzo GF, Shattock M, Mackay I, Zimmet P, et al. Genetic heterogeneity of autoimmune diabetes: age of presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS 43). UK Prospective Diabetes Study (UKPDS) Group. Diabetologia. 1999;42(5):608–16. Epub 1999/05/20
Houde AA, Guay SP, Desgagne V, Hivert MF, Baillargeon JP, St-Pierre J, et al. Adaptations of placental and cord blood ABCA1 DNA methylation profile to maternal metabolic status. Epigenetics. 2013;8(12):1289–302.
Howson JM, Rosinger S, Smyth DJ, Boehm BO, Todd JA. Genetic analysis of adult-onset autoimmune diabetes. Diabetes. 2011;60(10):2645–53. Epub 2011/08/30
Huang YC, Lin JM, Lin HJ, Chen CC, Chen SY, Tsai CH, et al. Genome-wide association study of diabetic retinopathy in a Taiwanese population. Ophthalmology. 2011;118(4):642–8. Epub 2011/02/12
Huopio H, Cederberg H, Vangipurapu J, Hakkarainen H, Paakkonen M, Kuulasmaa T, et al. Association of risk variants for type 2 diabetes and hyperglycemia with gestational diabetes. Eur J Endocrinol. 2013;169(3):291–7.
Huyghe JR, et al. Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. Nat Genet. 2013;45(2):197–201.
Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. Diabetes. 2003;52(4):1052–5.
Ilonen J, Hammais A, Laine A-P, Lempainen J, Vaarala O, Veijola R, et al. Patterns of β-cell autoantibody appearance and genetic associations during the first years of life. Diabetes. 2013;62(10):3636–40.
Imamura M, Maeda S, Yamauchi T, Hara K, Yasuda K, Morizono T, et al. A single-nucleotide polymorphism in ANK1 is associated with susceptibility to type 2 diabetes in Japanese populations. Hum Mol Genet. 2012;21(13):3042–9.
International HapMap Consortium. The International HapMap Project. Nature. 2003;426 (6968):789–96. Epub 2003/12/20
Kahara T, Takamura T, Hayakawa T, Nagai Y, Yamaguchi H, Katsuki T, et al. PPARgamma gene polymorphism is associated with exercise-mediated changes of insulin resistance in healthy men. Metabolism. 2003;52(2):209–12.

Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, et al. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia. 1992;35(11):1060–7. Epub 1992/11/01

Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care. 2000;23(10):1516–26. Epub 2000/10/07

Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet. 2017;18(6):331–44. Epub 2017/03/14

Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care. 2002;25(10):1862–8.

Kleinberger JW, Pollin TI. Undiagnosed MODY: time for action. Curr Diab Rep. 2015;15(12):110.

Köbberling J, Tillil H. Empirical risk figures for first-degree relatives of non-insulin dependent diabetics. In: Köbberling J, Tattersall R, editors. The genetics of diabetes mellitus. London: Academic; 1982. p. 201–9.

Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, et al. Parental origin of sequence variants associated with complex diseases. Nature. 2009;462(7275):868–74.

Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, et al. Genome-wide association study in individuals of south Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet. 2011;43(10):984–9.

Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR. The changing natural history of nephropathy in type I diabetes. Am J Med. 1985;78(5):785–94. Epub 1985/05/01

Krolewski AS, Poznik GD, Placha G, Canani L, Dunn J, Walker W, et al. A genome-wide linkage scan for genes controlling variation in urinary albumin excretion in type II diabetes. Kidney Int. 2006;69(1):129–36. Epub 2005/12/24

Kuhl C. Glucose metabolism during and after pregnancy in normal and gestational diabetic women. 1. Influence of normal pregnancy on serum glucose and insulin concentration during basal fasting conditions and after a challenge with glucose. Acta Endocrinol. 1975;79(4):709–19.

Kulkarni H, Kos MZ, Neary J, Dyer TD, Kent JW Jr, Goring HH, et al. Novel epigenetic determinants of type 2 diabetes in Mexican-American families. Hum Mol Genet. 2015;24(18):5330–44. Epub 2015/06/24

Kwak SH, Kim SH, Cho YM, Go MJ, Cho YS, Choi SH, et al. A genome-wide association study of gestational diabetes mellitus in Korean women. Diabetes. 2012;61(2):531–41.

Kyvik KO, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. BMJ. 1995;311(7010):913–7. Epub 1995/10/07

Langefeld CD, Beck SR, Bowden DW, Rich SS, Wagenknecht LE, Freedman BI. Heritability of GFR and albuminuria in Caucasians with type 2 diabetes mellitus. Am J Kidney Dis. 2004;43(5):796–800. Epub 2004/04/28

Lauenborg J, Grarup N, Damm P, Borch-Johnsen K, Jorgensen T, Pedersen O, et al. Common type 2 diabetes risk gene variants associate with gestational diabetes. J Clin Endocrinol Metab. 2009;94(1):145–50.

Laugesen E, Ostergaard JA, Leslie RD, Danish Diabetes Academy Workshop and Workshop Speakers. Latent autoimmune diabetes of the adult: current knowledge and uncertainty. Diabet Med. 2015;32(7):843–52. Epub 2015/01/21

Lesseur C, Armstrong DA, Paquette AG, Li Z, Padbury JF, Marsit CJ. Maternal obesity and gestational diabetes are associated with placental leptin DNA methylation. Am J Obstet Gynecol. 2014;211(6):654 e1–9.

Li H, Gan W, Lu L, Dong X, Han X, Hu C, et al. A genome-wide association study identifies GRK5 and RASGRP1 as type 2 diabetes loci in Chinese Hans. Diabetes. 2013;62(1):291–8.

Looker HC, Nelson RG, Chew E, Klein R, Klein BE, Knowler WC, et al. Genome-wide linkage analyses to identify Loci for diabetic retinopathy. Diabetes. 2007;56(4):1160–6. Epub 2007/03/31

Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, et al. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes. 2001;50(3):686–9.

Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA. Clan genomics and the complex architecture of human disease. Cell. 2011;147(1):32–43. Epub 2011/10/04

Lyssenko V, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med. 2008;359(21):2220–32.

Ma RC, et al. Genome-wide association study in a Chinese population identifies a susceptibility locus for type 2 diabetes at 7q32 near PAX4. Diabetologia. 2013;56(6):1291–305.

Manning AK, et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet. 2012;44(6):659–69.

Martin AO, Simpson JL, Ober C, Freinkel N. Frequency of diabetes mellitus in mothers of probands with gestational diabetes: possible maternal influence on the predisposition to gestational diabetes. Am J Obstet Gynecol. 1985;151(4):471–5.

Medici F, Hawa M, Ianari A, Pyke DA, Leslie RD. Concordance rate for type II diabetes mellitus in monozygotic twins: actuarial analysis. Diabetologia. 1999;42(2):146–50. Epub 1999/03/04

Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes. 2000;49(12):2201–7. Epub 2000/12/16

Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R. Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. Diabetes. 2008;57(12):3189–98.

Miao F, Chen Z, Zhang L, Liu Z, Wu X, Yuan YC, et al. Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. J Biol Chem. 2012;287(20): 16335–45.

Michalczyk AA, Dunbar JA, Janus ED, Best JD, Ebeling PR, Ackland MJ, et al. Epigenetic markers to predict conversion from gestational diabetes to type 2 diabetes. J Clin Endocrinol Metab. 2016;101(6):2396–404.

Minton JA, et al. Association studies of genetic variation in the WFS1 gene and type 2 diabetes in U.K. populations. Diabetes. 2002;51(4):1287–90.

Moltke I, Grarup N, Jorgensen ME, Bjerregaard P, Treebak JT, Fumagalli M, et al. A common Greenlandic TBC1D4 variant confers muscle insulin resistance and type 2 diabetes. Nature. 2014;512(7513):190–3.

Moore T, Haig D. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet. 1991;7(2):45–9.

Moran VA, Perera RJ, Khalil AM. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. Nucleic Acids Res. 2012;40(14):6391–400.

Morris AP, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012a;44(9):981–90.

Morris AP, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012b;44(9):981–90.

Murphy R, Turnbull DM, Walker M, Hattersley AT. Clinical features, diagnosis and management of maternally inherited diabetes and deafness (MIDD) associated with the 3243A>G mitochondrial point mutation. Diabet Med. 2008a;25(4):383–99.

Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab. 2008b;4(4):200–13. Epub 2008/02/28

Nerup J, Platz P, Andersen OO, Christy M, Lyngse J, Poulsen JE, et al. HL-A antigens and diabetes mellitus. Lancet. 1974;304(7885):864–6.

Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia. 1987;30(10):763–8. Epub 1987/10/01

Ng MC, et al. Meta-analysis of genome-wide association studies in African Americans provides insights into the genetic architecture of type 2 diabetes. PLoS Genet. 2014;10(8):e1004517.

Nikzamir A, Nakhjavani M, Esteghamati A, Rashidi A. Correlates of ACE activity in macroalbuminuric type 2 diabetic patients treated with chronic ACE inhibition. Nephrol Dial Transplant. 2008;23(4):1274–7.

Njolstad PR, Sagen JV, Bjorkhaug L, Odili S, Shehadeh N, Bakry D, et al. Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. Diabetes. 2003;52(11):2854–60.

Noble JA. Immunogenetics of type 1 diabetes: a comprehensive review. J Autoimmun. 2015;64:101–12.

O'Sullivan JB. Diabetes mellitus after GDM. Diabetes. 1991;40(Suppl 2):131–5.

Palmer ND, McDonough CW, Hicks PJ, Roh BH, Wing MR, An SS, et al. A genome-wide association search for type 2 diabetes genes in African Americans. PLoS One. 2012;7(1): e29202.

Parra EJ, Below JE, Krithika S, Valladares A, Barta JL, Cox NJ, et al. Genome-wide association study of type 2 diabetes in a sample from Mexico City and a meta-analysis of a Mexican-American sample from Starr County, Texas. Diabetologia. 2011;54(8):2038–46.

Pasquali L, Gaulton KJ, Rodriguez-Segui SA, Mularoni L, Miguel-Escalada I, Akerman I, et al. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. Nat Genet. 2014;46(2):136–43.

Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G, Group ES. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. Lancet. 2009;373(9680):2027–33. Epub 2009/06/02

Pearson ER, Starkey BJ, Powell RJ, Gribble FM, Clark PM, Hattersley AT. Genetic cause of hyperglycaemia and response to treatment in diabetes. Lancet. 2003;362(9392):1275–81.

Perkins BA, Ficociello LH, Roshan B, Warram JH, Krolewski AS. In patients with type 1 diabetes and new-onset microalbuminuria the development of advanced chronic kidney disease may not require progression to proteinuria. Kidney Int. 2010;77(1):57–64. Epub 2009/10/23

Perry JR, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet. 2012a;8(5): e1002741.

Perry JR, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet. 2012b;8(5): e1002741.

Pettitt DJ, Nelson RG, Saad MF, Bennett PH, Knowler WC. Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. Diabetes Care. 1993;16(1):310–4.

Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. Nat Rev Genet. 2009;10(12):872–8.

Pociot F, Lernmark A. Genetic risk factors for type 1 diabetes. Lancet. 2016;387(10035):2331–9. Epub 2016/06/16

Pociot F, Norgaard K, Hobolth N, Andersen O, Nerup J. A nationwide population-based study of the familial aggregation of type 1 (insulin-dependent) diabetes mellitus in Denmark. Danish Study Group of Diabetes in Childhood. Diabetologia. 1993;36(9):870–5. Epub 1993/09/01

Pociot F, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, et al. Genetics of type 1 diabetes: what's next? Diabetes. 2010;59(7):1561–71. Epub 2010/07/01

Polak M, Cave H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis. 2007;2:12. Epub 2007/03/14

Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance–a population-based twin study. Diabetologia. 1999;42(2):139–45. Epub 1999/03/04

Prasad RB, Lessmark A, Almgren P, Kovacs G, Hansson O, Oskolkov N, et al. Excess maternal transmission of variants in the THADA gene to offspring with type 2 diabetes. Diabetologia. 2016a;59(8):1702–13. Epub 2016/05/09

Prasad RB, Lessmark A, Almgren A, Kovacs G, Oskolkov, N, Vitai M, Ladenvall C, Kovacs P, Fadista J, Lachmann M, Zhou Y, Hansson O, Sonestedt E, Poon W, Wolheim C, Orho-Melander M, Stumvoll M, Tuomi T, Pääbo S, Koranyi L, Groop L. Genetics of type 2 diabetes—Pitfalls and possibilities. Genes (Basel). 2015;6(1):87–123.

Prokopenko I, et al. Variants in MTNR1B influence fasting glucose levels. Nat Genet. 2009;41 (1):77–81.
Pugliese A. The insulin gene in type 1 diabetes. IUBMB Life. 2005;57(7):463–8. Epub 2005/08/06
Qi L, et al. Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. Hum Mol Genet. 2010;19(13):2706–15.
Qi L, Qi Q, Prudente S, Mendonca C, Andreozzi F, di Pietro N, et al. Association between a genetic variant related to glutamic acid metabolism and coronary heart disease in individuals with type 2 diabetes. JAMA. 2013;310(8):821–8.
Qiu M, Xiong W, Liao H, Li F. VEGF -634G>C polymorphism and diabetic retinopathy risk: a meta-analysis. Gene. 2013;518(2):310–5. Epub 2013/01/29
Rani PK, Raman R, Gupta A, Pal SS, Kulothungan V, Sharma T. Albuminuria and diabetic retinopathy in type 2 diabetes mellitus sankara nethralaya diabetic retinopathy epidemiology and molecular genetic study (SN-DREAMS, report 12). Diabetol Metab Syndr. 2011;3(1):9. Epub 2011/05/27
Replication DIG, Meta-analysis C, Asian Genetic Epidemiology Network Type 2 Diabetes C, South Asian Type 2 Diabetes C, Mexican American Type 2 Diabetes C, Type 2 Diabetes Genetic Exploration by Nex-generation sequencing in muylti-Ethnic Samples C, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014;46(3):234–44. Epub 2014/02/11
Reynisdottir I, Thorleifsson G, Benediktsson R, Sigurdsson G, Emilsson V, Einarsdottir AS, et al. Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34-q35.2. Am J Hum Genet. 2003;73(2):323–35. Epub 2003/07/10
Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86(4):1343–6.
Ritz E, Zeng XX, Rychlik I. Clinical manifestation and natural history of diabetic nephropathy. Contrib Nephrol. 2011;170:19–27. Epub 2011/06/11
Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. Genet Med. 2008;10(4):240–50.
Rosengren AH, et al. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science. 2010;327(5962):217–20.
Ruggenenti P, Remuzzi G. Nephropathy of type 1 and type 2 diabetes: diverse pathophysiology, same treatment? Nephrol Dial Transplant. 2000;15(12):1900–2. Epub 2000/11/30
Rung J, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet. 2009;41(10):1110–5.
Sackton TB, Hartl DL. Genotypic context and epistasis in individuals and populations. Cell. 2016;166(2):279–87. Epub 2016/07/16
Said G. Diabetic neuropathy – a review. Nat Clin Pract Neurol. 2007;3(6):331–40.
Salonen JT, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. Am J Hum Genet. 2007;81(2):338–45.
Sandholm N, Salem RM, McKnight AJ, Brennan EP, Forsblom C, Isakova T, et al. New susceptibility Loci associated with kidney disease in type 1 diabetes. PLoS Genet. 2012;8(9): e1002921. Epub 2012/10/03
Sandholm N, McKnight AJ, Salem RM, Brennan EP, Forsblom C, Harjutsalo V, et al. Chromosome 2q31.1 associates with ESRD in women with type 1 diabetes. J Am Soc Nephrol. 2013;24(10): 1537–43. Epub 2013/09/14
Sandhu MS, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet. 2007;39(8):951–3.
Sanjeevi CB, Lybrand TP, DeWeese C, Landin-Olsson M, Kockum I, Dahlquist G, et al. Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of IDDM. Members of the Swedish Childhood Diabetes Study. Diabetes. 1995;44(1):125–31. Epub 1995/01/01

Saxena R, et al. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes. 2006;55(10):2890–5.
Saxena R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316(5829):1331–6.
Saxena R, et al. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet. 2010;42(2):142–8.
Saxena R, et al. Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. Am J Hum Genet. 2012;90(3):410–25.
Saxena R, et al. Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. Diabetes. 2013;62(5):1746–55.
Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007;316(5829):1341–5.
Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet. 2012;44(9):991–1005.
Scott RA, et al. An expanded genome-wide association study of type 2 diabetes in Europeans. Diabetes. 2017;66(11):2888–902.
Segerstolpe A, Palasantza A, Eliasson P, Andersson EM, Andreasson AC, Sun X, et al. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metab. 2016;24(4):593–607.
Shaat N, Ekelund M, Lernmark A, Ivarsson S, Nilsson A, Perfekt R, et al. Genotypic and phenotypic differences between Arabian and Scandinavian women with gestational diabetes mellitus. Diabetologia. 2004;47(5):878–84.
Shepherd M, Shields B, Ellard S, Rubio-Cabezas O, Hattersley AT. A genetic diagnosis of HNF1A diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. Diabet Med. 2009;26(4):437–41. Epub 2009/04/25
Sheu WH, Kuo JZ, Lee IT, Hung YJ, Lee WJ, Tsai HY, et al. Genome-wide association study in a Chinese population with diabetic retinopathy. Hum Mol Genet. 2013;22(15):3165–73.
Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia. 2010;53(12):2504–8. Epub 2010/05/26
Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, et al. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet. 2010;6(9):e1001127.
SIGMA Type 2 Diabetes Consortium, et al. Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature. 2014a;506(7486):97–101.
SIGMA Type 2 Diabetes Consortium, et al. Association of a low-frequency variant in HNF1A with type 2 diabetes in a Latino population. JAMA. 2014b;311(22):2305–14.
Silverman BL, Rizzo T, Green OC, Cho NH, Winter RJ, Ogata ES, et al. Long-term prospective evaluation of offspring of diabetic mothers. Diabetes. 1991;40(Suppl 2):121–5.
Sim X, et al. Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. PLoS Genet. 2011;7(4):e1001363.
Singal DP, Blajchman MA. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes. 1973;22(6):429–32. Epub 1973/06/01
Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445(7130):881–5.
Small KS, Hedman AK, Grundberg E, Nica AC, Thorleifsson G, Kong A, et al. Identification of an imprinted master trans regulator at the KLF14 locus related to multiple metabolic phenotypes. Nat Genet. 2011;43(6):561–4. Epub 2011/05/17
Smyth DJ, Cooper JD, Howson JM, Walker NM, Plagnol V, Stevens H, et al. PTPN22 Trp620 explains the association of chromosome 1p13 with type 1 diabetes and shows a statistical interaction with HLA class II genotypes. Diabetes. 2008;57(6):1730–7. Epub 2008/02/29

Sonestedt E, Lyssenko V, Ericson U, Gullberg B, Wirfalt E, Groop L, et al. Genetic variation in the glucose-dependent insulinotropic polypeptide receptor modifies the association between carbohydrate and fat intake and risk of type 2 diabetes in the Malmo Diet and Cancer cohort. J Clin Endocrinol Metab. 2012;97(5):E810–8.

Steinke JM, Sinaiko AR, Kramer MS, Suissa S, Chavers BM, Mauer M, et al. The early natural history of nephropathy in Type 1 Diabetes: III. Predictors of 5-year urinary albumin excretion rate patterns in initially normoalbuminuric patients. Diabetes. 2005;54(7):2164–71. Epub 2005/06/29

Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet. 2007;39(6):770–5.

Steinthorsdottir V, Thorleifsson G, Sulem P, Helgason H, Grarup N, Sigurdsson A, et al. Identification of low-frequency and rare sequence variants associated with elevated or reduced risk of type 2 diabetes. Nat Genet. 2014;46(3):294–8.

Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet. 1997;15:106–10.

Stoy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci U S A. 2007;104(38):15040–4.

Strawbridge RJ, et al. Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. Diabetes. 2011;60(10):2624–34.

Tabassum R, et al. Genome-wide association study for type 2 diabetes in Indians identifies a new susceptibility locus at 2q21. Diabetes. 2013;62(3):977–86.

Takeuchi F, et al. Confirmation of multiple risk Loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. Diabetes. 2009;58(7):1690–9.

Taneera J, Fadista J, Ahlqvist E, Atac D, Ottosson-Laakso E, Wollheim CB, et al. Identification of novel genes for glucose metabolism based upon expression pattern in human islets and effect on insulin secretion and glycemia. Hum Mol Genet. 2015;24(7):1945–55. Epub 2014/12/10

Tattersall RB. Mild familial diabetes with dominant inheritance. Q J Med. 1974;43(170):339–57.

Temple IK, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, et al. An imprinted gene(s) for diabetes? Nat Genet. 1995;9(2):110–2.

Temple IK, Gardner RJ, Robinson DO, Kibirige MS, Ferguson AW, Baum JD, et al. Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22-q23. Hum Mol Genet. 1996;5(8):1117–21.

Thamotharampillai K, Chan AK, Bennetts B, Craig ME, Cusumano J, Silink M, et al. Decline in neurophysiological function after 7 years in an adolescent diabetic cohort and the role of aldose reductase gene polymorphisms. Diabetes Care. 2006;29(9):2053–7.

Thorleifsson G, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet. 2009;41(1):18–24.

Timpson NJ, et al. Adiposity-related heterogeneity in patterns of type 2 diabetes susceptibility observed in genome-wide association data. Diabetes. 2009;58(2):505–10.

Tong Z, Yang Z, Patel S, Chen H, Gibbs D, Yang X, et al. Promoter polymorphism of the erythropoietin gene in severe diabetic eye and kidney complications. Proc Natl Acad Sci U S A. 2008;105(19):6998–7003. Epub 2008/05/07

Tong Y, Lin Y, Zhang Y, Yang J, Liu H, Zhang B. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large Human Genome Epidemiology (HuGE) review and meta-analysis. BMC Med Genet. 2009;10:15. Epub 2009/02/21

Toperoff G, Aran D, Kark JD, Rosenberg M, Dubnikov T, Nissan B, et al. Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum Mol Genet. 2012;21(2):371–83. Epub 2011/10/14

Travers ME, Mackay DJ, Dekker Nitert M, Morris AP, Lindgren CM, Berry A, et al. Insights into the molecular mechanism for type 2 diabetes susceptibility at the KCNQ1 locus from temporal changes in imprinting status in human islets. Diabetes. 2013;62(3):987–92. Epub 2012/11/10

Tsai FJ, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet. 2010;6(2):e1000847.
Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes. 1993a;42(2):359–62. Epub 1993/02/01
Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes. 1993b;42(2):359–62.
Unoki H, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet. 2008;40(9):1098–102.
van den Ouweland JM, Lemkes HH, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PA, et al. Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet. 1992;1(5):368–71.
Visscher PM, Hill WG, Wray NR. Heritability in the genomics era–concepts and misconceptions. Nat Rev Genet. 2008;9(4):255–66. Epub 2008/03/06
Viswanath K, McGavin DD. Diabetic retinopathy: clinical findings and management. Community Eye Health/Int Centre Eye Health. 2003;16(46):21–4.
Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010;42(7):579–89.
Volkov P, Bacos K, Ofori JK, Esguerra JL, Eliasson L, Ronn T, et al. Whole-genome bisulfite sequencing of human pancreatic islets reveals novel differentially methylated regions in type 2 diabetes pathogenesis. Diabetes. 2017;66(4):1074–85.
von Muhlendahl KE, Herkenhoff H. Long-term course of neonatal diabetes. N Engl J Med. 1995;333(11):704–8.
Wang F, Fang Q, Yu N, Zhao D, Zhang Y, Wang J, et al. Association between genetic polymorphism of the angiotensin-converting enzyme and diabetic nephropathy: a meta-analysis comprising 26,580 subjects. J Renin-Angiotensin-Aldosterone Syst. 2012;13(1):161–74. Epub 2011/08/04
Wang YJ, Schug J, Won KJ, Liu C, Naji A, Avrahami D, et al. Single-cell transcriptomics of the human endocrine pancreas. Diabetes. 2016;65(10):3028–38.
Weedon MN, et al. Meta-analysis and a large association study confirm a role for calpain-10 variation in type 2 diabetes susceptibility. Am J Hum Genet. 2003;73(5):1208–12.
Wei WH, Hemani G, Haley CS. Detecting epistasis in human complex traits. Nat Rev Genet. 2014;15(11):722–33. Epub 2014/09/10
Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–78.
Wellcome Trust Case Control C, Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, et al. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. Nature. 2010;464(7289):713–20.
White AJ, Sandler DP, Bolick SC, Xu Z, Taylor JA, DeRoo LA. Recreational and household physical activity at different time points and DNA global methylation. Eur J Cancer. 2013;49(9):2199–206.
WHO. WHO report. 2014. http://www.who.int/gho/publications/world_health_statistics/en/.
Willer CJ, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008;40(2):161–9.
Williams MA, Qiu C, Dempsey JC, Luthy DA. Familial aggregation of type 2 diabetes and chronic hypertension in women with gestational diabetes mellitus. J Reprod Med. 2003;48(12):955–62.
Williams WW, Salem RM, McKnight AJ, Sandholm N, Forsblom C, Taylor A, et al. Association testing of previously reported variants in a large case-control meta-analysis of diabetic nephropathy. Diabetes. 2012;61(8):2187–94. Epub 2012/06/23
Winckler W, et al. Association of common variation in the HNF1alpha gene region with risk of type 2 diabetes. Diabetes. 2005a;54(8):2336–42.

Winckler W, et al. Association testing of variants in the hepatocyte nuclear factor 4alpha gene with risk of type 2 diabetes in 7,883 people. Diabetes. 2005b;54(3):886–92.
Wolf JB, Hager R. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. PLoS Biol. 2006;4(12):e380.
Writing Team for the Diabetes C, Complications Trial/Epidemiology of Diabetes I, Complications Research G. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA. 2002;287(19):2563–9. Epub 2002/05/22
Writing Team for the Diabetes C, Complications Trial/Epidemiology of Diabetes I, Complications Research G. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA. 2003;290(16):2159–67. Epub 2003/10/23
Wu P, Farrell WE, Haworth KE, Emes RD, Kitchen MO, Glossop JR, et al. Maternal genome-wide DNA methylation profiling in gestational diabetes shows distinctive disease-associated changes relative to matched healthy pregnancies. Epigenetics. 2018;13(2):122–128. https://doi.org/10.1080/15592294.2016.1166321.
Xin Y, Kim J, Okamoto H, Ni M, Wei Y, Adler C, et al. RNA sequencing of single human islet cells reveals type 2 diabetes genes. Cell Metab. 2016;24(4):608–15.
Yamauchi T, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet. 2010;42(10):864–8.
Yasuda K, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet. 2008;40(9):1092–7.
Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35(3):556–64. Epub 2012/02/04
Young BC, Ecker JL. Fetal macrosomia and shoulder dystocia in women with gestational diabetes: risks amenable to treatment? Curr Diab Rep. 2013;13(1):12–8.
Young AI, Wauthier F, Donnelly P. Multiple novel gene-by-environment interactions modify the effect of FTO variants on body mass index. Nat Commun. 2016;7:12724.
Zeggini E, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007;316(5829):1336–41.
Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. 2008;40(5):638–45.
Zhao T, Zhao J. Association between the -634C/G polymorphisms of the vascular endothelial growth factor and retinopathy in type 2 diabetes: a meta-analysis. Diabetes Res Clin Pract. 2010;90(1):45–53. Epub 2010/07/02
Zheng Y, Wang Z, Zhou Z. miRNAs: novel regulators of autoimmunity-mediated pancreatic beta-cell destruction in type 1 diabetes. Cell Mol Immunol. 2017;14(6):488–96.
Zhernakova A, van Diemen CC, Wijmenga C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. Nat Rev Genet. 2009;10(1):43–55. Epub 2008/12/19
Zullo A, Sommese L, Nicoletti G, Donatelli F, Mancini FP, Napoli C. Epigenetics and type 1 diabetes: mechanisms and translational applications. Transl Res. 2017;185:85–93. Epub 2017/05/30

Pathogenesis of Type 1 Diabetes

6

Alberto Pugliese

Contents

Abstract

Type 1 diabetes (T1D) is considered a multifactorial, chronic, autoimmune disease in which autoreactive T-lymphocytes cause severe loss of pancreatic beta cells. Much progress has been done in the discovery of disease-predisposing genes, the identification of islet cell autoantigens, and key features of islet autoimmune responses. There is growing evidence for contributing

A. Pugliese (✉)
Department of Medicine, Division of Diabetes, Endocrinology and Metabolism, Department of Microbiology and Immunology, Diabetes Research Institute, Leonard Miller School of Medicine, University of Miami, Miami, FL, USA
e-mail: apuglies@med.miami.edu

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_7

environmental factors, including viruses, the microbiome, and dietary factors. Recent studies suggest that autoimmune-mediated beta cell destruction is likely the key pathogenic mechanism, but over a prolonged period of time, extending beyond clinical diagnosis. It is becoming evident that chronic beta cell inflammation is also a key component of the disease pathogenesis. Moreover, beta cell dysfunction is likely to precede and coexist with beta cell destruction, and it is emerging as a significant contributor to the onset of symptomatic diabetes. Thus, chronic islet inflammation and beta cell dysfunction should be considered critical therapeutic targets, together with improved immunoregulation. Growing evidence that beta cell destruction at diagnosis may only be partial in many patients is raising questions about the dynamics of the autoimmune process; the persistence of beta cells, insulin secretion, and disease activity for years after diagnosis point at the chronicity of T1D and suggest that therapeutic intervention to halt the disease process may be possible beyond the traditional, but arbitrary, immediate postdiagnosis period.

Keywords
Autoimmunity · Virus · Inflammation · Insulin resistance · Beta cell · Dysfunction · HLA · Insulin · Type 1 diabetes

T1D Is a Multifactorial, Chronic, Heterogeneous Disease

T1D is a multifactorial disease commonly diagnosed in children and adolescents, but it develops in adult age as well (Maahs et al. 2010). Both a genetic predisposing background, inclusive of multiple gene variants, and exposure to environmental factors may promote the development of chronic autoimmune responses to multiple autoantigens expressed by pancreatic beta cells. Over time, chronic islet autoimmunity leads to severe beta cell loss and in turn, severe insulin deficiency (Eisenbarth 1986).

Gepts provided an initial description of the T1D pancreas more than 60 years ago (Gepts 1965), but the study of the pancreas pathology in T1D has been limited by scarce access to tissue from patients. In the mid-1980s, studies in the UK examined autopsy specimens obtained from patients deceased near the time of diagnosis (Bottazzo et al. 1985; Foulis and Farquharson 1986; Foulis et al. 1986). In 2007 the JDRF supported the launch of the Network for the Pancreatic Organ Donor with Diabetes (nPOD), to recover pancreas and other tissues from organ donors with T1D (Pugliese et al. 2014) across the natural history of the disease, thus covering the preclinical stages and a broad range of disease duration after diagnosis (Campbell-Thompson et al. 2016). Access to the pancreas of living patients through biopsy has been rarely performed and is limited by safety and ethical considerations (Atkinson 2014), but important information has been learned from biopsies performed in Japan (Imagawa et al. 2001) and in Norway by the DiViD Study (Krogvold et al. 2014).

There is much heterogeneity in the disease progression, clinical manifestations, and severity at diagnosis, as well as in the clinical course after diagnosis (Pugliese

2013). Recent findings emphasize the chronic nature of the disease and its pathogenic mechanisms, with genetic factors and environmental factors modulating disease mechanisms: while islet autoimmunity has been considered the sole driver of beta cell destruction, there is growing evidence that chronic islet inflammation and beta cell dysfunction are critical components of the disease pathogenesis. Critical to a more comprehensive understanding is the recognition that various disease mechanisms are chronic, most often coexist, and yet their activity and severity is heterogeneous, with beta cell destruction proceeding asynchronously throughout the pancreas over a prolonged period of time. It remains unclear to what extent beta cell destruction is a process that proceeds chronically and progressively or whether it has a relapsing-remitting course as many other autoimmune diseases (Atkinson et al. 2014).

It is key to consider that multiple disease mechanisms are operative, not just autoimmunity. Indeed, it is becoming evident that beta cell dysfunction is a critical component of a disease process that over time evolves towards severe beta cell loss. The ability to distinguish the relative contributions of each at various disease stages is quite limited, but the current knowledge support a model in which dysfunction would co-exist with destruction early on, with beta cell destruction mediated by chronic autoimmunity becoming more prominent over time. This chapter integrates the current knowledge about pancreas pathology, genetic factors, environmental exposures, and disease mechanisms, into a comprehensive view of the disease pathogenesis. Figure 1 presents an integrated view of the natural history and pathogenesis of T1D.

Genetic Predisposition

While most patients with T1D lack a family history for the disease when diagnosed, first-degree relatives have higher probability of developing disease than the general population. The frequency of T1D among siblings of affected individuals is on average 6% compared to about 0.4% in the US Caucasian population. Thus, the disease is about 15 times more likely in siblings of individuals with T1D than in the general population. The offspring of affected individuals also have higher T1D risk, approximately 3–6%; however, the offspring of affected mothers tend to have lower risk than the offspring of affected fathers (Warram et al. 1984; Tuomilehto et al. 1995; Harjutsalo et al. 2006). The degree of allele sharing or genetic identity with the proband also correlates with disease risk (Aly et al. 2006). Disease concordance rates in monozygotic twins (100% shared genes) are reported at around 30–50% (Redondo et al. 2001, 2008), but higher rates were observed with prolonged follow-up: autoantibody-positive monozygotic twins have an estimated 89% risk of developing T1D within 16 years from the first autoantibody-positive test (Redondo et al. 2008). To further highlight the importance of genetic identity, disease concordance rates in dizygotic twins (50% sharing) are much lower, at around 10% (Redondo et al. 2004).

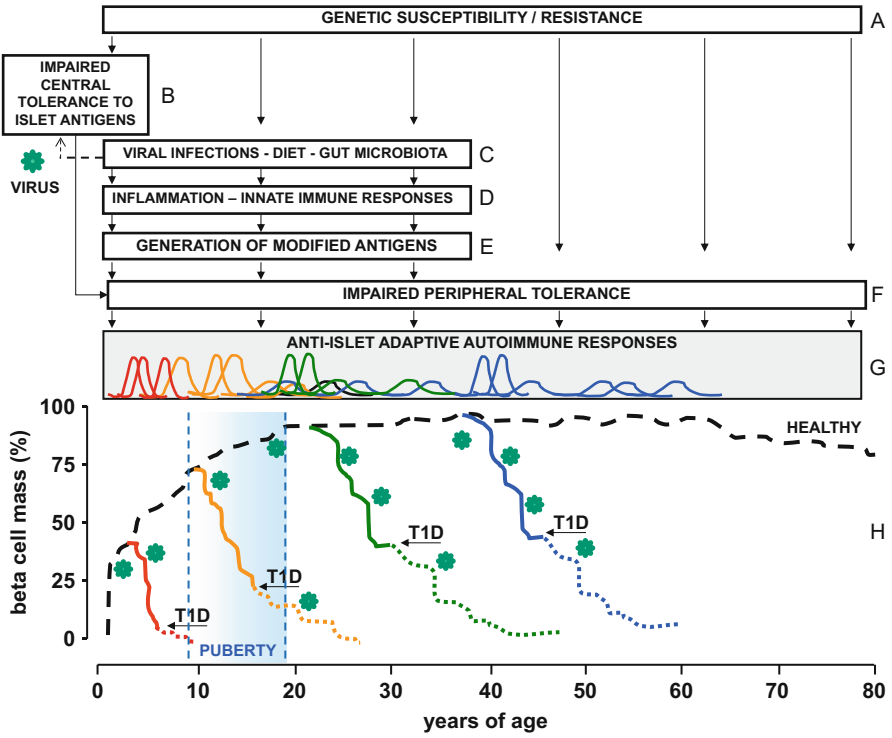


Fig. 1 A model for the natural history and pathogenesis of type 1 diabetes. The figure illustrates a theoretical model for T1D natural history and pathogenesis: (**A**) Genetic factors influence multiple pathways throughout the life of the individual, albeit selected genetic influences may be more active at certain time points. Genetic factors confer both susceptibility and resistance to T1D. (**B**) Incomplete central tolerance for islet autoantigens is the earliest abnormality, setting the stage by generating a T cell repertoire that contains one or more autoreactive T cells, which could be triggered later in life. Hypothetically, neonatal viral infections could infect the thymus and further impair thymic selection processes. (**C**, **D**) Viral infections are believed to infect beta cells, and multiple infection and/or chronic infections may chronically stimulate autoimmunity. The gut microbiota, which is linked to diet and altered gut permeability, can promote inflammation and enhance innate immune responses with potentially deleterious effects on immune regulation and increased likelihood of triggering adaptive immune responses. Dysregulation of innate immunity can also favor infections and inflammation. (**E**) Beta cell inflammation may facilitate the formation of posttranslationally modified antigens hybrid peptides, which may be major driver of islet autoimmunity, since there may not be proper tolerance for such epitopes. (**F**) The triggering of autoreactive T cells that escaped thymic selection or to modified epitopes is facilitated by impaired peripheral immune regulation, which is in part genetically determined. Peripheral tolerance may be further impaired by environmental exposures. In a vicious circle, defects in peripheral tolerance may help sustaining inflammation and enhance innate immune responses. (**G**) Autoimmunity may have a remitting/relapsing course and may persist beyond the time of diagnosis. The severity and dynamics of adaptive T cell responses may be heterogeneous, perhaps more aggressive in younger subjects, perhaps in relation to beta cell mass. (**H**) Approximate representation of beta cell mass throughout life, and plots beta cell loss beginning at different ages. The solid lines are based on the model proposed by Tsai et al. (2006), and the dotted lines plot the hypothetical loss after diagnosis, marked by arrows. The amount of beta cell mass may determine time to diabetes development; it is

Overall, gene variants explain ~80% of T1D inheritability; there is growing evidence that epigenetic changes (Javierre et al. 2011; Rakyan et al. 2011; Miao et al. 2012; Belot et al. 2013; Stankov et al. 2013; Cepek et al. 2016; Elboudwarej et al. 2016; Rui et al. 2016; Wu et al. 2016), as well as regulation of gene expression by microRNAs and long-noncoding RNAs (Guay et al. 2012; Floyel et al. 2015; Osmai et al. 2016; Seyhan et al. 2016), also contributes to modulate T1D risk. Importantly, these may be mechanisms of genetic regulation that evolve over time and may affect the function of genes that control immune function and/or insulin secretion and survival of beta cells.

The inheritability of T1D does not fit Mendelian patterns. The best model to explain susceptibility involves a single, major locus, together with several other genes conferring additional smaller effects on risk (Rich 1990). The results of large efforts conducted over the last two decades to map genetic susceptibility are consistent with this model (Barrett et al. 2009; Rich et al. 2009; Pociot et al. 2010; Cooper et al. 2012; Onengut-Gumuscu et al. 2015).

The primary genetic locus is located in the HLA (Human Leukocyte Antigen) complex. The HLA region confers 50–60% of the overall genetic risk (Noble et al. 2010) from inherited alleles. Within the HLA complex, loci more strongly linked to T1D are those coding for the HLA class II (HLA-DR, HLA-DQ) and class I (HLA-A, HLA-B) histocompatibility antigens. These molecules present vesicular peptide antigens to CD4 (class II) T-lymphocytes or cytosolic peptide antigens to CD8 (class I) T-lymphocytes, respectively. This presentation is important both in the context of thymic selection, relevant to the maturation of the adaptive immune system, and later during the activation of the immune response. This genetic system is highly polymorphic; genetic variation affects the coding sequences and in turn the structure and function of the pockets in which peptide antigens are bound and displayed for presentation to the T-lymphocytes. Selected HLA class I alleles contribute to the genetic risk for T1D, especially HLA-A2, HLA-A24, and HLA-B39 (Nejentsev et al. 2007; Noble et al. 2010). However, stronger predisposition comes from the class II alleles HLA-DRB1*03:01 (DR3), DQA1*05:01-DQB1*02:01 (DQ2) and HLA-DRB1*04 (DR4), DQA1*03:01-DQB1*03:02 (DQ8). Among T1D patients, approximately 80–90% carry at least one of these high-risk haplotypes and 30–50% carry both (Erlich et al. 2008); the heterozygous genotype confers the strongest risk for T1D. It is believed that this higher risk may be explained by the formation of a trans-complementing HLA-DQ heterodimer that present epitopes from islet autoantigens (van Lummel et al. 2012). HLA-DR4 subtypes (e.g., DRB1*0401, DRB1*0404) vary in their association with T1D, even when in linkage with the high-risk DQA1*0301-DQB1*0302; the HLA-DRB1 chain is also key for antigen presentation and different variants may differ in their ability to present islet

Fig. 1 (continued) unclear whether rates of progression are affected by age. At least in a proportion of patients, especially those with obesity, insulin resistance can be further amplified during puberty and represents a contributing factor to disease progression, possibly by increasing functional demands on pancreatic beta cells. (Modified from Pugliese 2013)

self-antigens (Erlich et al. 2008). These classical HLA associations have been shifting in recent decades; several populations show declining proportions of patients carrying the heterozygous HLA-DR3/DR4 genotype (except young children) and an increase among patients of HLA types linked to moderate risk (Hermann et al. 2003; Gillespie et al. 2004; Fourlanos et al. 2008; Resic-Lindehammer et al. 2008; Vehik et al. 2008). The HLA-DRB1*15:01 (DR2), DQA1*01:02-DQB1*06:02 haplotype affords strong protection from T1D (Baisch et al. 1990); protection is also reported in relatives with autoantibodies who carry this haplotype (Pugliese et al. 1995, 2016). Typically these relatives show more limited autoimmune responses, mostly expressing a single autoantibody and predominantly against the GAD65 autoantigen.

Over 50 risk loci have been identified throughout the genome and most confer much smaller proportions of risk (Pociot et al. 2010; Cooper et al. 2012). For most non-HLA risk loci, genetic variation occurs in noncoding regions, especially regulatory regions, for example, lymphoid gene enhancers (Onengut-Gumuscu et al. 2015). Regulatory variants modulate insulin gene (INS) transcription in the thymus (Pugliese et al. 1997), with implications for immunological self-tolerance to insulin, a key autoantigen (Sosinowski and Eisenbarth 2013). Many non-HLA T1D-associated gene variants modulate key aspects of immune regulation and peripheral immune tolerance; for example, IL2RA (Interleukin-2 receptor subunit alpha) and PTPN2 (protein tyrosine phosphatase, non-receptor type 2) gene variants modulate regulatory T-lymphocytes (Long et al. 2010, 2011).

There is growing evidence that both HLA and many non-HLA risk genes are active not just in immune cells but also in pancreatic beta cells (Floyel et al. 2015), where they influence key aspects of endocrine function and responses to innate stimuli (Zipris 2011), inflammation, and other stressors and may ultimately favor dysfunction and apoptosis of beta cells (Santin et al. 2011; Eizirik et al. 2012; Marroqui et al. 2014). A few T1D risk genes are also associated with type 2 diabetes, such as GLIS3 (GLIS family zinc finger 3) and TCF7L2 (transcription factor 7 like 2) (Barrett et al. 2009; Redondo et al. 2014). The association with the IFIH1 (interferon induced with helicase C domain 1), TYK2 (tyrosine kinase 2), and PTPN2 genes also supports a role for viral infections of beta cells in T1D pathogenesis (Colli et al. 2010; Marroqui et al. 2015). Figure 2 illustrates key mechanisms of action of selected T1D risk genes. Prospective follow-up studies of at-risk relatives reveal genetic influences on the risk of triggering islet autoimmunity and disease progression (Steck et al. 2012; Torn et al. 2015), and assessment of genetic risk is being exploited for risk stratification and prevention strategies (Giannopoulou et al. 2015; Insel et al. 2015).

Insulitis

The pathological hallmark of T1D has long been considered the lymphocytic infiltration of the pancreatic islets (Pugliese 2016). In this lesion, termed insulitis, immune, and inflammatory cells are detected within and around the islets (In't Veld

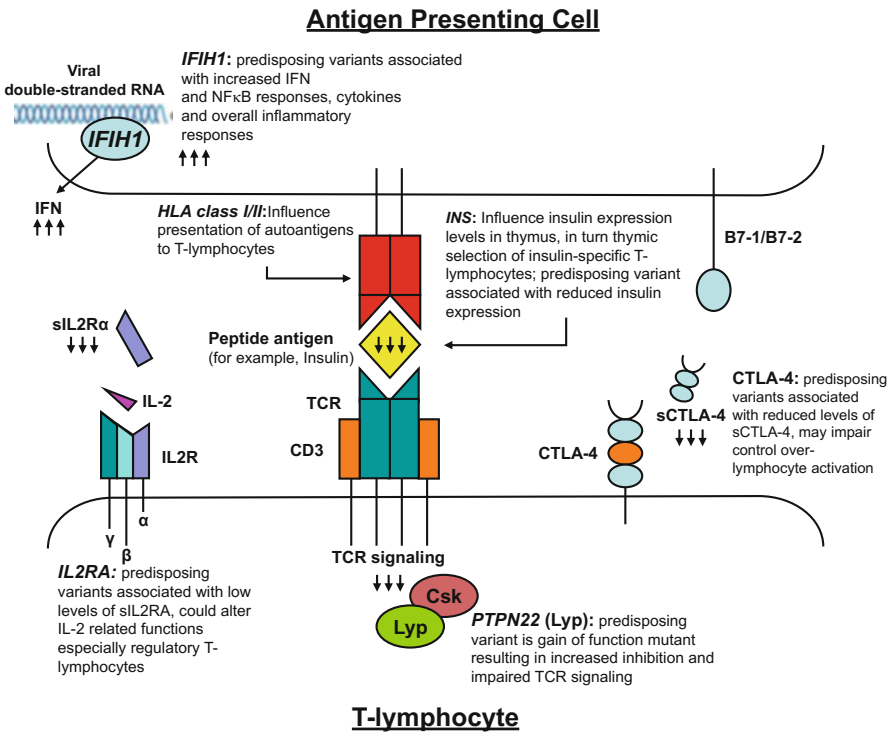


Fig. 2 Key molecular pathways modulated by selected T1D susceptibility loci. The figure depicts the interface between the antigen presenting cell (APC) and the T-lymphocyte, and the molecules that are influenced by selected genetic polymorphisms linked to T1D susceptibility. HLA molecules influence the presentation of peptide antigens to T-lymphocytes. Importantly, especially for class I antigens, this may also take place in inflamed islet cells that hyper-express HLA class I molecules and may present viral and/or self-peptides. In the case of insulin, polymorphisms at the insulin gene (INS) locus play a critical role in regulating levels of insulin expression in the thymus and in turn thymic selection processes that establish self-tolerance. CTLA-4, PTPN22, and IL2RA influence critical pathways of T cell activation, function, and regulation. Through its effects on TCR signaling, PTPN22 may also influence the selection of autoreactive T cells during thymic selection and may have synergistic effects with HLA and INS. Polymorphisms in the CTLA-4 and IL2RA genes are associated with reduced levels of the soluble forms of CTLA-4 and IL2RA. CTLA-4 and IL2RA polymorphisms may impair regulation of T-lymphocyte responses and the function of regulatory T-lymphocytes. The IFIH1 locus links innate responses to viruses to inflammation and possibly the triggering of adaptive responses

2011a); insulitis is considered the manifestation of the autoimmune attack against beta cells. It is typically observed in insulin-positive islets and the infiltrates clear out after beta cells have been destroyed (Fig. 3). The key elements of the 2013 consensus definition of the insulitis lesion (Campbell-Thompson et al. 2013) include the presence of a predominantly lymphocytic infiltration of at minimum three pancreatic islets, consisting of at least 15 CD45+ cells/islet, and the presence of insulin-deficient (pseudo-atrophic) islets.

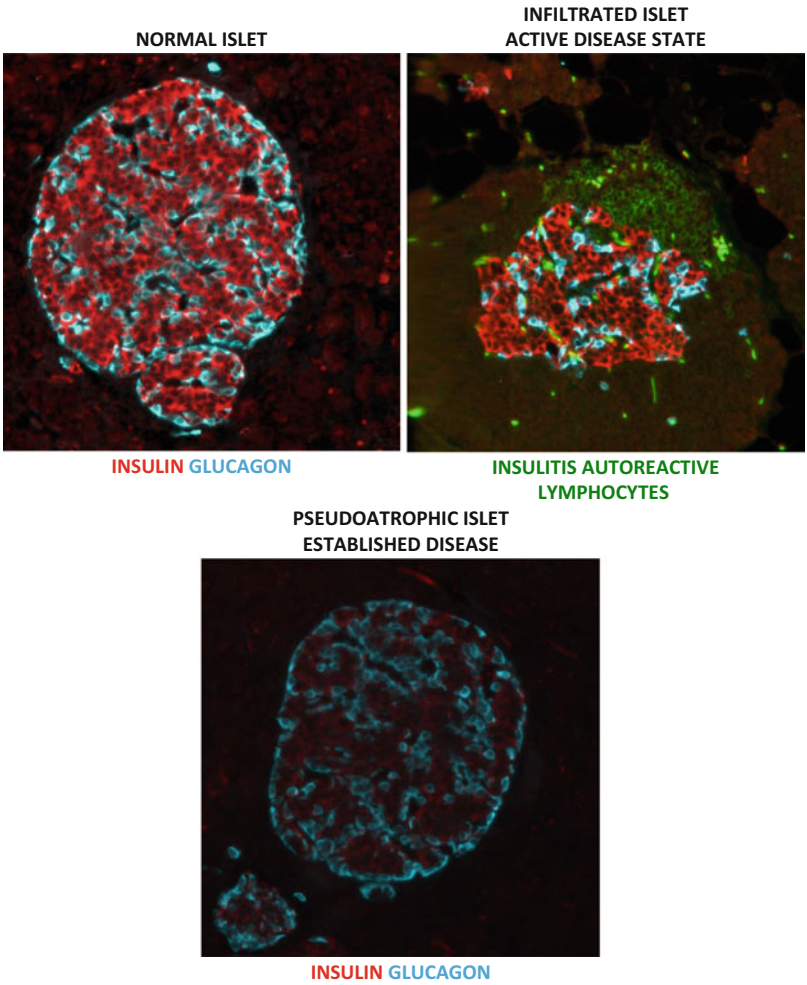


Fig. 3 Insulitis. The left panel shows a normal islet, which can be contrasted with an islet affected by insulitis in the center panel. The lesion has been considered to be more commonly present during the prediabetic phase and around the time of onset, but recent evidence suggests that it may be more chronic and observed even at later stages, as the lesion is asynchronous throughout the pancreas and affecting a limited proportion of islets at any given time. The right panel shows an islet that has lost much of its insulin staining, representing beta cell destruction, the final outcome of the disease process

It is critical to recognize that insulitis in the human pancreas with T1D is much less severe than in rodent experimental models of autoimmune diabetes, such as the nonobese diabetic (NOD) mouse (Anderson and Bluestone 2005); it more frequently observed in the islet periphery, and thus termed peri-insulitis, and it often exhibits focal aggregation at one pole of the islet; insulitis in the human pancreas less frequently shows presence of lymphocytes within the islet (intrainsulitis) (Reddy et al. 2015; Campbell-Thompson et al. 2016; Krogvold et al. 2016).

Insulitis is considered the pathognomonic pathological manifestation of T1D, yet its frequency in the human pancreas with T1D is low, reportedly affecting 10–30% of the islets at any given time, even at diagnosis. Insulitis is more prevalent in younger patients and in those whose pancreas was examined shortly after clinical diagnosis; approximately 30% of insulin-positive islets are infiltrated in recently diagnosed, young patients (In't Veld 2011a). In the DiViD Study, biopsies were performed in 6 recently diagnosed patients, aged 24–25 years: the proportions of islets with insulitis ranged widely between 5% and 58%. Importantly, only 1 of the DiViD Study patients had insulitis in more than 50% of the islets examined (Krogvold et al. 2014, 2016); on average, only 11% of the islets examined in the DiViD Study had insulitis in the biopsies obtained shortly after the time of diagnosis. Among 80 nPOD organ donors with a wide range of disease duration, insulitis was observed in 17 donors: the disease duration for these donors ranged from diagnosis up to 12 years after onset, and the age of these donors at diagnosis ranged between 4 and 28 years (Campbell-Thompson et al. 2016). In this study, the frequency of insulitis had limited inverse correlation with diabetes duration and no correlation with age at onset. Insulitis predominantly affected insulin-positive islets (33% compared to 2% of insulin-negative islets), suggesting that the presence of beta cells may be a driver for infiltrating T-lymphocytes. Both insulitis and residual beta cells were observed in many patients even many years after diagnosis, highlighting the chronic nature of the disease process in both children and young adults (Campbell-Thompson et al. 2016). All studies concur that insulitis does not affect all islets at the same time, suggesting that this is a process that evolves over time. The patchy distribution of the insulitis resembles that of vitiligo (Eisenbarth 2010).

Cellular composition of the insulitis. Studies of the cellular composition of the infiltrating cells in the insulitis lesion reveal heterogeneous profiles, which may influence disease severity and progression. Cytotoxic CD8 T-lymphocytes are the predominant T-lymphocyte type in the insulitis; CD4 T-lymphocytes are also observed but in lower proportions. B-lymphocytes are also present. B-lymphocytes are emerging as important in the pathogenesis of T1D, as suggested both by experimental studies (O'Neill et al. 2009) and by the preservation of insulin secretion reported in newly diagnosed patients treated with a B-lymphocyte depleting agent, a treatment that was most effective in children (Pescovitz et al. 2009). Additional evidence for a role of B-lymphocytes comes from studies of insulin-specific B-lymphocytes, which show changes during disease progression (Smith et al. 2015); a loss of insulin-specific, anergic B-lymphocytes during the prediabetic period has been linked to the activation of these cells.

The analysis of autopsy samples from UK patients with recent-onset T1D, the nPOD cohort, and the DiViD biopsy specimens concurs in defining two patterns of insulitis CD20 high and CD20-low) according to the prevalence of CD20-positive B-lymphocytes (Arif et al. 2014; Leete et al. 2016); the CD20-high insulitis was linked with early age of diagnosis (7 years), and patients diagnosed after age 13 had insulitis with low prevalence of B-lymphocytes. These findings suggest heterogeneity in the contribution of B-lymphocytes to disease pathogenesis and that higher proportions of B-lymphocytes in the insulitis lesion may possibly reflect an earlier

triggering of islet autoimmunity and/or a more aggressive disease. This may also explain the more pronounced therapeutic effect of B-lymphocyte depletion observed in children compared to older patients (Pescovitz et al. 2009).

Recent reports also identify the presence of immune cells, especially CD8 T-cells (Rodriguez-Calvo et al. 2014) and neutrophils (Valle et al. 2013), in the exocrine pancreas of donors with T1D. The significance of these findings and the function of these cells are still unclear; together with suggestive evidence that the exocrine pancreas may be reduced in size not just after (Williams et al. 2012) but also before diagnosis (Campbell-Thompson et al. 2012), it is being proposed that the exocrine pancreas may also be affected during the development of T1D (Campbell-Thompson et al. 2015).

Abnormalities of Extracellular Matrix Components

Extensive studies of nPOD pancreata identified additional important features of T1D pathology in the pancreas. We highlight here the emerging role of extracellular matrix components (Bogdani et al. 2014b), as these may influence the progression of insulitis and beta cell survival. Deposition of pro-inflammatory hyaluronan and hyaluronan binding proteins is reported around islet cells and infiltrating lymphocytes in insulitic islets (Bogdani et al. 2014a); it is also observed in the spleen and pancreatic lymph nodes from T1D organ donors. These molecules promote lymphocyte adhesion and migration, and interfering with hyaluronan deposition prevents autoimmune diabetes in mice (Nagy et al. 2015; Bogdani 2016; Kuipers et al. 2016). Cathepsins were described in the insulitis lesion near areas of disruption of the peri-islet basement membrane, suggesting that these enzymes favor lymphocyte penetration of the islets by degrading the peri-islet membrane (Korpos et al. 2013). Additionally, loss of heparan sulfate is being reported in the T1D pancreas; heparan sulfate is detected in beta and not alpha cells, and its loss reduces the viability of islet cells (Ziolkowski et al. 2012; Simeonovic et al. 2013). Thus, loss of heparan sulfate may contribute to beta cell loss.

Autoantigens, Humoral, and Cellular Autoimmune Responses

Several autoantigens have been identified during the last few decades. These include insulin itself, the 65 kDa isoform of glutamic acid decarboxylase (GAD65), the tyrosine phosphatase-like protein insulinoma-associated antigen 2 (IA-2), the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), the cation efflux transporter ZnT8 (Roep and Peakman 2012), and more recently peripherin (Doran et al. 2016). With the development of specific assays, both autoantibody and T-lymphocyte responses can be shown in most patients around the time of disease onset. Autoantibodies to insulin, GAD65, IA-2, and ZnT8 are considered diagnostic at disease onset and predict future disease development in longitudinal studies of

relatives, with multiple autoantibodies conferring much higher risk than a single autoantibody (Verge et al. 1996; Vehik et al. 2011; Ziegler et al. 2013).

Much progress has been made in the identification of epitopes targeted by autoreactive CD4 and CD8 T-lymphocytes. Assays to study such cells have improved steadily over the years. Given the rarity of autoreactive T-lymphocytes in the circulation, some assays are based on in vitro stimulation with autoantigen or peptides to induce proliferation and measure production of cytokines (Schloot et al. 2003; Martinuzzi et al. 2011); the development of HLA class II and class I chimeric molecules (tetramers, multimers) loaded with antigenic peptides allows studying antigen-specific T-lymphocytes directly ex vivo, so that their functional and phenotypic characteristics can be examined without the changes induced by culture stimulation (Velthuis et al. 2010; James et al. 2011). The multiplicity of autoantigens and epitopes targeted reflects the heterogeneity of the disease process as well as disease progression; as autoimmunity progresses, epitope spreading leads to the targeting of multiple targets (Brooks-Worrell et al. 2001).

In addition, there is growing appreciation that several autoantigenic epitopes are subject to posttranslational modification, which increases their antigenicity (Mannering et al. 2005; McGinty et al. 2014, 2015; Nguyen and James 2016). Recent studies have further shown that autoreactive T-lymphocytes can target hybrid peptides resulting from the fusion of two proteins, for example, insulin and chromogranin (Delong et al. 2016). These observations are of fundamental importance: posttranslational modification of peptides and the formation of hybrid peptides are processes that generate new target epitopes towards which central tolerance may not be possible to be achieved. Future studies of these posttranslationally modified and hybrid peptides may show them to be critical autoantigens for disease development.

Studies of autoreactive T-lymphocytes have been largely limited to the analysis of peripheral blood samples, which do not prove that the same response is or was active in the pancreas. Direct analysis of autoreactive lymphocytes at critical sites of the autoimmune response has been traditionally limited by sporadic access to the pancreas or the pancreatic lymph nodes from patients. Studies that validate the role of purified antigen-specific T-lymphocytes in T1D includes the detection of insulin or GAD-specific CD4 T-lymphocytes in the pancreatic lymph nodes obtained from T1D patients (Kent et al. 2005) and from T1D recipients of pancreas transplants with recurrent diabetes (Vendrame et al. 2010); furthermore, GAD-reactive CD4 T-lymphocytes from these pancreas transplant recipients were shown to kill human beta cells in vivo, after co-transplantation with human islets in immunodeficient mice (Vendrame et al. 2010). Elegant studies also showed that proinsulin-specific CD8 T-lymphocytes kill beta cells in vitro through the perforin pathway (Skowera et al. 2008; Kronenberg et al. 2012). Beta cell damage has also been modeled in humanized mice (Unger et al. 2012; Viehmann Milam et al. 2014).

As noted, access to tissues from T1D patients is becoming more common following the systematic effort to recover tissues from organ donors with T1D (Pugliese et al. 2014); studies of nPOD pancreata demonstrated autoantigen-specific T-lymphocytes in the insulitis lesion using tetramers (Coppieters et al. 2012), which

links those CD8 T-lymphocytes to disease pathogenesis. Infiltrated islets in pancreata with recent onset disease only contained autoreactive CD8 T-cells with a single antigen specificity; in contrast, inflamed islets from patients with longer disease duration contained multiple islet-reactive specificities. These findings support the concept of epitope spreading and the evolution towards multiple antigen responses as the disease progresses, but also suggest chronicity of the disease process even beyond the clinical diagnosis. Moreover, these observations directly connect autoreactive CD8 T-lymphocytes previously identified in studies of peripheral blood samples to the insulitis and, therefore, to disease pathogenesis.

Furthermore, a variety of autoreactive CD8 and CD4 T-lymphocytes were recently isolated and characterized from the infiltrated islets of organ donors who had had T1D for several years (Babon et al. 2016), further linking autoreactive T-lymphocytes initially identified through studies of peripheral blood samples to the disease process in the pancreas. Of note, these studies provided additional evidence for responses against posttranslationally modified and hybrid peptides (Delong et al. 2016).

A critical phenotype of autoreactive T-lymphocyte populations in T1D is their enrichment in memory cells; autoreactive memory T-lymphocytes are reported in patients but not in healthy subjects (Monti et al. 2009). Consistent with the notion that autoimmunity may persist chronically for years after diagnosis, at least 30–40% of prospective islet or pancreas transplant recipients have one or more autoantibodies, typically many years from diagnosis (Jaeger et al. 2000; Vendrame et al. 2016); patients with autoreactive CD8 T-lymphocytes detectable prior to islet transplantation have higher risk of subsequent graft failure (Hilbrands et al. 2009). It is also known that chronic immunosuppression in transplant recipients induces lymphopenia and increased levels of IL-7 and IL-15, cytokines that promote homeostatic proliferation of memory populations. In patients with an underlying autoimmune disease, memory lymphocyte populations are likely to be enriched in autoreactive cells (Monti et al. 2009). Thus, the persistence or the reactivation of memory responses following islet or pancreas transplantation represents a significant challenge to curing diabetes through transplantation. Moreover, chronic immunosuppression that prevents rejection does not always control the reactivation of islet autoimmune responses (Vendrame et al. 2010, 2016). The importance of memory cells in T1D is also supported by the positive impact of therapy with an anti-memory cell agent in a clinical trial of patients with recently diagnosed T1D (Rigby et al. 2013, 2015).

Impaired Central Tolerance

A number of large, longitudinal natural history studies involving relatives and subjects from the general population with increased genetic risk have shown that islet autoimmunity – as assessed by the seroconversion for autoantibodies to islet cell autoantigens – may be triggered early, often during the first 2 years of life; however, the triggering of autoimmune responses is also observed later in life (Vehik

et al. 2011; Ziegler et al. 2011, 2013; Insel et al. 2015). Islet autoimmune responses are typically detected months to years prior to the diagnosis and are heterogeneous with respect to the autoantigens as well number of autoantigens that may be targeted in an individual. Over time, the response can spread to additional autoantigens and in general the number of autoantibodies correlates strongly with risk of future clinical disease (Ziegler et al. 2013; Insel et al. 2015).

Despite most self-molecules are expressed in the thymus to establish central tolerance (Kyewski and Klein 2006) in early life, some may become targets of autoimmunity. It is believed that genetic mechanisms can lead to suboptimal thymic expression and in turn imperfect tolerance. In fact, allelic variation, alternative splicing, and epigenetic regulation can affect both levels and which epitopes are presented to developing lymphocytes in the thymus. Such mechanisms have been identified for several T1D autoantigens: for example, the thymic expression of insulin is influenced by allelic variants of the insulin gene, with evidence for further epigenetic effects suppressing T1D-protective insulin gene variants associated with higher transcriptional levels in the thymus (Pugliese et al. 1997; Vafiadis et al. 1997, 2001). Alternative mRNA splicing resulting in mismatched expression patterns in pancreas and thymus has been reported for the autoantigens IA-2 and IGRP; the immune system may not be tolerant to alternatively spliced forms of these molecules that are expressed in pancreas but not in the thymus (Diez et al. 2001; Dogra et al. 2006). Ultimately, suboptimal tolerization allows for the emergence of a T-lymphocyte repertoire harboring autoreactive T-lymphocytes, as shown for insulin both in several mouse models of autoimmune diabetes (Chentoufi and Polychronakos 2002; Moriyama et al. 2003; Thebault-Baumont et al. 2003; Fan et al. 2009; Jarchum and DiLorenzo 2010) and in patients (Durinovic-Bello et al. 2005, 2010).

The thymic expression and tolerogenic presentation of self-molecules is mediated by a subset of medullary thymic epithelial cells involved in the negative selection of self-reactive T-lymphocytes (Derbinski and Kyewski 2010). This process occurs under the direction of the autoimmune regulator transcription factor (*AIRE*) (Anderson et al. 2002). Thymic dendritic cells were also reported to express insulin and other self-molecules and may play a role in self-tolerance (Throsby et al. 1998; Pugliese et al. 2001; Zhu et al. 2006); self-molecules are also expressed in peripheral lymphoid tissues for the maintenance of self-tolerance. Multiple cell types reportedly mediate the tolerogenic expression of self-molecules in the periphery, including dendritic cells, stromal cells (Gardner et al. 2008) recently discovered to be of bone marrow-origin and to resemble features of dendritic cells (Gardner et al. 2013), lymph node-resident lymphatic endothelial cells (Cohen et al. 2010), and lymph node fibroblastic reticular cells (Fletcher et al. 2010). Insulin expression by AIRE-expressing dendritic cells is critical to maintain peripheral tolerance to this autoantigen (Grupillo et al. 2012). Thus, it appears that multiple cell types can produce self-molecules in redundant and complementary fashion to support a comprehensive representation of self and promote peripheral tolerance. Some of these cells transcribe self-molecule genes under AIRE control, but others use other transcription factors, such as Deaf1. Alternative splicing of Deaf1 generates a less active form that has been associated with decreased insulin gene expression in

pancreatic lymph nodes as NOD mice progress towards diabetes (Kodama et al. 2008); this mechanisms has been observed in pancreatic lymph nodes of nPOD donors with T1D (Yip et al. 2009) and has been associated with inflammation, via the impaired expression of the eukaryotic translation initiation factor 4 gamma 3 (Eif4g3) (Yip et al. 2013); these observations provide a link between inflammation and impaired peripheral tolerance.

As noted, HLA molecules present self-antigens to T-lymphocytes in both the thymus and peripheral lymphoid tissues. T1D-predisposing HLA molecules may lead to less than ideal presentation of islet cell autoantigenic epitopes and in turn inefficient negative selection of autoreactive T-lymphocytes in the thymus. For example, weak interactions between a T1D-associated preproinsulin peptide and the HLA-A2 molecule lead to suboptimal presentation to the responding TCR (Bulek et al. 2012), which may allow disease-associated CD8 T-lymphocytes to escape negative selection. A similar mechanism was described in the NOD mouse, in which the insulin B9-23 peptide is a critical target of CD4 T-lymphocytes that is essential for disease development (Nakayama et al. 2005); the B9-23 peptide binds the I-Ag7 MHC (major histocompatibility complex) class II molecule of NOD mice in multiple registers (Nakayama et al. 2015b); in the thymus, a register binding is used that leads to impaired negative selection of insulin B9-23 reactive CD4 T-lymphocytes; in the periphery the same peptide is presented in a different binding register that instead promotes activation of pathogenic T-lymphocytes (Stadinski et al. 2010; Mohan et al. 2011). Of note, the NOD mouse I-Ag7 molecule is strikingly similar to the human HLA-DQ8 molecule, a major genetic risk factor for T1D, including binding features for insulin peptides (Suri et al. 2005); it is plausible that similar mechanisms may operate in patients in whom insulin-specific HLA-DQ8-restricted CD4 T-lymphocytes are being described (Nakayama et al. 2015a) and highlight how different HLA-peptide complex interactions determine the activation and phenotypes of responding T-lymphocytes. Of note, such HLA influences can synergize with reduced insulin expression in the thymus associated with predisposing insulin gene variants. CD4 T-lymphocytes isolated from T1D patients show abnormalities in the immunological synapsis that may also promote the escape from thymic negative selection and promote effector functions upon encounter with their target antigen (Schubert et al. 2012). Overall, there is growing evidence that HLA-encoded genetic predisposition effects are operative in very early life, when they play a key role in determining the efficacy of thymic selection processes for insulin and likely other autoantigens.

Impaired Immune Regulation Promotes Islet Autoimmunity

Multiple defects have been described that impair immune regulation. Both impaired function of regulatory T-lymphocytes and an imbalance with proinflammatory Th17 T-lymphocytes have been reported in the pancreatic lymph nodes of T1D patients (Buckner 2010; Ferraro et al. 2011). Moreover, effector T-lymphocytes are resistant to the suppression mediated by regulatory T-lymphocytes in patients with T1D

(Schneider et al. 2008). Several of the known T1D risk genes may predispose to increased reactivity and impaired regulation (Pociot et al. 2010); however, the effects mediated by alleles at these loci are not disease-specific, and in fact many of these loci are involved in the genetic risk of many autoimmune disorders. Here we will focus on the effects of a selected few risk genes, which modulate T-lymphocyte activation, function, and regulation.

The PTPN22 (protein tyrosine phosphatase, nonreceptor type 22) gene encodes for an intracellular, lymphocyte-specific tyrosine phosphatase (Lyp) and a negative regulator of TCR signaling; T1D risk derives from a gain of function variant associated with suppression of TCR signaling (Bottini et al. 2004; Vang et al. 2005), which would favor the survival of autoreactive T-lymphocytes in the thymus. PTPN22 has also been associated with effects on the function of effector T-lymphocytes, regulatory T-lymphocytes, and B-lymphocytes in the periphery (Vang et al. 2007; Menard et al. 2011). As discussed previously, multiple lines of evidence demonstrate a role for B-lymphocytes in T1D (O'Neill et al. 2009; Pescovitz et al. 2009) as well as defects in B lymphocyte regulation (Cox and Silveira 2009; Smith et al. 2015).

Activated CD4 and CD8 T-lymphocytes express CTLA-4 (Cytotoxic T lymphocytes- antigen-4; CD152), a well-known inhibitor of T-lymphocyte responses. While CTLA-4 polymorphisms have very small effect on genetic predisposition, they are linked reduced levels of a soluble form of CTLA-4 with a negative effect on regulatory T-lymphocyte function (Gerold et al. 2011). A recent clinical trial showed that CTLA4-Ig therapy mitigated loss of insulin secretion in patients with new onset T1D (Orban et al. 2011, 2014).

The IL2RA gene encodes the alpha chain of the Interleukin-2 receptor (IL-2Rα, or CD25) and is a susceptibility locus for T1D and other autoimmune diseases (Lowe et al. 2007). IL-2 is of critical importance for the development and function of regulatory T-lymphocytes (Malek and Castro 2010). Predisposing IL-2RA alleles have been associated with lower circulating levels of soluble IL-2 receptor (Lowe et al. 2007), reduced STAT5 response to IL-2 in antigen-experienced CD4 T-lymphocytes, lower levels of expression of the Foxp3 transcription factor, and impaired suppression function of regulatory T-lymphocytes (Garg et al. 2012). In a similar fashion, the IL-2 gene is a susceptibility locus in NOD mice and influences diabetes development through impaired function of regulatory T-lymphocytes (Yamanouchi et al. 2007). In NOD mice, intraislet regulatory T-lymphocytes express reduced amounts of IL2RA, which favors their apoptosis and impairs regulation of effector T-lymphocyte promoting disease progression (Tang et al. 2008). Such defects are corrected with low dose IL-2 therapy, which reverses diabetes in NOD mice (Grinberg-Bleyer et al. 2010). Recently conducted clinical trials with low-dose IL-2 have shown clinical benefit in graft versus host disease and hepatitis C virus-induced vasculitis (Koreth et al. 2011; Saadoun et al. 2011). A safety trial in T1D patients demonstrated no significant untoward effects and dose-dependent increases of regulatory T-lymphocytes and a number of regulatory changes in the immune system, but it only involved a short course therapy and was not designed to impact metabolic function (Hartemann et al. 2013; Rosenzwajg et al. 2015).

Environmental Factors

Several environmental exposures have been linked to T1D, including toxins, viruses, dietary factors, and more recently the microbiome (Eringsmark Regnell and Lernmark 2013; Davis-Richardson and Triplett 2015; Gulden et al. 2015; Mejia-Leon and Barca 2015; Endesfelder et al. 2016; Hyoty 2016; Knip and Siljander 2016; Paun et al. 2016; Rewers and Ludvigsson 2016). These are described in greater detail in Chapter 4. Here, we will describe some putative pathogenic mechanisms by which viral infections, in particular enterovirus infections, may play a role in T1D development.

Since enteroviruses can infect the thymus, maternal or early life enterovirus infections may disrupt mechanisms of central tolerance (Jaidane et al. 2012) and perhaps synergize with the genetically encoded mechanisms previously discussed to promote loss of tolerance and the triggering of autoimmune responses in early life (Lonnrot et al. 1998; Roivainen et al. 1998). Moreover, this may also explain the association of maternal enterovirus infections with increased prevalence of T1D in the offspring (Viskari et al. 2012). A seminal study in a UK collection of archived pancreata from newly diagnosed patients found evidence of viral protein at much higher frequency in T1D pancreata compared to pancreata from nondiabetic donors or donors with type 2 diabetes (T2D) (Richardson et al. 2009). Moreover, a high prevalence of viral markers was found in the pancreatic islets in the DiViD study, in which patients with recent onset T1D underwent biopsy; however, a virus could be isolated and propagated from the islets examined (Krogvold et al. 2015a). Viral RNA in the circulation and viral infection of the gut mucosa has also been linked to T1D development (Oikarinen et al. 2011, 2012).

Enteroviruses can infect and damage beta cells; enterovirus infections have been shown to severely impair insulin secretion (Gallagher et al. 2015), to alter gene expression and microRNA regulation (Kim et al. 2016), induce inflammatory responses which contribute to mediate beta cell stress, dysfunction, and apoptosis (Marroqui et al. 2014, 2015; de Beeck and Eizirik 2016), and to some extent may induce beta cell replication (In't Veld 2011b; Willcox et al. 2011). Enteroviruses may possibly promote beta cell destruction that triggers autoimmunity, via presentation of self-molecules in an inflammatory context, or by molecular mimicry (Afonso and Mallone 2013).

It is unclear whether multiple acute and/or chronic viral infections may occur in the pancreas. It has been proposed that chronic infections may be the result of modifications of the viruses, which upon infection become replication defective and may therefore persist for a long time (Chapman et al. 2008). At any rate, chronic or multiple infections could over time trigger autoimmunity. The ongoing studies of the nPOD cohort, which includes T1D donors with a wide range of disease duration, support the concept that enterovirus infections may be chronic (Richardson et al. 2013). Viral infections may affect different regions of the pancreas over time, and this could help explaining the lobular distribution observed for insulitis and beta cell loss. As discussed in the next section, viral infections can also induce hyper-expression of HLA class I antigens and alpha-interferon (Foulis et al. 1987a, b;

Dotta et al. 2007; Richardson et al. 2016), which amplify inflammation and promote the triggering of islet autoimmunity.

The mapping of susceptibility loci to the IFIH1, TYK2, and PTPN2 genes (Colli et al. 2010; Marroqui et al. 2015) provides a genetic basis for viruses to play a role in the pathogenesis of T1D (Smyth et al. 2006; Nejentsev et al. 2009; Chistiakov 2010). IFIH1 encodes for a helicase (melanoma differentiation-associated gene 5, MDA5) that recognizes double-stranded RNA following the intracellular replication of enteroviruses. This recognition triggers antiviral responses that include interferon and other inflammatory cytokines, responses that are reported as more intense in individuals carrying T1D-predisposing IFIH1 variants. More intense antiviral responses could enhance exposure of self-antigens and the triggering of autoimmune responses, with more pronounced deleterious impact on beta cell function leading to apoptosis (Colli et al. 2010). In contrast, those individuals with *IFIH1* variants associated with genetic resistance to the development of T1D exhibit less intense inflammation following viral infection (Nejentsev et al. 2009). TYK2 may modulate human pancreatic beta cell apoptosis and production of proinflammatory cytokines mediators; the silencing of TYK2 in human beta cells exposed to surrogates of double-stranded RNA molecules as produced during viral infection resulted in reduced type I interferon pathway activation and lower levels of interferon-alpha and the chemokine CXCL10, which plays a role in the recruitment of T-lymphocytes to the pancreatic islets (Antonelli et al. 2014; Marroqui et al. 2015). These manipulated cells also showed had lower expression of HLA class I antigens. The inhibition of TYK2 also prevented beta cell apoptosis upon exposure to the viral mimic. The PTPN2 gene also affects beta cell responses to double-stranded RNA and may modulate resistance to apoptosis (Colli et al. 2010).

Hyper-expression of Histocompatibility Antigens By Pancreatic Islet Cells

The hyper-expression of HLA antigens by islet cells is another typical feature of the T1D pancreas. This phenomenon was initially reported in the mid-1980s, both for HLA class I and class II molecules (Bottazzo et al. 1985; Foulis and Farquharson 1986; Foulis et al. 1986, 1987a). While both HLA class I and class II hyper-expression have been the subject of controversy (Allison et al. 1988; Harrison et al. 1989; Lafferty and Wang 1990; Skog et al. 2015), recent studies have provided robust validation with a variety of techniques that indeed hyper-expression of HLA class I molecules is a true phenomenon and a key feature in the pathology of T1D that highlights a chronic inflammatory state (Richardson et al. 2016); of note, hyper-expression of HLA class I molecules is often associated with insulitis; like insulitis, it is essentially limited to insulin-containing islets and continues to be observed for years after diagnosis. Hyper-expression of class I molecules (and class II molecules) may be induced by viral infections, which are postulated to play a key role in T1D pathogenesis (Hyoty 2016). Moreover, hyper-expression of class I molecules is often associated with the presence of markers of viral infection in beta cells, although not

necessarily in the same islets (Richardson et al. 2009; Richardson et al. 2013; Krogvold et al. 2015a). It is presently unknown whether islet-infiltrating CD8 T-lymphocytes can target viral epitopes presented by infected beta cells on their HLA class I molecules, but if this were true it would have profound implications for our understanding of the disease pathogenesis by supporting a direct role of viral infections in the triggering of beta cell destruction.

Inflammation of Pancreatic Beta Cells

Inflammation is emerging as a major factor in the pathogenesis of T1D, and it plays a significant role in beta cell dysfunction as well as beta cell death. Inflammation is likely to results from a combination of genetic and environmental factors (Colli et al. 2010; Eizirik et al. 2012). Evidence for inflammation can be found in peripheral blood signatures for IL-1 (Jia et al. 2011; Chen et al. 2014) and interferon (Ferreira et al. 2014), which were reported in relatives or genetically at risk children (Cabrera et al. 2016). These signatures precede the triggering of islet autoimmunity and could also be linked to viral infections. Genetically at-risk children reportedly undergo metabolic changes prior to the triggering of islet autoimmunity, as shown by alterations of metabolite profiles (amino acids, lipids, fatty acids), which could be related to inflammation and apoptosis in the pancreas (Oresic et al. 2008; Pflueger et al. 2011; Overgaard et al. 2016). As previously discussed, there is evidence that the *IFIH1* and *PTPN2* susceptibility genes play a role in mediating beta cell apoptosis during inflammation (Colli et al. 2010; Marroqui et al. 2014). Several more genes linked to T1D risk have been found to be expressed by beta cells (Floyel et al. 2015), highlighting the role that beta cell responses have in their own demise.

Besides enterovirus infections which may promote inflammation in the gut (Oikarinen et al. 2012), there has been increased interest in the role of the gut flora (Dunne et al. 2014), with growing evidence that dietary habits can influence intestinal permeability, flora composition, and in turn the immune system (Brown et al. 2011), such as the production of cytokines and chemokines (Sarkar et al. 2012), innate immune responses (Zipris 2011; Alkanani et al. 2012), and the function of gut-associated lymphocytes, including those with regulatory function (Mizrahi and Ilan 2009). Overall, growing evidence points at inflammation and metabolic changes as significant factors in T1D pathogenesis; these likely originate from the interplay between genetic and environmental factors, can promote chronic immune dysregulation through effects on both innate and adaptive immune responses, and are likely to precede the triggering of islet autoimmunity and persist chronically as well. Both inflammatory and metabolic changes can induce significant stress in beta cells, facilitate protein misfolding, and ultimately lead to impaired function (Fu et al. 2013).

There is also evidence that endoplasmic reticulum stress impairs beta cell function in T1D (Marhfour et al. 2012; Eizirik et al. 2013; Burch et al. 2015; Grzesik et al. 2015; Krogvold et al. 2015b); both saturated fats and inflammatory cytokines can trigger the unfolded protein response in pancreatic beta cells; in turn, this potentiates

activation of nuclear factor κB. Inflammation also results in the production of several chemokines, which were detected in T1D pancreata (Sarkar et al. 2012) and may be induced by proinflammatory Th17 T-lymphocytes (Grieco et al. 2014) or in response to enterovirus infections (Schulte et al. 2012). The activation of the unfolded protein response sensitizes pancreatic beta cells to the effects of proinflammatory cytokines, which besides promoting inflammation may also contribute to amplify and sustain insulitis. Consistent with this, the pancreas of nPOD T1D donors exhibits elevated islet cell expression of endoplasmic reticulum stress and the unfolded protein response, especially in insulin-positive, infiltrated islets (Marhfour et al. 2012; Eizirik et al. 2013), and may be linked to insulin secretory abnormalities reported during the prediabetic phase in at-risk relatives (Sims et al. 2016).

It is possible that beta cell inflammation and stress may also play a role in the formation of posttranslationally modified and hybrid autoantigen peptides that are likely to play a key role in breaking self-tolerance and promoting chronic islet autoimmunity (Marre et al. 2015). Endoplasmic reticulum stress was experimentally shown to alter the endomembrane distribution of the GAD65 autoantigen, and the palmitoylated form of this molecule accumulated in trans-Golgi membranes. This abnormal distribution was also observed in beta cells, by examination of pancreas sections from nPOD organ donors with T1D who had residual beta cells and insulitis and from nondiabetic donors with GAD65 autoantibodies (Phelps et al. 2016). Of note, the palmitoylated GAD65 has higher immunogenicity and ability to be uptaken by antigen-presenting cells and stimulate T-lymphocytes. Thus, inflammation leading to beta cell endoplasmic reticulum stress can also induce aberrant accumulation of more immunogenic GAD65 autoantigen in Golgi membranes and promote the triggering of islet autoimmune responses.

The Extent of Beta Cell Loss

Beta cell loss is the predominant feature of the T1D pancreas and it is eventually almost complete in many patients. However, both recent findings from the nPOD and DiViD studies and a meta-analysis of earlier data (Klinke 2008, 2011) challenge the traditional belief that 90% of the beta cell mass is already lost by the time of diagnosis. Younger children appear to have lower residual beta cell mass when diagnosed, possibly because at young age the pancreas has not fully grown and reached the adult level beta cell mass. Patients diagnosed when teenagers or older had at least 40–60% of their islets staining positive for insulin, including the findings from the UK and DiViD new-onset cohorts, and the nPOD cohort (Klinke 2008; Campbell-Thompson et al. 2016; Krogvold et al. 2016). As noted, the 6 DiViD biopsies were performed in young adult (mid-20s) patients with recent onset T1D: 18–66% and on average 36% of the islets examined stained for insulin (Krogvold et al. 2016). An analysis of 80 T1D nPOD donors, of whom only a few were recently diagnosed, reported residual beta cells in all 17 T1D donors with insulitis with disease duration extending to 12 years; the donors with insulitis had on average tenfold higher beta cell mass than donors without insulitis, and no correlation was

observed between beta cell mass and insulitis, disease duration, and age of onset (Campbell-Thompson et al. 2016). Persistence of insulin-positive beta cells is reported even decades after diagnosis (Meier et al. 2005a, b; Keenan et al. 2010) along with the expression of glucose transporters (Coppieters et al. 2011, 2012). The observation of low-level beta cell apoptosis in pancreata with long duration of T1D implies the existence of some beta cell turnover (Meier et al. 2005a, b, Keenan et al. 2010). The persistence of beta cells in the T1D pancreas for years after diagnosis and the incomplete destruction observed at onset support the chronicity of the disease process.

Concordant with these pathology observations, the assessment of functional beta cell function and response to stimulation in living patients shows the persistence of both fasting and increased stimulated C-peptide responses in many patients, not just around the time of diagnosis but even decades later (Sherry et al. 2005; Tsai et al. 2006; Greenbaum et al. 2009, 2012; Sherr et al. 2014). A prospective, postdiagnosis evaluation of C-peptide responses involving newly diagnosed patients has shown that 88% of patients at 1 year and 66% at 2 years have stimulated C-peptide responses that would meet the threshold for randomization in a new-onset clinical trial (>0.2 pmol/ml); the decline in insulin secretion is slower after the first year and there is also individual heterogeneity in the rates of decline (Greenbaum et al. 2012); with 4 years of postdiagnosis of follow-up demonstrating further decline, this study also shows that C-peptide responses and beta cell function are lower in younger children; however, this may be a reflection of age-related lower beta cell mass rather than rate of beta cell loss after onset, as this was largely similar among the age groups compared (Hao et al. 2016). Several studies have reported that most patients may secrete low amount of C-peptide (microsecretors) even decades after diagnosis; about 80% respond with increased levels upon stimulation (Keenan et al. 2010; Wang et al. 2012; Oram et al. 2014, 2015), and a significant proportion may still have peak C-peptide responses >0.2 pmol/ml regardless of long disease duration. Of note, this is the threshold for inclusion in a clinical trial at disease onset.

Beta Cell Dysfunction and Insulin Resistance

The modest frequency of insulitis and the partial loss of beta cells observed in many patients at diagnosis cannot fully explain the diabetes symptoms and severe impairment of insulin secretion that are typical of newly diagnosed T1D. Partial beta cell loss and modest frequency of insulitis has also been noted in recipients of pancreas transplants who had developed T1D recurrence in their grafts after years of normal function and in the absence of rejection (Vendrame et al. 2010); yet these patients showed severely impaired insulin secretion. Moreover, islets isolated from the pancreas obtained via biopsy in newly diagnosed T1D patients in the DiViD Study were shown to recover function in culture. Thus, islet dysfunction may be corrected and islet function may be recoverable (Krogvold et al. 2015b). The above implies that therapeutic intervention to reverse diabetes at diagnosis may have limited success if this is limited to the sole targeting of islet autoimmunity, given the modest

proportion of islets affected by insulitis at any given time, and that immunomodulation therapy may not directly correct beta cell dysfunction. Combinatorial therapies should therefore address autoimmunity, inflammation, and beta cell dysfunction (Skyler 2015).

It is also well known that excessive body weight, obesity, and insulin resistance have become more prevalent in western society, so that even T2D is becoming more common in youth; obesity and insulin resistance have become more prevalent also in children with T1D (Liu et al. 2010). Insulin resistance is reported in approximately 20% of young T1D patients (Pang and Narendran 2008), in particular during puberty; insulin resistance precedes T1D diagnosis and promotes the progression of islet autoimmunity (Fourlanos et al. 2004; Weir and Bonner-Weir 2004; Dabelea et al. 2006; Bingley et al. 2008; Ferrannini et al. 2010). Thus, insulin resistance can be a contributing factor to islet stress and dysfunction during the progression to overt disease.

The Prediabetic Period

Understanding the disease processes during the prediabetic period is of fundamental importance for unveiling key triggers and disease mechanism, and ultimately for T1D prevention and cure. In 2015, the JDRF, the Endocrine Society, and the American Diabetes Association have proposed a classification of the prediabetic phase. This classification is based on data from natural history studies of subjects at genetic risk. Three stages have been proposed:

- Stage 1: The subject lacks any symptoms and has normal glucose tolerance, but beta cell autoimmunity is present, as evidenced by two or more autoantibodies.
- Stage 2: In addition to beta cell autoimmunity, dysglycemia is present while subjects remains asymptomatic.
- Stage 3: This represents the onset of symptomatic disease.

The adoption of this classification provides a useful framework for the design of prevention trials and for an optimized benefit/risk ratio that will facilitate regulatory approval and clinical translation of therapies in the early stages of T1D to prevent symptomatic disease. It is important to recognize that Stage 2 is essentially a disease state, even if asymptomatic.

However, we must recognize that very little is known about the pancreas pathology T1D during the prediabetic period. While autoantibody conversion is considered the initial triggering of islet autoimmunity, whether this is temporally associated with insulitis and beta cell loss is essentially unknown. Because many individuals have autoantibodies for years prior to disease onset, we need to understand whether this chronicity is due to a slow destructive process affecting a minority of islets over time or rather some precipitating event triggers insulitis and beta cell destruction at a later stage. Prospective studies have shown that autoantibody-positive relatives may have normal metabolic measures for years, with abnormalities in glucose metabolism and

insulin secretion becoming apparent closer to diagnosis, usually between 1 and 2 years and about 6 months prior to symptomatic disease (Sosenko et al. 2006; Ferrannini et al. 2010; Sosenko et al. 2010). These observations suggest the hypothesis that the immune-mediated destruction of beta cells may be triggered nearer to the time of diagnosis, and autoantibody positivity may not necessarily indicate insulitis and beta cell loss (Diedisheim et al. 2016).

Such key questions may be addressed if robust measures of islet inflammation, insulitis, beta cell mass, and death can be developed an applied in longitudinal studies. Addressing these questions will require a combination on laboratory and imaging approaches (Gaglia et al. 2011; Reiner et al. 2011; Herold et al. 2015) and, critically, the study of nondiabetic organ donors with autoantibodies. It is feasible to screen organ donors for autoantibodies (Gianani et al. 2006; Tauriainen et al. 2010) and a large-scale screening effort is ongoing in the USA (Pugliese et al. 2014; Wasserfall et al. 2016). Yet identifying such donors remains a challenge, as these donors are quite rare in the general population. So far, pancreata from 18 nPOD donors tested positive for autoantibodies and were recovered; 13 expressed a single autoantibody, typically against the glutamic acid decarboxylase autoantigen (Campbell-Thompson et al. 2016); these donors lacked HLA genes associated with increased T1D risk and do not appear to have insulitis (Oikarinen et al. 2008; Campbell-Thompson et al. 2016); however, there are increasing reports that their pancreas may have signs of islet inflammation and stress (Pugliese et al. 2014). nPOD also identified 5 donors with multiple autoantibodies, of whom 2 donors were found to have insulitis in association with high-risk HLA types (Campbell-Thompson et al. 2016).

Retrospective autoantibody screening of donors whose pancreas had been used for islet cell isolation was conducted in Europe; these studies only allow for pathology studies to be conducted in a small portion of the pancreas. Of 62 autoantibody-positive donors identified in such a study from Belgium, insulitis was reported in 2 of 3 donors expressing multiple autoantibodies; of note, these donors carried high-risk HLA types (In't Veld et al. 2007). Likewise, a study from Scandinavia reported 32 autoantibody-positive donors; 9 donors had multiple autoantibodies, but none had high-risk HLA alleles and insulitis was not detected (in some cases, this could be a sampling issue) (Wiberg et al. 2015).

While these results are too limited and cannot support firm conclusions, there seems to be concordance with the clinical observation that multiple autoantibodies are associated with higher risk of T1D than single autoantibody positivity (Ziegler et al. 2013). The antigen specificity of the autoimmune responses may be a factor in determining the presence of insulitis, but the number of autoantibody-positive donors reported in the literature is too small to fully inform as whether particular autoantibody responses are closely associated with insulitis. There is a need to study more of these rare cases, as the relation between insulitis, beta cell loss, genetic predisposition, and autoantibodies is critical to understand during the prediabetic period. It is hoped that future studies of nondiabetic, autoantibody-positive organ donors will establish if insulitis is closely associated with the appearance of single and/or multiple autoantibodies, and/or the presence of autoreactive T-lymphocytes in peripheral blood (Burke et al. 2016).

Conclusive Remarks

The clinical diagnosis has been traditionally considered a moment in the disease natural history at which the consequences of the chronic autoimmune responses and other pathogenic mechanisms are severe enough that the disease becomes clinically manifest. For many years, it has been thought that diagnosis represented the time point at which about 90% of the beta cell mass had been lost to autoimmunity. However, growing evidence that in many patients the actual beta cell loss at diagnosis may be much less severe is challenging this notion. Moreover, longitudinal studies of autoantibody-positive relatives during the prediabetic period have shown that signs of impaired glucose metabolism and insulin secretion may appear after many years of autoantibody positivity, usually during the 1–2 years preceding clinical onset. Emerging data support the possibility that perhaps autoantibody positivity does not imply that autoreactive T-lymphocyte responses coexist at all times; perhaps the actual triggering of cellular responses is a late event, more proximal to the time of diagnosis. This could explain the observation that beta cell loss at diagnosis is only partial in many patients, and thus clinical onset may not represent a time point of severe destruction beyond the threshold that allows normoglycemia to be maintained; rather, the clinical onset and its acute manifestations could be driven by a superimposed mechanism that leads to severe and, without proper intervention, permanent beta cell dysfunction. While T-lymphocyte-mediated autoimmunity is the main and likely the final effector of beta cell death, growing evidence implicates additional factors in disease pathogenesis and progression. We are currently limited in our ability to further test this concept, since we cannot concurrently estimate insulin secretory responses together with physical beta cell mass, disease activity, and beta cell death. Future advances in our understanding of the disease pathogenesis are likely to require robust imaging technologies and biomarkers of beta cell death and immune activity. Improved understanding of the chronic and multifactorial nature of T1D pathogenesis and its clinical course is required to develop efficacious preventative and therapeutic options. However, increasing appreciation for the chronic nature of the disease process and of the persistence of insulin secretion for years after diagnosis suggests that the time window for meaningful intervention is wider than previously thought.

Summary

T1D is a multifactorial, chronic, autoimmune disease leading to the destruction of pancreatic beta cells. Multiple genes control diabetes risk and modulate disease mechanisms, which effects on immune cells and beta cells. There is growing evidence that environmental factors contribute to the pathogenesis, including viruses, the microbiome, and dietary factors, by possibly promoting a pro-inflammatory milieu. Autoimmune-mediated beta cell destruction is considered the key pathogenic mechanism and, over a prolonged period of time, the final effector of beta cell destruction. Chronic beta cell inflammation is also a key component of the

disease pathogenesis. Beta cell dysfunction is likely an even that precedes and coexists with beta cell destruction, and may help explaining the appearance of clinical symptoms when growing evidence suggest that beta cell loss is partial in many patients. This observation raises questions about the dynamics of the autoimmune process; the persistence of beta cells, insulin secretion, and disease activity for years after diagnosis point at the chronicity of the disease pathogenesis, both before and after clinical onset.

References

Afonso G, Mallone R. Infectious triggers in type 1 diabetes: is there a case for epitope mimicry? Diabetes Obes Metab. 2013;15(Suppl 3):82–8.

Alkanani AK, Rewers M, Dong F, Waugh K, Gottlieb PA, Zipris D. Dysregulated Toll-like receptor-induced interleukin-1beta and interleukin-6 responses in subjects at risk for the development of type 1 diabetes. Diabetes. 2012;61(10):2525–33.

Allison J, Campbell IL, Morahan G, Mandel TE, Harrison LC, Miller JFAP. Diabetes in transgenic mice resulting resulting from over-expression of class I histocompatibility molecules in pancreatic B-cells. Nature. 1988;333:529–33.

Aly TA, Ide A, Jahromi MM, Barker JM, Fernando MS, Babu SR, Yu L, Miao D, Erlich HA, Fain PR, Barriga KJ, Norris JM, Rewers MJ, Eisenbarth GS. Extreme genetic risk for type 1A diabetes. Proc Natl Acad Sci U S A. 2006;103(38):14074–9.

Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. Annu Rev Immunol. 2005;23:447–85.

Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D. Projection of an immunological self shadow within the thymus by the aire protein. Science. 2002;298(5597):1395–401.

Antonelli A, Ferrari SM, Corrado A, Ferrannini E, Fallahi P. CXCR3, CXCL10 and type 1 diabetes. Cytokine Growth Factor Rev. 2014;25(1):57–65.

Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estorninho M, Kronenberg-Versteeg D, Bingley PJ, Todd JA, Guy C, Dunger DB, Powrie J, Willcox A, Foulis AK, Richardson SJ, de Rinaldis E, Morgan NG, Lorenc A, Peakman M. Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes. 2014;63(11):3835–45.

Atkinson MA. Pancreatic biopsies in type 1 diabetes: revisiting the myth of Pandora's box. Diabetologia. 2014;57(4):656–9.

Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383(9911):69–82.

Babon JA, DeNicola ME, Blodgett DM, Crevecoeur I, Buttrick TS, Maehr R, Bottino R, Naji A, Kaddis J, Elyaman W, James EA, Haliyur R, Brissova M, Overbergh L, Mathieu C, Delong T, Haskins K, Pugliese A, Campbell-Thompson M, Mathews C, Atkinson MA, Powers AC, Harlan DM, Kent SC. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nat Med. 2016. https://doi.org/10.1038/nm.4203. [Epub ahead of print].

Baisch JM, Weeks T, Giles R, Hoover M, Stastny P, Capra JD. Analysis of HLA-DQ genotypes and susceptibility in insulin- dependent diabetes mellitus. N Engl J Med. 1990;322(26):1836–41.

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009;464(7289):713–20.

Belot MP, Fradin D, Mai N, Le Fur S, Zelenika D, Kerr-Conte J, Pattou F, Lucas B, Bougneres P. CpG methylation changes within the IL2RA promoter in type 1 diabetes of childhood onset. PLoS One. 2013;8(7):e68093.

Bingley PJ, Mahon JL, Gale EA. Insulin resistance and progression to type 1 diabetes in the European Nicotinamide Diabetes Intervention Trial (ENDIT). Diabetes Care. 2008; 31(1):146–50.

Bogdani M. Thinking outside the cell: a key role for hyaluronan in the pathogenesis of human type 1 diabetes. Diabetes. 2016;65(8):2105–14.

Bogdani M, Johnson PY, Potter-Perigo S, Nagy N, Day AJ, Bollyky PL, Wight TN. Hyaluronan and hyaluronan-binding proteins accumulate in both human type 1 diabetic islets and lymphoid tissues and associate with inflammatory cells in insulitis. Diabetes. 2014a;63(8):2727–43.

Bogdani M, Korpos E, Simeonovic CJ, Parish CR, Sorokin L, Wight TN. Extracellular matrix components in the pathogenesis of type 1 diabetes. Curr Diab Rep. 2014b;14(12):552.

Bottazzo GF, Dean BM, McNally JM, Mackay EH, Swift PG, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med. 1985;313(6):353–60.

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337–8.

Brooks-Worrell B, Gersuk VH, Greenbaum C, Palmer JP. Intermolecular antigen spreading occurs during the preclinical period of human type 1 diabetes. J Immunol. 2001;166(8):5265–70.

Brown CT, vis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, Casella G, Drew JC, Ilonen J, Knip M, Hyoty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One. 2011;6(10):e25792.

Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010;10(12):849–59.

Bulek AM, Cole DK, Skowera A, Dolton G, Gras S, Madura F, Fuller A, Miles JJ, Gostick E, Price DA, Drijfhout JW, Knight RR, Huang GC, Lissin N, Molloy PE, Wooldridge L, Jakobsen BK, Rossjohn J, Peakman M, Rizkallah PJ, Sewell AK. Structural basis for the killing of human beta cells by CD8(+) T cells in type 1 diabetes. Nat Immunol. 2012;13(3):283–9.

Burch TC, Morris MA, Campbell-Thompson M, Pugliese A, Nadler JL, Nyalwidhe JO. Proteomic analysis of disease stratified human pancreas tissue indicates unique signature of type 1 diabetes. PLoS One. 2015;10(8):e0135663.

Burke GW 3rd, Posgai AL, Wasserfall CH, Atkinson MA, Pugliese A. Raising awareness: the need to promote allocation of pancreata from rare nondiabetic donors with pancreatic islet autoimmunity to type 1 diabetes research. Am J Transplant. 2016. https://doi.org/10.1111/ajt.13983. [Epub ahead of print].

Cabrera SM, Chen YG, Hagopian WA, Hessner MJ. Blood-based signatures in type 1 diabetes. Diabetologia. 2016;59(3):414–25.

Campbell-Thompson M, Wasserfall C, Montgomery EL, Atkinson MA, Kaddis JS. Pancreas organ weight in individuals with disease-associated autoantibodies at risk for type 1 diabetes. JAMA. 2012;308(22):2337–9.

Campbell-Thompson ML, Atkinson MA, Butler AE, Chapman NM, Frisk G, Gianani R, Giepmans BN, von Herrath MG, Hyoty H, Kay TW, Korsgren O, Morgan NG, Powers AC, Pugliese A, Richardson SJ, Rowe PA, Tracy S, In't Veld PA. The diagnosis of insulitis in human type 1 diabetes. Diabetologia. 2013;56(11):2541–3.

Campbell-Thompson M, Rodriguez-Calvo T, Battaglia M. Abnormalities of the exocrine pancreas in type 1 diabetes. Curr Diab Rep. 2015;15(10):79.

Campbell-Thompson M, Fu A, Kaddis JS, Wasserfall C, Schatz DA, Pugliese A, Atkinson MA. Insulitis and beta-cell mass in the natural history of type 1 diabetes. Diabetes. 2016;65 (3):719–31.

Cepek P, Zajacova M, Kotrbova-Kozak A, Silhova E, Cerna M. DNA methylation and mRNA expression of HLA-DQA1 alleles in type 1 diabetes mellitus. Immunology. 2016;148(2):150–9.

Chapman NM, Kim KS, Drescher KM, Oka K, Tracy S. 5′ terminal deletions in the genome of a coxsackievirus B2 strain occurred naturally in human heart. Virology. 2008;375(2):480–91.

Chen YG, Cabrera SM, Jia S, Kaldunski ML, Kramer J, Cheong S, Geoffrey R, Roethle MF, Woodliff JE, Greenbaum CJ, Wang X, Hessner MJ. Molecular signatures differentiate immune states in type 1 diabetic families. Diabetes. 2014;63(11):3960–73.

Chentoufi AA, Polychronakos C. Insulin expression levels in the thymus modulate insulin-specific autoreactive T-cell tolerance: the mechanism by which the IDDM2 locus may predispose to diabetes. Diabetes. 2002;51(5):1383–90.

Chistiakov DA. Interferon induced with helicase C domain 1 (IFIH1) and virus-induced autoimmunity: a review. Viral Immunol. 2010;23(1):3–15.

Cohen JN, Guidi CJ, Tewalt EF, Qiao H, Rouhani SJ, Ruddell A, Farr AG, Tung KS, Engelhard VH. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med. 2010;207(4):681–8.

Colli ML, Moore F, Gurzov EN, Ortis F, Eizirik DL. MDA5 and PTPN2, two candidate genes for type 1 diabetes, modify pancreatic beta-cell responses to the viral by-product double-stranded RNA. Hum Mol Genet. 2010;19(1):135–46.

Cooper JD, Howson JM, Smyth D, Walker NM, Stevens H, Yang JH, She JX, Eisenbarth GS, Rewers M, Todd JA, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Pociot F, Rich SS. Confirmation of novel type 1 diabetes risk loci in families. Diabetologia. 2012;55(4):996–1000.

Coppieters KT, Wiberg A, Amirian N, Kay TW, von Herrath MG. Persistent glucose transporter expression on pancreatic beta cells from longstanding type 1 diabetic individuals. Diabetes Metab Res Rev. 2011;27(8):746–54.

Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med. 2012;209(1):51–60.

Cox SL, Silveira PA. Emerging roles for B lymphocytes in type 1 diabetes. Expert Rev Clin Immunol. 2009;5(3):311–24.

Dabelea D, D'Agostino RB Jr, Mayer-Davis EJ, Pettitt DJ, Imperatore G, Dolan LM, Pihoker C, Hillier TA, Marcovina SM, Linder B, Ruggiero AM, Hamman RF. Testing the accelerator hypothesis: body size, beta-cell function, and age at onset of type 1 (autoimmune) diabetes. Diabetes Care. 2006;29(2):290–4.

Davis-Richardson AG, Triplett EW. A model for the role of gut bacteria in the development of autoimmunity for type 1 diabetes. Diabetologia. 2015;58(7):1386–93.

de Beeck AO, Eizirik DL. Viral infections in type 1 diabetes mellitus – why the beta cells? Nat Rev Endocrinol. 2016;12(5):263–73.

Delong T, Wiles TA, Baker RL, Bradley B, Barbour G, Reisdorph R, Armstrong M, Powell RL, Reisdorph N, Kumar N, Elso CM, DeNicola M, Bottino R, Powers AC, Harlan DM, Kent SC, Mannering SI, Haskins K. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. Science. 2016;351(6274):711–4.

Derbinski J, Kyewski B. How thymic antigen presenting cells sample the body's self-antigens. Curr Opin Immunol. 2010;22(5):592–600.

Diedisheim M, Mallone R, Boitard C, Larger E. Beta-cell mass in nondiabetic autoantibody-positive subjects: an analysis based on the network for pancreatic organ donors database. J Clin Endocrinol Metab. 2016;101(4):1390–7.

Diez J, Park Y, Zeller M, Brown D, Garza D, Ricordi C, Hutton J, Eisenbarth GS, Pugliese A. Differential splicing of the IA-2 mRNA in pancreas and lymphoid organs as a permissive genetic mechanism for autoimmunity against the IA-2 type 1 diabetes autoantigen. Diabetes. 2001;50(4):895–900.

Dogra RS, Vaidyanathan P, Prabakar KR, Marshall KE, Hutton JC, Pugliese A. Alternative splicing of G6PC2, the gene coding for the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), results in differential expression in human thymus and spleen compared with pancreas. Diabetologia. 2006;49(5):953–7.

Doran TM, Morimoto J, Simanski S, Koesema EJ, Clark LF, Pels K, Stoops SL, Pugliese A, Skyler JS, Kodadek T. Discovery of phosphorylated peripherin as a major humoral autoantigen in type 1 diabetes mellitus. Cell Chem Biol. 2016;23(5):618–28.

Dotta F, Censini S, van Halteren AG, Marselli L, Masini M, Dionisi S, Mosca F, Boggi U, Muda AO, Prato SD, Elliott JF, Covacci A, Rappuoli R, Roep BO, Marchetti P. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A. 2007;104(12):5115–20.

Dunne JL, Triplett EW, Gevers D, Xavier R, Insel R, Danska J, Atkinson MA. The intestinal microbiome in type 1 diabetes. Clin Exp Immunol. 2014;177(1):30–7.

Durinovic-Bello I, Jelinek E, Schlosser M, Eiermann T, Boehm BO, Karges W, Marchand L, Polychronakos C. Class III alleles at the insulin VNTR polymorphism are associated with regulatory T-cell responses to proinsulin epitopes in HLA-DR4, DQ8 individuals. Diabetes. 2005;54(Suppl 2):S18–24.

Durinovic-Bello I, Wu RP, Gersuk VH, Sanda S, Shilling HG, Nepom GT. Insulin gene VNTR genotype associates with frequency and phenotype of the autoimmune response to proinsulin. Genes Immun. 2010;11(2):188–93.

Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med. 1986;314: 1360–8.

Eisenbarth GS. Banting lecture 2009: an unfinished journey: molecular pathogenesis to prevention of type 1A diabetes. Diabetes. 2010;59(4):759–74.

Eizirik DL, Sammeth M, Bouckenooghe T, Bottu G, Sisino G, Igoillo-Esteve M, Ortis F, Santin I, Colli ML, Barthson J, Bouwens L, Hughes L, Gregory L, Lunter G, Marselli L, Marchetti P, McCarthy MI, Cnop M. The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet. 2012;8(3): e1002552.

Eizirik DL, Miani M, Cardozo AK. Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia. 2013;56(2): 234–41.

Elboudwarej E, Cole M, Briggs FB, Fouts A, Fain PR, Quach H, Quach D, Sinclair E, Criswell LA, Lane JA, Steck AK, Barcellos LF, Noble JA. Hypomethylation within gene promoter regions and type 1 diabetes in discordant monozygotic twins. J Autoimmun. 2016;68:23–9.

Endesfelder D, Engel M, Castell WZ. Gut immunity and type 1 diabetes: a melange of microbes, diet, and host interactions? Curr Diab Rep. 2016;16(7):60.

Eringsmark Regnell S, Lernmark A. The environment and the origins of islet autoimmunity and type 1 diabetes. Diabet Med. 2013;30(2):155–60.

Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, Lavant E, Louey A, Moonsamy P. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008;57(4):1084–92.

Fan Y, Rudert WA, Grupillo M, He J, Sisino G, Trucco M. Thymus-specific deletion of insulin induces autoimmune diabetes. EMBO J. 2009;28(18):2812–24.

Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS. Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes. 2010;59(3):679–85.

Ferraro A, Socci C, Stabilini A, Valle A, Monti P, Piemonti L, Nano R, Olek S, Maffi P, Scavini M, Secchi A, Staudacher C, Bonifacio E, Battaglia M. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. Diabetes. 2011;60(11):2903–13.

Ferreira RC, Guo H, Coulson RM, Smyth DJ, Pekalski ML, Burren OS, Cutler AJ, Doecke JD, Flint S, McKinney EF, Lyons PA, Smith KG, Achenbach P, Beyerlein A, Dunger DB, Clayton DG, Wicker LS, Todd JA, Bonifacio E, Wallace C, Ziegler AG. A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. Diabetes. 2014;63(7):2538–50.

Fletcher AL, Lukacs-Kornek V, Reynoso ED, Pinner SE, Bellemare-Pelletier A, Curry MS, Collier AR, Boyd RL, Turley SJ. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. J Exp Med. 2010;207(4):689–97.

Floyel T, Kaur S, Pociot F. Genes affecting beta-cell function in type 1 diabetes. Curr Diab Rep. 2015;15(11):97.

Foulis AK, Farquharson MA. Aberrant expression of HLA-DR antigens by insulin containing beta cells in recent onset type I (insulin dependent) diabetes mellitus. Diabetes. 1986;35: 1215–26.

Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia. 1986;29(5):267–74.

Foulis AK, Farquharson MA, Hardman R. Aberrant expression of class II major histocompatibility complex molecules by beta cells and hyperexpression of class I major histocompatibility complex molecules by insulin containing islets in type I (insulin dependent) diabetes mellitus. Diabetologia. 1987a;30:333–43.

Foulis AK, Farquharson MA, Meager A. Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. Lancet. 1987b;2(8573):1423–7.

Fourlanos S, Narendran P, Byrnes GB, Colman PG, Harrison LC. Insulin resistance is a risk factor for progression to type 1 diabetes. Diabetologia. 2004;47(10):1661–7.

Fourlanos S, Varney MD, Tait BD, Morahan G, Honeyman MC, Colman PG, Harrison LC. The rising incidence of type 1 diabetes is accounted for by cases with lower-risk human leukocyte antigen genotypes. Diabetes Care. 2008;31(8):1546–9.

Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic beta-cell dysfunction in diabetes. Curr Diabetes Rev. 2013;9(1):25–53.

Gaglia JL, Guimaraes AR, Harisinghani M, Turvey SE, Jackson R, Benoist C, Mathis D, Weissleder R. Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. J Clin Invest. 2011;121(1):442–5.

Gallagher GR, Brehm MA, Finberg RW, Barton BA, Shultz LD, Greiner DL, Bortell R, Wang JP. Viral infection of engrafted human islets leads to diabetes. Diabetes. 2015;64(4):1358–69.

Gardner JM, DeVoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X, Johannes KP, Su MA, Chang HY, Krummel MF, Anderson MS. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science. 2008;321(5890):843–7.

Gardner JM, Metzger TC, McMahon EJ, Au-Yeung BB, Krawisz AK, Lu W, Price JD, Johannes KP, Satpathy AT, Murphy KM, Tarbell KV, Weiss A, Anderson MS. Extrathymic Aire-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of CD4(+) T cells. Immunity. 2013;39(3):560–72.

Garg G, Tyler JR, Yang JH, Cutler AJ, Downes K, Pekalski M, Bell GL, Nutland S, Peakman M, Todd JA, Wicker LS, Tree TI. Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. J Immunol. 2012;188 (9):4644–53.

Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes. 1965;14 (10):619–33.

Gerold KD, Zheng P, Rainbow DB, Zernecke A, Wicker LS, Kissler S. The soluble CTLA-4 splice variant protects from type 1 diabetes and potentiates regulatory T-cell function. Diabetes. 2011;60(7):1955–63.

Gianani R, Putnam A, Still T, Yu L, Miao D, Gill RG, Beilke J, Supon P, Valentine A, Iveson A, Dunn S, Eisenbarth GS, Hutton J, Gottlieb P, Wiseman A. Initial results of screening of nondiabetic organ donors for expression of islet autoantibodies. J Clin Endocrinol Metab. 2006;91(5):1855–61.

Giannopoulou EZ, Winkler C, Chmiel R, Matzke C, Scholz M, Beyerlein A, Achenbach P, Bonifacio E, Ziegler AG. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. Diabetologia. 2015;58(10):2317–23.

Gillespie KM, Bain SC, Barnett AH, Bingley PJ, Christie MR, Gill GV, Gale EA. The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. Lancet. 2004;364(9446):1699–700.

Greenbaum CJ, Anderson AM, Dolan LM, Mayer-Davis EJ, Dabelea D, Imperatore G, Marcovina S, Pihoker C. Preservation of beta-cell function in autoantibody-positive youth with diabetes. Diabetes Care. 2009;32(10):1839–44.

Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, Lachin JM, McGee P, Palmer JP, Pescovitz MD, Krause-Steinrauf H, Skyler JS, Sosenko JM. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite type 1 diabetes TrialNet data. Diabetes. 2012;61(8):2066–73.

Grieco FA, Moore F, Vigneron F, Santin I, Villate O, Marselli L, Rondas D, Korf H, Overbergh L, Dotta F, Marchetti P, Mathieu C, Eizirik DL. IL-17A increases the expression of pro-inflammatory chemokines in human pancreatic islets. Diabetologia. 2014;57(3):502–11.

Grinberg-Bleyer Y, Baeyens A, You S, Elhage R, Fourcade G, Gregoire S, Cagnard N, Carpentier W, Tang Q, Bluestone J, Chatenoud L, Klatzmann D, Salomon BL, Piaggio E. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. J Exp Med. 2010;207(9):1871–8.

Grupillo M, Gualtierotti G, He J, Sisino G, Bottino R, Rudert WA, Trucco M, Fan Y. Essential roles of insulin expression in Aire+ tolerogenic dendritic cells in maintaining peripheral self-tolerance of islet beta-cells. Cell Immunol. 2012;273(2):115–23.

Grzesik WJ, Nadler JL, Machida Y, Nadler JL, Imai Y, Morris MA. Expression pattern of 12-lipoxygenase in human islets with type 1 diabetes and type 2 diabetes. J Clin Endocrinol Metab. 2015;100(3):E387–95.

Guay C, Jacovetti C, Nesca V, Motterle A, Tugay K, Regazzi R. Emerging roles of non-coding RNAs in pancreatic beta-cell function and dysfunction. Diabetes Obes Metab. 2012;14(Suppl 3):12–21. https://doi.org/10.1111/j.1463-1326.2012.01654.x.

Gulden E, Wong FS, Wen L. The gut microbiota and type 1 diabetes. Clin Immunol. 2015; 159(2):143–53.

Hao W, Gitelman S, DiMeglio LA, Boulware D, Greenbaum CJ. Fall in C-peptide during first 4 years from diagnosis of type 1 diabetes: variable relation to age, HbA1c, and insulin dose. Diabetes Care. 2016;39(10):1664–70.

Harjutsalo V, Reunanen A, Tuomilehto J. Differential transmission of type 1 diabetes from diabetic fathers and mothers to their offspring. Diabetes. 2006;55(5):1517–24.

Harrison LC, Campbell IL, Allison J, Miller JFAP. MHC molecules and cell destruction. Immune and nonimmune mechanisms. Diabetes. 1989;38:815–8.

Hartemann A, Bensimon G, Payan CA, Jacqueminet S, Bourron O, Nicolas N, Fonfrede M, Rosenzwajg M, Bernard C, Klatzmann D. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2013;1(4):295–305.

Hermann R, Knip M, Veijola R, Simell O, Laine AP, Akerblom HK, Groop PH, Forsblom C, Pettersson-Fernholm K, Ilonen J. Temporal changes in the frequencies of HLA genotypes in patients with type 1 diabetes – indication of an increased environmental pressure? Diabetologia. 2003;46(3):420–5.

Herold KC, Usmani-Brown S, Ghazi T, Lebastchi J, Beam CA, Bellin MD, Ledizet M, Sosenko JM, Krischer JP, Palmer JP. Beta cell death and dysfunction during type 1 diabetes development in at-risk individuals. J Clin Invest. 2015;125(3):1163–73.

Hilbrands R, Huurman VA, Gillard P, Velthuis JH, De WM, Mathieu C, Kaufman L, Pipeleers-Marichal M, Ling Z, Movahedi B, Jacobs-Tulleneers-Thevissen D, Monbaliu D, Ysebaert D, Gorus FK, Roep BO, Pipeleers DG, Keymeulen B. Differences in baseline lymphocyte counts and autoreactivity are associated with differences in outcome of islet cell transplantation in type 1 diabetic patients. Diabetes. 2009;58(10):2267–76.

Hyoty H. Viruses in type 1 diabetes. Pediatr Diabetes. 2016;17(Suppl 22):56–64.

Imagawa A, Hanafusa T, Tamura S, Moriwaki M, Itoh N, Yamamoto K, Iwahashi H, Yamagata K, Waguri M, Nanmo T, Uno S, Nakajima H, Namba M, Kawata S, Miyagawa JI, Matsuzawa Y. Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. Diabetes. 2001;50(6):1269–73.

In't Veld P. Insulitis in human type 1 diabetes: the quest for an elusive lesion. Islets. 2011a; 3(4):131–8.

In't Veld P. Insulitis in the human endocrine pancreas: does a viral infection lead to inflammation and beta cell replication? Diabetologia. 2011b;54(9):2220–2.

In't Veld P, Lievens D, De Grijse J, Ling Z, Van der Auwera B, Pipeleers-Marichal M, Gorus F, Pipeleers D. Screening for insulitis in adult autoantibody-positive organ donors. Diabetes. 2007;56(9):2400–4.

Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark A, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015;38(10):1964–74.

Jaeger C, Brendel MD, Eckhard M, Bretzel RG. Islet autoantibodies as potential markers for disease recurrence in clinical islet transplantation. Exp Clin Endocrinol Diabetes. 2000;108(5):328–33.

Jaidane H, Sane F, Hiar R, Goffard A, Gharbi J, Geenen V, Hober D. Immunology in the clinic review series; focus on type 1 diabetes and viruses: enterovirus, thymus and type 1 diabetes pathogenesis. Clin Exp Immunol. 2012;168(1):39–46.

James EA, Mallone R, Schloot NC, Gagnerault MC, Thorpe J, Fitzgerald-Miller L, Reichow J, Wagner R, Pham MN, Jospe N, Lou O, Gottlieb PA, Brooks-Worrell BM, Durinovic-Bello I. Immunology of diabetes society T-cell workshop: HLA class II tetramer-directed epitope validation initiative. Diabetes Metab Res Rev. 2011;27(8):727–36.

Jarchum I, DiLorenzo TP. Ins2 deficiency augments spontaneous HLA-A*0201-restricted T cell responses to insulin. J Immunol. 2010;184(2):658–65.

Javierre BM, Hernando H, Ballestar E. Environmental triggers and epigenetic deregulation in autoimmune disease. Discov Med. 2011;12(67):535–45.

Jia S, Kaldunski M, Jailwala P, Geoffrey R, Kramer J, Wang X, Hessner MJ. Use of transcriptional signatures induced in lymphoid and myeloid cell lines as an inflammatory biomarker in type 1 diabetes. Physiol Genomics. 2011;43(11):697–709.

Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL. Residual insulin production and pancreatic ss-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes. 2010;59(11):2846–53.

Kent SC, Chen Y, Bregoli L, Clemmings SM, Kenyon NS, Ricordi C, Hering BJ, Hafler DA. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature. 2005;435(7039):224–8.

Kim KW, Ho A, Alshabee-Akil A, Hardikar AA, Kay TW, Rawlinson WD, Craig ME. Coxsackievirus B5 infection induces dysregulation of microRNAs predicted to target known type 1 diabetes risk genes in human pancreatic islets. Diabetes. 2016;65(4):996–1003.

Klinke DJ. Extent of beta cell destruction is important but insufficient to predict the onset of type 1 diabetes mellitus. PLoS One. 2008;3(1):e1374.

Klinke DJ. Age-corrected beta cell mass following onset of type 1 diabetes mellitus correlates with plasma C-peptide in humans. PLoS One. 2011;6(11):e26873.

Knip M, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. Nat Rev Endocrinol. 2016;12(3):154–67.

Kodama K, Butte AJ, Creusot RJ, Su L, Sheng D, Hartnett M, Iwai H, Soares LR, Fathman CG. Tissue- and age-specific changes in gene expression during disease induction and progression in NOD mice. Clin Immunol. 2008;129(2):195–201.

Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP III, Armand P, Cutler C, Ho VT, Treister NS, Bienfang DC, Prasad S, Tzachanis D, Joyce RM, Avigan DE, Antin JH, Ritz J, Soiffer RJ. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med. 2011;365(22):2055–66.

Korpos E, Kadri N, Kappelhoff R, Wegner J, Overall CM, Weber E, Holmberg D, Cardell S, Sorokin L. The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. Diabetes. 2013;62(2):531–42.

Krogvold L, Edwin B, Buanes T, Ludvigsson J, Korsgren O, Hyoty H, Frisk G, Hanssen KF, Dahl-Jorgensen K. Pancreatic biopsy by minimal tail resection in live adult patients at the onset of type 1 diabetes: experiences from the DiViD study. Diabetologia. 2014;57(4):841–3.

Krogvold L, Edwin B, Buanes T, Frisk G, Skog O, Anagandula M, Korsgren O, Undlien D, Eike MC, Richardson SJ, Leete P, Morgan NG, Oikarinen S, Oikarinen M, Laiho JE, Hyoty H, Ludvigsson J, Hanssen KF, Dahl-Jorgensen K. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. Diabetes. 2015a;64(5):1682–7.

Krogvold L, Skog O, Sundstrom G, Edwin B, Buanes T, Hanssen KF, Ludvigsson J, Grabherr M, Korsgren O, Dahl-Jorgensen K. Function of isolated pancreatic islets from patients at onset of type 1 diabetes: insulin secretion can be restored after some days in a nondiabetogenic environment in vitro: results from the DiViD study. Diabetes. 2015b;64(7):2506–12.

Krogvold L, Wiberg A, Edwin B, Buanes T, Jahnsen FL, Hanssen KF, Larsson E, Korsgren O, Skog O, Dahl-Jorgensen K. Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. Diabetologia. 2016;59(3):492–501.

Kronenberg D, Knight RR, Estorninho M, Ellis RJ, Kester MG, de Ru A, Eichmann M, Huang GC, Powrie J, Dayan CM, Skowera A, van Veelen PA, Peakman M. Circulating preproinsulin signal peptide-specific CD8 T cells restricted by the susceptibility molecule HLA-A24 are expanded at onset of type 1 diabetes and Kill beta-cells. Diabetes. 2012;61(7):1752–9.

Kuipers HF, Rieck M, Gurevich I, Nagy N, Butte MJ, Negrin RS, Wight TN, Steinman L, Bollyky PL. Hyaluronan synthesis is necessary for autoreactive T-cell trafficking, activation, and Th1 polarization. Proc Natl Acad Sci U S A. 2016;113(5):1339–44.

Kyewski B, Klein L. A central role for central tolerance. Annu Rev Immunol. 2006;24:571–606.

Lafferty KJ, Wang Y. Ectopic class II MHC antigen expression and the development of diabetes. J Autoimmun. 1990;3:75–80.

Leete P, Willcox A, Krogvold L, Dahl-Jorgensen K, Foulis AK, Richardson SJ, Morgan NG. Differential insulitic profiles determine the extent of beta-cell destruction and the age at onset of type 1 diabetes. Diabetes. 2016;65(5):1362–9.

Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, Pihoker C, Dabelea D, Hamman R, Waitzfelder B, Kahn HS. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. Pediatr Diabetes. 2010;11(1):4–11.

Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, Zhang ZY, Pihoker C, Sanda S, Greenbaum C, Buckner JH. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes. 2010;59(2):407–15.

Long SA, Cerosaletti K, Wan JY, Ho JC, Tatum M, Wei S, Shilling HG, Buckner JH. An autoimmune-associated variant in PTPN2 reveals an impairment of IL-2R signaling in CD4 (+) T cells. Genes Immun. 2011;12(2):116–25.

Lonnrot M, Knip M, Roivainen M, Koskela P, Akerblom HK, Hyoty H. Onset of type 1 diabetes mellitus in infancy after enterovirus infections. Diabet Med. 1998;15(5):431–4.

Lowe CE, Cooper JD, Brusko T, Walker NM, Smyth DJ, Bailey R, Bourget K, Plagnol V, Field S, Atkinson M, Clayton DG, Wicker LS, Todd JA. Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. Nat Genet. 2007;39(9):1074–82.

Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am. 2010;39(3):481–97.

Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity. 2010;33(2):153–65.

Mannering SI, Harrison LC, Williamson NA, Morris JS, Thearle DJ, Jensen KP, Kay TW, Rossjohn J, Falk BA, Nepom GT, Purcell AW. The insulin A-chain epitope recognized by human T cells is posttranslationally modified. J Exp Med. 2005;202(9):1191–7.

Marhfour I, Lopez XM, Lefkaditis D, Salmon I, Allagnat F, Richardson SJ, Morgan NG, Eizirik DL. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia. 2012;55(9):2417–20.

Marre ML, James EA, Piganelli JD. Beta cell ER stress and the implications for immunogenicity in type 1 diabetes. Front Cell Dev Biol. 2015;3:67.

Marroqui L, Santin I, Dos Santos RS, Marselli L, Marchetti P, Eizirik DL. BACH2, a candidate risk gene for type 1 diabetes, regulates apoptosis in pancreatic beta-cells via JNK1 modulation and crosstalk with the candidate gene PTPN2. Diabetes. 2014;63(7):2516–27.

Marroqui L, Dos Santos RS, Floyel T, Grieco FA, Santin I, Op de Beeck A, Marselli L, Marchetti P, Pociot F, Eizirik DL. TYK2, a candidate gene for type 1 diabetes, modulates apoptosis and the innate immune response in human pancreatic beta-cells. Diabetes. 2015;64(11):3808–17.

Martinuzzi E, Afonso G, Gagnerault MC, Naselli G, Mittag D, Combadiere B, Boitard C, Chaput N, Zitvogel L, Harrison LC, Mallone R. acDCs enhance human antigen-specific T-cell responses. Blood. 2011;118(8):2128–37.

McGinty JW, Chow IT, Greenbaum C, Odegard J, Kwok WW, James EA. Recognition of post-translationally modified GAD65 epitopes in subjects with type 1 diabetes. Diabetes. 2014;63 (9):3033–40.

McGinty JW, Marre ML, Bajzik V, Piganelli JD, James EA. T cell epitopes and post-translationally modified epitopes in type 1 diabetes. Curr Diab Rep. 2015;15(11):90.

Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia. 2005a;48(11):2221–8.

Meier JJ, Ritzel RA, Maedler K, Gurlo T, Butler PC. Increased vulnerability of newly forming beta cells to cytokine-induced cell death. Diabetologia. 2005b;49:83–9.

Mejia-Leon ME, Barca AM. Diet, microbiota and immune system in type 1 diabetes development and evolution. Nutrients. 2015;7(11):9171–84.

Menard L, Saadoun D, Isnardi I, Ng YS, Meyers G, Massad C, Price C, Abraham C, Motaghedi R, Buckner JH, Gregersen PK, Meffre E. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. J Clin Invest. 2011; 121(9):3635–44.

Miao F, Chen Z, Zhang L, Liu Z, Wu X, Yuan YC, Natarajan R. Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. J Biol Chem. 2012; 287(20):16335–45.

Mizrahi M, Ilan Y. The gut mucosa as a site for induction of regulatory T-cells. Curr Pharm Des. 2009;15(11):1191–202.

Mohan JF, Petzold SJ, Unanue ER. Register shifting of an insulin peptide-MHC complex allows diabetogenic T cells to escape thymic deletion. J Exp Med. 2011;208(12):2375–83.

Monti P, Heninger AK, Bonifacio E. Differentiation, expansion, and homeostasis of autoreactive T cells in type 1 diabetes mellitus. Curr Diab Rep. 2009;9(2):113–8.

Moriyama H, Abiru N, Paronen J, Sikora K, Liu E, Miao D, Devendra D, Beilke J, Gianani R, Gill RG, Eisenbarth GS. Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. Proc Natl Acad Sci U S A. 2003;100(18):10376–81.

Nagy N, Kaber G, Johnson PY, Gebe JA, Preisinger A, Falk BA, Sunkari VG, Gooden MD, Vernon RB, Bogdani M, Kuipers HF, Day AJ, Campbell DJ, Wight TN, Bollyky PL. Inhibition of hyaluronan synthesis restores immune tolerance during autoimmune insulitis. J Clin Invest. 2015;125(10):3928–40.

Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, Yu L, Wegmann DR, Hutton JC, Elliott JF, Eisenbarth GS. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature. 2005;435(7039):220–3.

Nakayama M, McDaniel K, Fitzgerald-Miller L, Kiekhaefer C, Snell-Bergeon JK, Davidson HW, Rewers M, Yu L, Gottlieb P, Kappler JW, Michels A. Regulatory vs. inflammatory cytokine T-cell responses to mutated insulin peptides in healthy and type 1 diabetic subjects. Proc Natl Acad Sci U S A. 2015a;112(14):4429–34.

Nakayama M, Simmons KM, Michels AW. Molecular interactions governing autoantigen presentation in type 1 diabetes. Curr Diab Rep. 2015b;15(12):113.

Nejentsev S, Howson JM, Walker NM, Szeszko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD,

Clayton DG, Todd JA. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. Nature. 2007;450(7171):887–92.
Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science. 2009;324(5925):387–9.
Nguyen H, James EA. Immune recognition of citrullinated epitopes. Immunology. 2016;149(2): 131–8.
Noble JA, Valdes AM, Varney MD, Carlson JA, Moonsamy P, Fear AL, Lane JA, Lavant E, Rappner R, Louey A, Concannon P, Mychaleckyj JC, Erlich HA. HLA class I and genetic susceptibility to type 1 diabetes: results from the type 1 diabetes genetics consortium. Diabetes. 2010;59(11):2972–9.
O'Neill SK, Liu E, Cambier JC. Change you can B(cell)eive in: recent progress confirms a critical role for B cells in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes. 2009;16(4):293–8.
Oikarinen M, Tauriainen S, Honkanen T, Vuori K, Karhunen P, Vasama-Nolvi C, Oikarinen S, Verbeke C, Blair GE, Rantala I, Ilonen J, Simell O, Knip M, Hyoty H. Analysis of pancreas tissue in a child positive for islet cell antibodies. Diabetologia. 2008;51(10):1796–802.
Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H, Ilonen J, Veijola R, Simell O, Knip M, Hyoty H. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes. 2011;60(1):276–9.
Oikarinen M, Tauriainen S, Oikarinen S, Honkanen T, Collin P, Rantala I, Maki M, Kaukinen K, Hyoty H. Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes. 2012;61(3):687–91.
Onengut-Gumuscu S, Chen WM, Burren O, Cooper NJ, Quinlan AR, Mychaleckyj JC, Farber E, Bonnie JK, Szpak M, Schofield E, Achuthan P, Guo H, Fortune MD, Stevens H, Walker NM, Ward LD, Kundaje A, Kellis M, Daly MJ, Barrett JC, Cooper JD, Deloukas P, Todd JA, Wallace C, Concannon P, Rich SS. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. Nat Genet. 2015;47(4):381–6.
Oram RA, Jones AG, Besser RE, Knight BA, Shields BM, Brown RJ, Hattersley AT, McDonald TJ. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia. 2014;57(1):187–91.
Oram RA, McDonald TJ, Shields BM, Hudson MM, Shepherd MH, Hammersley S, Pearson ER, Hattersley AT. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. Diabetes Care. 2015;38(2):323–8.
Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Krischer JP, Skyler JS. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet. 2011;378(9789):412–9.
Orban T, Bundy B, Becker DJ, Dimeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Peakman M, Raskin P, Russell WE, Schatz D, Wherrett DK, Wilson DM, Krischer JP, Skyler JS. Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. Diabetes Care. 2014;37(4):1069–75.
Oresic M, Simell S, Sysi-Aho M, Nanto-Salonen K, Seppanen-Laakso T, Parikka V, Katajamaa M, Hekkala A, Mattila I, Keskinen P, Yetukuri L, Reinikainen A, Lahde J, Suortti T, Hakalax J, Simell T, Hyoty H, Veijola R, Ilonen J, Lahesmaa R, Knip M, Simell O. Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. J Exp Med. 2008;205(13):2975–84.
Osmai M, Osmai Y, Bang-Berthelsen CH, Pallesen EM, Vestergaard AL, Novotny GW, Pociot F, Mandrup-Poulsen T. MicroRNAs as regulators of beta-cell function and dysfunction. Diabetes Metab Res Rev. 2016;32(4):334–49.
Overgaard AJ, Kaur S, Pociot F. Metabolomic biomarkers in the progression to type 1 diabetes. Curr Diab Rep. 2016;16(12):127.

Pang TT, Narendran P. Addressing insulin resistance in type 1 diabetes. Diabet Med. 2008; 25(9):1015–24.

Paun A, Yau C, Danska JS. Immune recognition and response to the intestinal microbiome in type 1 diabetes. J Autoimmun. 2016;71:10–8.

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Lachin JM, Skyler JS. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009;361(22):2143–52.

Pflueger M, Seppanen-Laakso T, Suortti T, Hyotylainen T, Achenbach P, Bonifacio E, Oresic M, Ziegler AG. Age- and islet autoimmunity-associated differences in amino acid and lipid metabolites in children at risk for type 1 diabetes. Diabetes. 2011;60(11):2740–7.

Phelps EA, Cianciaruso C, Michael IP, Pasquier M, Kanaani J, Nano R, Lavallard V, Billestrup N, Hubbell JA, Baekkeskov S. Aberrant accumulation of the diabetes autoantigen GAD65 in Golgi membranes in conditions of er stress and autoimmunity. Diabetes. 2016;65(9):2686–99.

Pociot F, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, Nierras CR, Todd JA, Rich SS, Nerup J. Genetics of type 1 diabetes: what's next? Diabetes. 2010;59(7):1561–71.

Pugliese A. The multiple origins of type 1 diabetes. Diabet Med. 2013;30(2):135–46.

Pugliese A. Insulitis in the pathogenesis of type 1 diabetes. Pediatr Diabetes. 2016;17(Suppl 22):31–6.

Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, Jackson RA, Eisenbarth GS. HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. Diabetes. 1995; 44(6):608–13.

Pugliese A, Zeller M, Fernandez AJ, Zalcberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the *INS* VNTR-*IDDM2* susceptibility locus for type 1 diabetes. Nat Genet. 1997;15:293–7.

Pugliese A, Brown D, Garza D, Murchison D, Zeller M, Redondo MJ, Diez J, Eisenbarth GS, Patel DD, Ricordi C. Self-antigen presenting cells expressing IDDM-associated autoantigens exist in both thymus and peripheral lymphoid organs in humans. J Clin Investig. 2001;107:585–93.

Pugliese A, Yang M, Kusmarteva I, Heiple T, Vendrame F, Wasserfall C, Rowe P, Moraski JM, Ball S, Jebson L, Schatz DA, Gianani R, Burke GW, Nierras C, Staeva T, Kaddis JS, Campbell-Thompson M, Atkinson MA. The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD) Program: goals, operational model and emerging findings. Pediatr Diabetes. 2014;15(1):1–9.

Pugliese A, Boulware D, Yu L, Babu S, Steck AK, Becker D, Rodriguez H, DiMeglio L, Evans-Molina C, Harrison LC, Schatz D, Palmer JP, Greenbaum C, Eisenbarth GS, Sosenko JM. HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype protects autoantibody-positive relatives from type 1 diabetes throughout the stages of disease progression. Diabetes. 2016;65 (4):1109–19.

Rakyan VK, Beyan H, Down TA, Hawa MI, Maslau S, Aden D, Daunay A, Busato F, Mein CA, Manfras B, Dias KR, Bell CG, Tost J, Boehm BO, Beck S, Leslie RD. Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis. PLoS Genet. 2011;7(9):e1002300.

Reddy S, Zeng N, Al-Diery H, Jung D, Yeu C, Joret MO, Merrilees MJ, Wu F. Analysis of peri-islet CD45-positive leucocytic infiltrates in long-standing type 1 diabetic patients. Diabetologia. 2015;58(5):1024–35.

Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, Leslie RD. Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. Diabetologia. 2001;44(3):354–62.

Redondo MJ, Fain PR, Krischer JP, Yu L, Cuthbertson D, Winter WE, Eisenbarth GS. Expression of beta-cell autoimmunity does not differ between potential dizygotic twins and siblings of patients with type 1 diabetes. J Autoimmun. 2004;23(3):275–9.

Redondo MJ, Jeffrey J, Fain PR, Eisenbarth GS, Orban T. Concordance for islet autoimmunity among monozygotic twins. N Engl J Med. 2008;359(26):2849–50.

Redondo MJ, Muniz J, Rodriguez LM, Iyer D, Vaziri-Sani F, Haymond MW, Hampe CS, Metzker ML, Grant SF, Balasubramanyam A. Association of TCF7L2 variation with single islet autoantibody expression in children with type 1 diabetes. BMJ Open Diabetes Res Care. 2014;2(1):e000008.

Reiner T, Thurber G, Gaglia J, Vinegoni C, Liew CW, Upadhyay R, Kohler RH, Li L, Kulkarni RN, Benoist C, Mathis D, Weissleder R. Accurate measurement of pancreatic islet beta-cell mass using a second-generation fluorescent exendin-4 analog. Proc Natl Acad Sci U S A. 2011;108(31):12815–20.

Resic-Lindehammer S, Larsson K, Ortqvist E, Carlsson A, Cederwall E, Cilio CM, Ivarsson SA, Jonsson BA, Larsson HE, Lynch K, Neiderud J, Nilsson A, Sjoblad S, Lernmark A, Aili M, Baath LE, Carlsson E, Edenwall H, Forsander G, Granstro BW, Gustavsson I, Hanas R, Hellenberg L, Hellgren H, Holmberg E, Hornell H, Ivarsson SA, Johansson C, Jonsell G, Kockum K, Lindblad B, Lindh A, Ludvigsson J, Myrdal U, Neiderud J, Segnestam K, Sjoblad S, Skogsberg L, Stromberg L, Stahle U, Thalme B, Tullus K, Tuvemo T, Wallensteen M, Westphal O, Aman J. Temporal trends of HLA genotype frequencies of type 1 diabetes patients in Sweden from 1986 to 2005 suggest altered risk. Acta Diabetol. 2008;45(4):231–5.

Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet. 2016;387(10035):2340–8.

Rich SS. Mapping genes in diabetes. Genetic epidemiological perspective. Diabetes. 1990;39(11):1315–9.

Rich SS, Akolkar B, Concannon P, Erlich H, Hilner JE, Julier C, Morahan G, Nerup J, Nierras C, Pociot F, Todd JA. Overview of the type I Diabetes Genetics Consortium. Genes Immun. 2009;10(Suppl 1):S1–4.

Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia. 2009;52(6):1143–51.

Richardson SJ, Leete P, Bone AJ, Foulis AK, Morgan NG. Expression of the enteroviral capsid protein VP1 in the islet cells of patients with type 1 diabetes is associated with induction of protein kinase R and downregulation of Mcl-1. Diabetologia. 2013;56(1):185–93.

Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, Zeissler M, Leete P, Krogvold L, Dahl-Jorgensen K, von Herrath M, Pugliese A, Atkinson MA, Morgan NG. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016;59(11):2448–58.

Rigby MR, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, Patel CM, Griffin KJ, Tsalikian E, Gottlieb PA, Greenbaum CJ, Sherry NA, Moore WV, Monzavi R, Willi SM, Raskin P, Moran A, Russell WE, Pinckney A, Keyes-Elstein L, Howell M, Aggarwal S, Lim N, Phippard D, Nepom GT, McNamara J, Ehlers MR. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Diabetes Endocrinol. 2013;1(4):284–94.

Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, Griffin KJ, Tsalikian E, Gottlieb PA, Greenbaum CJ, Sherry NA, Moore WV, Monzavi R, Willi SM, Raskin P, Keyes-Elstein L, Long SA, Kanaparthi S, Lim N, Phippard D, Soppe CL, Fitzgibbon ML, McNamara J, Nepom GT, Ehlers MR. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. J Clin Invest. 2015;125(8):3285–96.

Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. Diabetes. 2014;63(11):3880–90.

Roep BO, Peakman M. Antigen targets of type 1 diabetes autoimmunity. Cold Spring Harb Perspect Med. 2012;2(4):a007781.

Roivainen M, Knip M, Hyoty H, Kulmala P, Hiltunen M, Vahasalo P, Hovi T, Akerblom HK. Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. Childhood Diabetes in Finland (DiMe) Study Group. J Med Virol. 1998;56(1):74–8.

Rosenzwajg M, Churlaud G, Mallone R, Six A, Derian N, Chaara W, Lorenzon R, Long SA, Buckner JH, Afonso G, Pham HP, Hartemann A, Yu A, Pugliese A, Malek TR, Klatzmann D. Low-dose interleukin-2 fosters a dose-dependent regulatory T cell tuned milieu in T1D patients. J Autoimmun. 2015;58:48–58.

Rui J, Deng S, Lebastchi J, Clark PL, Usmani-Brown S, Herold KC. Methylation of insulin DNA in response to proinflammatory cytokines during the progression of autoimmune diabetes in NOD mice. Diabetologia. 2016;59(5):1021–9.

Saadoun D, Rosenzwajg M, Joly F, Six A, Carrat F, Thibault V, Sene D, Cacoub P, Klatzmann D. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. N Engl J Med. 2011;365(22):2067–77.

Santin I, Moore F, Colli ML, Gurzov EN, Marselli L, Marchetti P, Eizirik DL. PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic beta-cell apoptosis via regulation of the BH3-only protein Bim. Diabetes. 2011;60(12):3279–88.

Sarkar SA, Lee CE, Victorino F, Nguyen TT, Walters JA, Burrack A, Eberlein J, Hildemann SK, Homann D. Expression and regulation of chemokines in murine and human type 1 diabetes. Diabetes. 2012;61(2):436–46.

Schloot NC, Meierhoff G, Karlsson FM, Ott P, Putnam A, Lehmann P, Gottlieb P, Roep BO, Peakman M, Tree T. Comparison of cytokine ELISpot assay formats for the detection of islet antigen autoreactive T cells. Report of the third immunology of diabetes society T-cell workshop. J Autoimmun. 2003;21(4):365–76.

Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C, Buckner JH. The effector T cells of diabetic subjects are resistant to regulation via $CD4^+$ $FOXP3^+$ regulatory T cells. J Immunol. 2008;181(10):7350–5.

Schubert DA, Gordo S, Sabatino JJ Jr, Vardhana S, Gagnon E, Sethi DK, Seth NP, Choudhuri K, Reijonen H, Nepom GT, Evavold BD, Dustin ML, Wucherpfennig KW. Self-reactive human CD4 T cell clones form unusual immunological synapses. J Exp Med. 2012;209(2):335–52.

Schulte BM, Lanke KH, Piganelli JD, Kers-Rebel ED, Bottino R, Trucco M, Huijbens RJ, Radstake TR, Engelse MA, de Koning EJ, Galama JM, Adema GJ, van Kuppeveld FJ. Cytokine and chemokine production by human pancreatic islets upon enterovirus infection. Diabetes. 2012;61(8):2030–6.

Seyhan AA, Nunez Lopez YO, Xie H, Yi F, Mathews C, Pasarica M, Pratley RE. Pancreas-enriched miRNAs are altered in the circulation of subjects with diabetes: a pilot cross-sectional study. Sci Rep. 2016;6:31479.

Sherr JL, Ghazi T, Wurtz A, Rink L, Herold KC. Characterization of residual beta cell function in long-standing type 1 diabetes. Diabetes Metab Res Rev. 2014;30(2):154–62.

Sherry NA, Tsai EB, Herold KC. Natural history of beta-cell function in type 1 diabetes. Diabetes. 2005;54(Suppl 2):S32–9.

Simeonovic CJ, Ziolkowski AF, Wu Z, Choong FJ, Freeman C, Parish CR. Heparanase and autoimmune diabetes. Front Immunol. 2013;4:471. eCollection@2013: 471

Sims EK, Chaudhry Z, Watkins R, Syed F, Blum J, Ouyang F, Perkins SM, Mirmira RG, Sosenko J, DiMeglio LA, Evans-Molina C. Elevations in the fasting serum proinsulin-to-C-peptide ratio precede the onset of type 1 diabetes. Diabetes Care. 2016;39(9):1519–26.

Skog O, Korsgren S, Wiberg A, Danielsson A, Edwin B, Buanes T, Krogvold L, Korsgren O, Dahl-Jorgensen K. Expression of human leukocyte antigen class I in endocrine and exocrine pancreatic tissue at onset of type 1 diabetes. Am J Pathol. 2015;185(1):129–38.

Skowera A, Ellis RJ, Varela-Calvino R, Arif S, Huang GC, Van-Krinks C, Zaremba A, Rackham C, Allen JS, Tree TI, Zhao M, Dayan CM, Sewell AK, Unger W, Drijfhout JW, Ossendorp F, Roep BO, Peakman M. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. J Clin Invest. 2008;118(10):3390–402.

Skyler JS. Prevention and reversal of type 1 diabetes – past challenges and future opportunities. Diabetes Care. 2015;38(6):997–1007.

Smith MJ, Packard TA, O'Neill SK, Henry Dunand CJ, Huang M, Fitzgerald-Miller L, Stowell D, Hinman RM, Wilson PC, Gottlieb PA, Cambier JC. Loss of anergic B cells in prediabetic and new-onset type 1 diabetic patients. Diabetes. 2015;64(5):1703–12.

Smyth DJ, Cooper JD, Bailey R, Field S, Burren O, Smink LJ, Guja C, Ionescu-Tirgoviste C, Widmer B, Dunger DB, Savage DA, Walker NM, Clayton DG, Todd JA. A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet. 2006;38(6):617–9.

Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, Krischer JP, Chase HP, White NH, Buckingham B, Herold KC, Cuthbertson D, Skyler JS. Patterns of metabolic progression to type 1 diabetes in the diabetes prevention trial-type 1. Diabetes Care. 2006;29(3):643–9.

Sosenko JM, Skyler JS, Krischer JP, Greenbaum CJ, Mahon J, Rafkin LE, Cuthbertson D, Cowie C, Herold K, Eisenbarth G, Palmer JP. Glucose excursions between states of glycemia with progression to type 1 diabetes in the diabetes prevention trial-type 1 (DPT-1). Diabetes. 2010;59(10):2386–9.

Sosinowski T, Eisenbarth GS. Type 1 diabetes: primary antigen/peptide/register/trimolecular complex. Immunol Res. 2013;55(1–3):270–6.

Stadinski BD, Zhang L, Crawford F, Marrack P, Eisenbarth GS, Kappler JW. Diabetogenic T cells recognize insulin bound to IAg7 in an unexpected, weakly binding register. Proc Natl Acad Sci U S A. 2010;107(24):10978–83.

Stankov K, Benc D, Draskovic D. Genetic and epigenetic factors in etiology of diabetes mellitus type 1. Pediatrics. 2013;132(6):1112–22.

Steck AK, Wong R, Wagner B, Johnson K, Liu E, Romanos J, Wijmenga C, Norris JM, Eisenbarth GS, Rewers MJ. Effects of non-HLA gene polymorphisms on development of islet autoimmunity and type 1 diabetes in a population with high-risk HLA-DR,DQ genotypes. Diabetes. 2012;61(3):753–8.

Suri A, Walters JJ, Gross ML, Unanue ER. Natural peptides selected by diabetogenic DQ8 and murine I-A(g7) molecules show common sequence specificity. J Clin Invest. 2005;115(8):2268–76.

Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo CA, Salomon BL, Bluestone JA. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity. 2008;28(5):687–97.

Tauriainen S, Salmela K, Rantala I, Knip M, Hyoty H. Collecting high-quality pancreatic tissue for experimental study from organ donors with signs of beta-cell autoimmunity. Diabetes Metab Res Rev. 2010;26(7):585–92.

Thebault-Baumont K, Dubois-Laforgue D, Krief P, Briand JP, Halbout P, Vallon-Geoffroy K, Morin J, Laloux V, Lehuen A, Carel JC, Jami J, Muller S, Boitard C. Acceleration of type 1 diabetes mellitus in proinsulin 2-deficient NOD mice. J Clin Invest. 2003;111(6):851–7.

Throsby M, Homo-Delarche F, Chevenne D, Goya R, Dardenne M, Pleau JM. Pancreatic hormone expression in the murine thymus: localization in dendritic cells and macrophages. Endocrinology. 1998;139(5):2399–406.

Torn C, Hadley D, Lee HS, Hagopian W, Lernmark A, Simell O, Rewers M, Ziegler A, Schatz D, Akolkar B, Onengut-Gumuscu S, Chen WM, Toppari J, Mykkanen J, Ilonen J, Rich SS, She JX, Steck AK, Krischer J. Role of type 1 diabetes-associated SNPs on risk of autoantibody positivity in the TEDDY study. Diabetes. 2015;64(5):1818–29.

Tsai EB, Sherry NA, Palmer JP, Herold KC. The rise and fall of insulin secretion in type 1 diabetes mellitus. Diabetologia. 2006;49:261–70.

Tuomilehto J, Podar T, Tuomilehto-Wolf E, Virtala E. Evidence for importance of gender and birth cohort for risk of IDDM in offspring of IDDM parents. Diabetologia. 1995;38(8):975–82.

Unger WW, Pearson T, Abreu JR, Laban S, van der Slik AR, der Kracht SM, Kester MG, Serreze DV, Shultz LD, Griffioen M, Drijfhout JW, Greiner DL, Roep BO. Islet-specific CTL cloned from a type 1 diabetes patient cause beta-cell destruction after engraftment into HLA-A2 transgenic NOD/scid/IL2RG null mice. PLoS One. 2012;7(11):e49213.

Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet. 1997;15(3):289–92.

Vafiadis P, Ounissi-Benkalha H, Palumbo M, Grabs R, Rousseau M, Goodyer CG, Polychronakos C. Class III alleles of the variable number of tandem repeat insulin polymorphism associated with silencing of thymic insulin predispose to type 1 diabetes. J Clin Endocrinol Metab. 2001; 86(8):3705–10.

Valle A, Giamporcaro GM, Scavini M, Stabilini A, Grogan P, Bianconi E, Sebastiani G, Masini M, Maugeri N, Porretti L, Bonfanti R, Meschi F, De Pellegrin M, Lesma A, Rossini S, Piemonti L, Marchetti P, Dotta F, Bosi E, Battaglia M. Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. Diabetes. 2013;62(6):2072–7.

van Lummel M, van Veelen PA, Zaldumbide A, de Ru A, Janssen GM, Moustakas AK, Papadopoulos GK, Drijfhout JW, Roep BO, Koning F. Type 1 diabetes-associated HLA-DQ8 transdimer accommodates a unique peptide repertoire. J Biol Chem. 2012;287(12):9514–24.

Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, Nika K, Tautz L, Tasken K, Cucca F, Mustelin T, Bottini N. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet. 2005;37(12):1317–9.

Vang T, Miletic AV, Bottini N, Mustelin T. Protein tyrosine phosphatase PTPN22 in human autoimmunity. Autoimmunity. 2007;40(6):453–61.

Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith GJ, Rewers M, Dabelea D. Trends in high-risk HLA susceptibility genes among Colorado youth with type 1 diabetes. Diabetes Care. 2008;31(7):1392–6.

Vehik K, Beam CA, Mahon JL, Schatz DA, Haller MJ, Sosenko JM, Skyler JS, Krischer JP. Development of autoantibodies in the TrialNet Natural History Study. Diabetes Care. 2011;34 (9):1897–901.

Velthuis JH, Unger WW, Abreu JR, Duinkerken G, Franken K, Peakman M, Bakker AH, Reker-Hadrup S, Keymeulen B, Drijfhout JW, Schumacher TN, Roep BO. Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. Diabetes. 2010;59(7):1721–30.

Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, Diamantopoulos S, Standifer N, Geubtner K, Falk BA, Ichii H, Takahashi H, Snowhite I, Chen Z, Mendez A, Chen L, Sageshima J, Ruiz P, Ciancio G, Ricordi C, Reijonen H, Nepom GT, Burke GW III, Pugliese A. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. Diabetes. 2010;59(4):947–57.

Vendrame F, Hopfner YY, Diamantopoulos S, Virdi SK, Allende G, Snowhite IV, Reijonen HK, Chen L, Ruiz P, Ciancio G, Hutton JC, Messinger S, Burke GW 3rd, Pugliese A. Risk factors for type 1 diabetes recurrence in immunosuppressed recipients of simultaneous pancreas-kidney transplants. Am J Transplant. 2016;16(1):235–45.

Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Chase HP, Eisenbarth GS. Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type I diabetes. J Autoimmun. 1996;9:379–83.

Viehmann Milam AA, Maher SE, Gibson JA, Lebastchi J, Wen L, Ruddle NH, Herold KC, Bothwell AL. A humanized mouse model of autoimmune insulitis. Diabetes. 2014; 63(5):1712–24.

Viskari H, Knip M, Tauriainen S, Huhtala H, Veijola R, Ilonen J, Simell O, Surcel HM, Hyoty H. Maternal enterovirus infection as a risk factor for type 1 diabetes in the exposed offspring. Diabetes Care. 2012;35(6):1328–32.

Wang L, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. Diabetes Care. 2012;35(3):465–70.

Warram HH, Krolewski AS, Gottlieb MS, Kahn CR. Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. N Engl J Med. 1984;311:149–52.

Wasserfall C, Montgomery E, Yu L, Michels A, Gianani R, Pugliese A, Nierras C, Kaddis JS, Schatz DA, Bonifacio E, Atkinson MA. Validation of a rapid type 1 diabetes autoantibody screening assay for community-based screening of organ donors to identify subjects at increased risk for the disease. Clin Exp Immunol. 2016;185(1):33–41.

Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes. 2004;53(Suppl 3):S16–21.

Wiberg A, Granstam A, Ingvast S, Harkonen T, Knip M, Korsgren O, Skog O. Characterization of human organ donors testing positive for type 1 diabetes-associated autoantibodies. Clin Exp Immunol. 2015;182(3):278–88.

Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Immunohistochemical analysis of the relationship between islet cell proliferation and the production of the enteroviral capsid protein, VP1, in the islets of patients with recent-onset type 1 diabetes. Diabetologia. 2011;54(9):2417–20.

Williams AJ, Thrower SL, Sequeiros IM, Ward A, Bickerton AS, Triay JM, Callaway MP, Dayan CM. Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. J Clin Endocrinol Metab. 2012;97(11):E2109–13.

Wu H, Zhao M, Yoshimura A, Chang C, Lu Q. Critical link between epigenetics and transcription factors in the induction of autoimmunity: a comprehensive review. Clin Rev Allergy Immunol. 2016;50(3):333–44.

Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, Gonzalez-Munoz A, Clark J, Veijola R, Cubbon R, Chen SL, Rosa R, Cumiskey AM, Serreze DV, Gregory S, Rogers J, Lyons PA, Healy B, Smink LJ, Todd JA, Peterson LB, Wicker LS, Santamaria P. Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. Nat Genet. 2007; 39(3):329–37.

Yip L, Su L, Sheng D, Chang P, Atkinson M, Czesak M, Albert PR, Collier AR, Turley SJ, Fathman CG, Creusot RJ. Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. Nat Immunol. 2009; 10(9):1026–33.

Yip L, Creusot RJ, Pager CT, Sarnow P, Fathman CG. Reduced DEAF1 function during type 1 diabetes inhibits translation in lymph node stromal cells by suppressing Eif4g3. J Mol Cell Biol. 2013;5(2):99–110.

Zhu M, Chin RK, Christiansen PA, Lo JC, Liu X, Ware C, Siebenlist U, Fu YX. NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. J Clin Invest. 2006;116(11):2964–71.

Ziegler AG, Meier-Stiegen F, Winkler C, Bonifacio E. Prospective evaluation of risk factors for the development of islet autoimmunity and type 1 diabetes during puberty – TEENDIAB: study design. Pediatr Diabetes. 2011. https://doi.org/10.1111/j.1399-5448.2011.00763.x.

Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309(23):2473–9.

Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ. Heparan sulfate and heparanase play key roles in mouse beta cell survival and autoimmune diabetes. J Clin Invest. 2012;122(1):132–41.

Zipris D. Innate immunity in type 1 diabetes. Diabetes Metab Res Rev. 2011;27(8):824–9.

Pathogenesis of Type 2 Diabetes Mellitus

7

Ralph A. DeFronzo

Contents

R. A. DeFronzo (✉)
Diabetes Division, Diabetes Research Unit, University of Texas Health Science Center, San Antonio, TX, USA
e-mail: defronzo@uthscsa.edu

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_8

Abstract

- Type 2 diabetes is characterized by multiple pathophysiologic abnormalities which collectively have been referred to as the Ominous Octet:
  - Muscle insulin resistance → reduced glucose uptake
  - Hepatic insulin resistance → excessive glucose production
  - Adipocyte insulin resistance → accelerated lipolysis and elevated circulating levels of FFA and insulin-resistance provoking adipocytokines
  - Progressive β-cell failure and apoptosis
  - Increased alpha cell secretion of glucagon and increased hepatic sensitivity to glucagon
  - Reduced incretin effect due to beta cell resistance to GLP-1 and GIP
  - Increased renal glucose production
  - Elevated renal tubular glucose reabsorption
  - Brain insulin resistance and altered neurotransmitter dysfunction leading to impaired appetite suppression and weight gain.
- Insulin resistance in muscle and liver are the earliest detectable abnormalities in the natural history of type 2 diabetes.
- With time, progressive β-cell failure ensues and, in the presence of insulin resistance, individuals progress from normal glucose tolerance to impaired glucose tolerance to overt type 2 diabetes.

Keywords

Pathophysiology of T2DM · Insulin resistance · Beta cell failure · Liver, muscle, adipocyte · Ominous octet

Key Points

- Type 2 diabetes is characterized by multiple pathophysiologic abnormalities which collectively have been referred to as the Ominous Octet:
  - Muscle insulin resistance → reduced glucose uptake
  - Hepatic insulin resistance → excessive glucose production
  - Adipocyte insulin resistance → accelerated lipolysis and elevated circulating levels of FFA and insulin-resistance provoking adipocytokines
  - Progressive β-cell failure and apoptosis

  - Increased alpha cell secretion of glucagon and increased hepatic sensitivity to glucagon
  - Reduced incretin effect due to beta cell resistance to GLP-1 and GIP
  - Increased renal glucose production
  - Elevated renal tubular glucose reabsorption
  - Brain insulin resistance and altered neurotransmitter dysfunction leading to impaired appetite suppression and weight gain.
- Insulin resistance in muscle and liver are the earliest detectable abnormalities in the natural history of type 2 diabetes.
- With time, progressive β-cell failure ensues and, in the presence of insulin resistance, individuals progress from normal glucose tolerance to impaired glucose tolerance to overt type 2 diabetes.

Maintenance of Normal Glucose Homeostasis

In order to appreciate the multiple pathophysiologic disturbances responsible for the development of impaired glucose metabolism in individuals with type 2 diabetes mellitus (T2DM), a review of the whole body, organ, and cellular mechanisms involved in the maintenance of normal glucose homeostasis in the postabsorptive state (10–12-h overnight fast) and following ingestion of a typical mixed meal is warranted (DeFronzo 1998, 1997, 2009; DeFronzo and Ferrannini 2010). During the sleeping and throughout the postabsorptive state, the great majority of total body glucose disposal takes place in insulin independent tissues, primarily the brain and other neural tissues which account for ~50% of all glucose utilization. Brain glucose utilization is insulin independent and saturates at a plasma glucose concentration of ~40 mg/dl (DeFronzo and Ferrannini 2010; Grill 1990). Since the normal fasting plasma glucose (FPG) concentration is ~70–80 mg/dl, this provides a large window of protection against cerebral neuroglycopenia. During the postabsorptive state, ~25% of glucose disposal takes in the splanchnic area (liver plus gastrointestinal tissues) and is insulin independent. Insulin-dependent tissues, primarily muscle and to a lesser extent adipose tissue, account for the remaining ~25% of glucose utilization. Basal glucose utilization averages ~2.0 mg/kg per min and is precisely matched by the rate of endogenous glucose production. Approximately 85% of endogenous glucose production is contributed by the liver and the remaining ~15% by the kidney. The ratio of insulin to glucagon in the portal circulation is the primary regulator of hepatic glucose production (Cherrington 1999), while in the kidney insulin is primary regulator of renal glucose production (Meyer et al. 1998a). Glucagon has been reported to have no effect on renal glucose production (Stumvoll et al. 1998). Glycogenolysis and gluconeogenesis contribute approximately equally to the basal rate of hepatic glucose production, while gluconeogenesis is responsible for all of renal glucose production (Cherrington 1999; Gerich et al. 2001).

Following ingestion of glucose or a mixed meal, the plasma glucose concentration rises resulting in the stimulation of insulin secretion by the pancreatic beta cells (DeFronzo and Ferrannini 2010; Ferrannini and DeFronzo 2015). The combination

of hyperinsulinemia and hyperglycemia (i) stimulates glucose uptake by splanchnic (liver and gut) and peripheral (muscle and adipose) tissues and (ii) suppresses endogenous (hepatic and renal) glucose production (DeFronzo 1998, 1997, 2009; DeFronzo and Ferrannini 2010, 1987; Ferrannini and DeFronzo 2015; DeFronzo et al. 1985, 1981; Ferrannini et al. 1985; Mandarino et al. 2001). Muscle accounts for the majority (~80–85%) of glucose uptake by peripheral tissues, with a small amount (~5%) being disposed of by adipocytes. Although fat accounts for only a small amount of glucose disposal, it contributes to the maintenance of total body glucose homeostasis by regulating the release of free fatty acids (FFA) from stored triglycerides and through the production of adipocytokines that influence insulin sensitivity in muscle and liver (Bays et al. 2004; Groop et al. 1989; Bergman 2000). Lipolysis is highly sensitive to insulin, and the rise in plasma insulin concentration following glucose/meal ingestion results in a decline in plasma FFA concentration (Groop et al. 1989). FFA inhibit glucose uptake in muscle and stimulate hepatic glucose production (Belfort et al. 2005; Bajaj et al. 2005; Groop et al. 1991). As the plasma FFA concentration declines following glucose/meal ingestion, muscle glucose uptake is increased and hepatic glucose production is inhibited. Thus, the reduction in plasma FFA concentration in response to the increases in plasma insulin and glucose concentrations plays an important role in the maintenance of normal glucose homeostasis (Bays et al. 2004; Groop et al. 1989; Bergman 2000; Belfort et al. 2005).

Glucagon secretion by the alpha cell also plays a central role in the regulation of fasting and postprandial glycemic (Cherrington 1999; Baron et al. 1987). During fasting conditions, approximately half of total hepatic glucose output is dependent upon glucagon, and inhibition of basal glucagon secretion with somatostatin reduces hepatic glucose output and plasma glucose concentration. After a meal glucagon secretion is inhibited by insulin, and the decline in plasma glucagon plays a pivotal role in the suppression of hepatic glucose production and maintenance of normal postprandial glucose tolerance. If, following a meal, glucose enters from both the liver and gastrointestinal tract, postprandial hyperglycemia will ensue. Within the pancreas, approximately 70% of the beta cells are in direct communication with nonbeta cells, including alpha cells, through gap junctions containing connexin proteins (Bosco et al. 2010; Orci et al. 1975; Benninger and Piston 2014). In addition, beta cells can influence alpha cell secretion via intraislet blood flow (Jain and Lammert 2009). Thus, the local paracrine effect of insulin, as well as the rise in circulating plasma insulin concentration, conspires to inhibit glucagon secretion.

Following oral glucose administration, the amount of insulin which is secreted is 2.5–3 fold greater than if glucose were given intravenously to mimic the plasma glucose concentration observed following glucose ingestion. This is referred to as the incretin effect and is related to the release of glucagon-like peptide-1 (GLP-1) from the L cells in the distal small bowel/large intestine and glucose-dependent insulinotropic polypeptide (previously called gastric inhibitory polypeptide) (GIP) from the K cells in the early part of the small intestine (Drucker 2006, 2013; Holst 2007; Nauck and Meier 2016). Collectively, GLP-1 plus GIP account for 60–70% of the insulin that is secreted during a meal. All nutrients (glucose, protein, fat) stimulate GLP-1 and GIP secretion, but glucose is the most potent. GLP-1, but not

GIP, also inhibits glucagon secretion, and the decline in plasma glucagon concentration contributes to suppression of hepatic glucose production following meal ingestion. Within minutes after ingestion of a meal, circulating levels of GLP-1 and GIP increase. This occurs long before nutrients can reach the K cells in the duodenum and the L cells in the more distal intestine. This rapid release of GLP-1 and GIP is mediated via neural impulses that are carried to the hypothalamus and back to the intestinal cells via the vagus nerve (Nauch and Meier 2016). GLP-1 and GIP bind to their respective receptors on the β cell, leading to activation of adenyl cyclase and an increase in insulin secretion (Drucker 2006, 2013; Holst 2007; Nauck and Meier 2016). Importantly, the stimulation of insulin secretion by GLP1 and GIP is glucose-dependent; that is, insulin release is augmented in the presence of hyperglycemia and wanes as the blood glucose concentration returns to normoglycemic levels. Similarly, the inhibitory effect of GLP-1 on glucagon secretion wanes as the plasma glucose concentration returns to its baseline level, allowing hepatic glucose production to increase, thereby preventing hypoglycemia.

The route of glucose entry into the body also plays an important role in glucose homeostasis (Cherrington 1999; DeFronzo et al. 1978a; Ferrannini et al. 1980). IV glucose exerts a modest effect to increase splanchnic glucose uptake, and the increase in SGU is directly proportional to the increase in plasma glucose concentration (DeFronzo et al. 1985). Similarly, intravenous insulin exerts only a small stimulatory effect on splanchnic (liver plus gut) glucose uptake. In contrast, when glucose is ingested, splanchnic glucose uptake increases markedly in direct proportion to the negative hepatic artery-portal vein glucose concentration gradient (Cherrington 1999). As this gradient widens, a neural reflex is activated in which vagal activity is enhanced and sympathetic nerves innervating the liver are inhibited. These neural changes stimulate hepatic glycogen synthase, inhibit glycogen phosphorylase, and augment liver glucose uptake and glycogen formation. Consequently, following oral glucose administration, splanchnic tissues remove ~30–40% of the ingested glucose. This is in marked contrast to IV glucose/insulin administration, where muscle accounts for the majority (~85%) of glucose disposal.

Natural History of Prediabetes and Type 2 Diabetes

Type 2 diabetes mellitus (T2DM) occurs in a two-step process in which insulin resistant-normal glucose tolerant (NGT) individuals progress to "prediabetes" (impaired glucose tolerance [IGT] and impaired fasting glucose [IFG]) and then to overt type 2 diabetes (DeFronzo 1998, 2009; Weyer et al. 1999; Lyssenko et al. 2005; Jallut et al. 1990; Saad et al. 1991; Kahn et al. 2014; Kanat et al. 2015). The progression from NGT to "prediabetes" to diabetes is characterized by worsening beta cell failure, and overt type 2 diabetes becomes established when the compensatory increase in insulin secretion no longer is sufficient to offset the underlying insulin resistance (DeFronzo 2009). Thus, in ethnic populations where insulin resistance is mild-moderate, i.e., Asians, beta cell failure must be quite severe before overt T2DM becomes manifest (Abdul-Ghani et al. 2007a; Yang and Weng 2014). In

contrast, in ethnic populations where insulin resistance is more severe, a more modest degree of beta cell failure will lead to the development of diabetes. It also should be emphasized that the cut points for the diagnosis of diabetes are quite arbitrary and not based upon the pathophysiologic disturbances that characterize the disease. Thus, the 2-h plasma glucose concentration of ≥200 mg/dl is based upon ~10% of the diabetic population having proliferative retinopathy. Although no one would argue that such individuals have diabetes, it is obvious that the diabetic state must have been present long before the onset of proliferative retinopathy. Consistent with this, prediabetic individuals have an incidence of peripheral neuropathy, microalbuminuria, and background retinopathy that ranges from 10% to 20% (Group DPPR 2007; Nagi et al. 1997; Plantinga et al. 2010; Bongaerts et al. 2012). Thus, the microvascular complications are present long before the diagnosis of diabetes is established by current criteria. A more rational approach would be to establish the diagnosis of diabetes based upon its pathophysiology (DeFronzo 2009), although the practicality of this approach is difficult since the requisite clinical tools to quantitate the underlying core defects, i.e., insulin resistance and beta cell failure, are not currently available in clinical practice.

The natural history of T2DM has been well described in multiple populations (DeFronzo 1998, 2009; Lyssenko et al. 2005; Jallut et al. 1990; Kahn et al. 2014; Saad et al. 1989, 1988; Martin et al. 1992; Haffner et al. 1995; Lillioja et al. 1993; Dowse et al. 1996; Weyer et al. 2001; Eriksson et al. 1989) and is reviewed in references (DeFronzo 2009; Kahn et al. 2014; and DeFronzo and Abdul-Ghani 2011). Individuals destined to develop T2DM inherit a set of genes from their parents that make their tissues resistant to insulin (DeFronzo 1998, 1997, 2009; Gulli et al. 1992; Groop and Lyssenko 2008; Pratipanawatr et al. 2001; Pendergrass et al. 2007; DeFronzo et al. 2015; Fuchsberger et al. 2016), although the genetic basis of the insulin resistance remains largely undefined. With the advent of genome-wide association studies, more than 100 SNPs (single nucleotide polymorphisms) in genes have been linked to T2DM (Morris et al. 2012). Most of these SNPs are in introns and would be best referred to as genetic loci rather than genes. The mechanisms by which these loci increase the risk for T2DM remain largely undefined. Thus, SNPs in the TCF7L2 gene most consistently have been found in T2DM patients (Grant et al. 2006; Lyssenko et al. 2007), yet it remains undefined how these SNPs disrupt glucose metabolism and cause diabetes. Exceptions are a few variants in exons which influence gene function, i.e., SLC30A8 (encodes a zinc transporter that is required for insulin storage in beta cells), KCNJ11 (encodes ATP-dependent potassium channel), GCKR (encodes a glucokinase regulatory protein), and PPARγ (encodes nuclear transcription factor that regulates genes involved in insulin action) (Morris et al. 2012; Flannick et al. 2014; Sladek et al. 2007; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT et al. 2007; Deeb et al. 1998). With the exception of the later, most of these known exonic SNPs are associated with beta cell function. When viewed in toto, these genetic variants account for, at most, only 10–20% of the risk for diabetes (DeFronzo et al. 2015; Morris et al. 2012). Further, 54% of nondiabetic individuals carry these risk variants for T2DM (Morris et al. 2012). In a prospective study of ~2700 individuals followed

for 8 years, all individuals became progressively more obese, but only those with high genetic risk (≥12 risk alleles) developed diabetes because of an inability to augment insulin secretion sufficiently to offset the obesity-associated insulin resistance (Lyssenko et al. 2008). Although genes play a major role in the development of T2DM, the majority of the heritability (85%) cannot be accounted for by currently known SNPs. Alternative explanations for the high heritability of T2DM include gene-environment interactions and epigenetic modifications (DNA methylation and chromatin modifications) (DeFronzo et al. 2015).

The insulin resistance involves the muscle, liver, and adipocytes (DeFronzo 1998, 1997, 2009). Hepatic insulin resistance is manifested by overproduction of glucose during the postabsorptive state despite the presence of fasting hyperinsulinemia (DeFronzo et al. 1989) and impaired suppression of hepatic glucose production (HGP) by insulin (Groop et al. 1989), as occurs following a meal (Ferrannini et al. 1988). Muscle insulin resistance (DeFronzo et al. 1985, 1979a; Groop et al. 1989; Pendergrass et al. 2007; Bajaj and DeFronzo 2003) is manifest by impaired glucose uptake following carbohydrate ingestion and results in postprandial hyperglycemia (Groop et al. 1989). Although the insulin resistance has a strong genetic background (DeFronzo 1997; Groop and Lyssenko 2008; Morino et al. 2005), the current explosion of diabetes that has enveloped Westernized countries primarily results from the epidemic of obesity and physical inactivity (James 2008). Both obesity (DeFronzo et al. 1978b) and physical inactivity (Koivisto and DeFronzo 1986) are insulin-resistant states and, when superimposed on the genetic component of insulin resistance, place a major stress on the pancreatic β cells to augment their secretion of insulin to offset the defect in insulin action (DeFronzo 1998, 1997, 2009; Saad et al. 1991; Kahn et al. 2014; Kanat et al. 2015; Diamond et al. 1995). Initially, the pancreatic β cells respond by augmenting their secretion of insulin to offset the insulin resistance and glucose tolerance remains normal. However, with time the β cells begin to fail, resulting in postprandial plasma hyperglycemia followed by fasting hyperglycemia and eventually overt diabetes (DeFronzo 1998, 1997, 2009; Saad et al. 1991; Kahn et al. 2014; Kanat et al. 2015; Bergman et al. 2002; Kahn 2003). Collectively, the insulin resistance in muscle and liver and β-cell failure comprise the Triumvirate (DeFronzo 1998). The resultant hyperglycemia (glucotoxicity) (Rossetti et al. 1990; Yki-Jarvinen and DA 2015) and accumulation of fat and toxic lipid metabolites in muscle/liver (lipotoxicity) (Bays et al. 2004, 2008) cause a further decline in insulin sensitivity, but it is the progressive β-cell failure that determines the rate of disease progression.

Over the last 50 years, the incidence of T2DM has increased epidemically. This cannot be explained by an abundance of novel genetic mutations and clearly is associated with the epidemic of obesity (James 2008) and lipotoxicity (Bays et al. 2004, 2008), which cause insulin resistance in muscle and liver and promote beta cell failure (DeFronzo 1998, 2009).

The relative contributions of insulin resistance and β-cell failure to the development of T2DM vary among different ethnic groups (Abdul-Ghani et al. 2007a; Yang and Weng 2014). However, progressive β-cell failure superimposed upon a background of genetic/acquired insulin resistance represents the core pathophysiologic

defects responsible for the development of overt diabetes (DeFronzo 1998, 1997, 2009; Kahn et al. 2014; Bergman et al. 2002; Kahn 2003).

The natural history of T2DM is depicted by a prospective 6-year study carried out by Felber and colleagues (Jallut et al. 1990) (Fig. 1). In this European population, subjects had a euglycemic insulin clamp to quantitate insulin sensitivity and an oral glucose tolerance test (OGTT) to characterize glucose tolerance and provide a measure of insulin secretion. Weight gain was associated with the development of insulin resistance, but glucose tolerance remained normal because of a compensatory increase in insulin secretion. With time the obese NGT individuals progressed to impaired glucose tolerance (IGT) in association with a further worsening of the insulin resistance. Although the rise in plasma glucose concentration is modest, people with IGT are in a very precarious position, since they are maximally/near-maximally insulin resistant and their β-cell function is severely impaired even though, in absolute terms, their plasma insulin response is increased (DeFronzo 1998, 2009). However, it is important not to equate insulin secretion with beta cell function. These are two very different physiologic parameters and this distinction will be discussed below. With time, the β cells cannot maintain their high insulin secretory rate and obese IGT individual progresses to overt diabetes as the result of a marked decrease in insulin secretion without further or minimal change in insulin sensitivity (Fig. 1). This inverted U-shaped curve describing the relationship between the plasma insulin response and increase in plasma glucose concentration has been referred to as Starling's curve of the pancreas (DeFronzo 1998) and is characteristic of the natural history of T2DM in many diverse ethnic populations (DeFronzo 1998; DeFronzo 2009; Lyssenko et al. 2005; Jallut et al. 1990; Kahn et al. 2014; Saad et al. 1989; Martin et al. 1992; Saad et al. 1988; Haffner et al. 1995; Lillioja et al. 1993; Dowse et al. 1996; Weyer et al. 2001; Eriksson et al. 1989; DeFronzo and Abdul-Ghani 2011; UK Prospective Diabetes Study (UKPDS) Group 1998; Levy et al. 1998) and during the development of diabetes in primates (Hansen and Bodkin 1986; Guardado-Mendoza et al. 2009).

Beta-Cell Function and Insulin Secretion

Early in the natural history of T2DM, the plasma insulin response to hyperglycemia is increased as the beta cells increase their secretion of insulin in an attempt to offset the underlying insulin resistance (Fig. 1). However, the hyperinsulinemic response should not be interpreted to mean that the β cell is functioning normally. To the contrary, it is now clear that the β-cell failure occurs much earlier in the natural history of T2DM and is more severe than previously appreciated. In the San Antonio Metabolism (SAM) study and Veterans Administration Genetic Epidemiology Study (VAGES) (DeFronzo 2009; Gastaldelli et al. 2004; Ferrannini et al. 2005; Abdul-Ghani et al. 2006a, b), NGT ($n = 318$), 259 IGT ($n = 259$), and T2DM ($n = 201$) subjects had an OGTT to evaluate overall glucose tolerance and insulin secretion and a euglycemic insulin clamp to measure insulin sensitivity. Simply measuring the plasma insulin response to a glucose challenge does not provide a valid index of β-

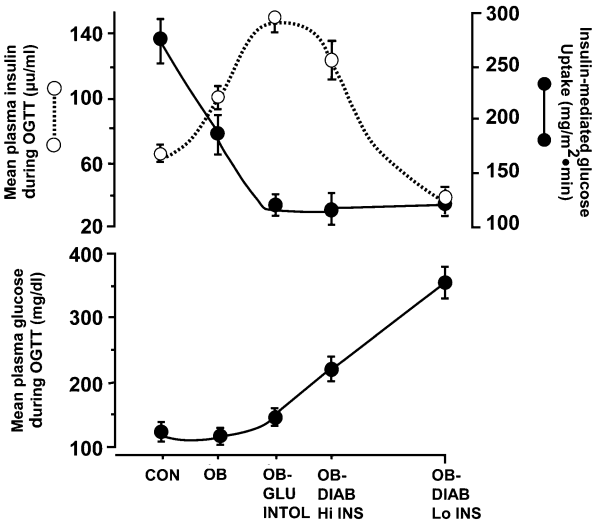


Fig. 1 Summary of the plasma insulin (top) (dashed line, open circles) and plasma glucose (bottom) responses during a 100-g OGTT and tissue sensitivity to insulin in obese (OB) normal glucose tolerant, obese glucose intolerant (OB-GLU INTOL), obese hyperinsulinemic diabetic (OB-DIAB Hi INS), and obese hypoinsulinemic diabetic subjects (OB-DIAB Lo INS). See text for a detailed discussion. (Source: DeFronzo RA. *Diabetes* 1988;**37**:667–687)

cell function (Ahrén and Taborsky 2003). The β cell responds to an *increment* in plasma glucose (ΔG) with an *increment* in plasma insulin (ΔI) (Ahrén and Taborsky 2003). Thus, a better measure of β-cell function is ΔI/ΔG. However, the β cell also recognizes the severity of insulin resistance and adjusts its secretion of insulin to offset the defect in insulin action (DeFronzo 1998; Kahn et al. 2014; Diamond et al. 1995; Ahrén and Taborsky 2003; Reaven et al. 1989; Bergman 1989). Thus, the gold standard measure of β-cell function is the insulin secretion/insulin resistance (ΔI/ΔG ÷ IR), or so-called disposition, index.

If one plots the insulin secretion/insulin resistance index (ΔI/ΔG ÷ IR) in NGT, IGT, and T2DM subjects as a function of the 2-h plasma glucose concentration (OGTT), it can be seen that the decline in beta cell function begins long before the onset of "prediabetes" (DeFronzo 2009; Gastaldelli et al. 2004; Ferrannini et al. 2005; Abdul-Ghani et al. 2006a, b). Subjects in the upper tertile of "normal" glucose tolerance (2-h PG = 120–139 mg/dl) have lost two-thirds of their β-cell function, while subjects in the upper tertile of IGT (2-h PG = 180–199 mg/dl) have lost ~80–85% of their β-cell function. Similar results have been described in other populations (Saad et al. 1989, 1988; Weyer et al. 2001; Ferrannini et al. 2011; American Diabetes Association 2008). Most biomedical phenomena occur as a log function (Fig. 2). When the natural log of the 2-h plasma glucose concentration (OGTT) is plotted against the natural log of the insulin secretion/insulin resistance (β-cell function) index, these two variables are strongly and linearly related ($r = 0.91$, $p < 0.00001$), and it is not possible to define cut points that distinguish NGT from IGT or IGT from T2DM. Rather, glucose intolerance is a continuum, and subjects move up and down this curve as a function of the insulin secretion/insulin resistance index. Therefore, the current diagnostic criteria (Zimmet et al. 1978) for IGT and T2DM are quite arbitrary and glucose tolerance should be viewed as a continuum of risk. The higher the 2-h plasma glucose concentration, even within the

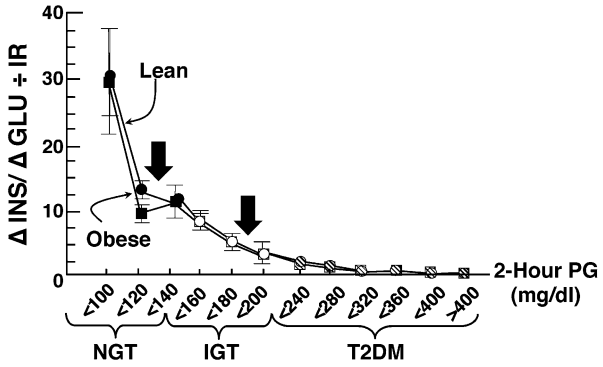


Fig. 2 Insulin secretion/insulin resistance (disposition) index (defined as increment in insulin/increment in glucose ÷ insulin resistance [ΔINS/ΔGLU ÷ IR]) in individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) as a function of the 2-h plasma glucose (PG) concentration in lean (circles) and obese (squares) subjects. (From *Diabetes* 58:773–795, 2008)

range of IGT, the greater is the risk for microvascular complications. Further, as a predictor of future development of T2DM, a 1-h plasma glucose concentration > 150 mg/dl during the OGTT is a much better predictor than the 2-h plasma glucose (Abdul-Ghani et al. 2006c, 2007b, 2009a; Abdul-Ghani and DeFronzo 2009).

Although the insulin secretion/insulin resistance (disposition) index has proven useful in understanding the progression from NGT to IGT to T2DM, it should be emphasized that the plasma insulin response during the OGTT is the composite of two variables which move in opposite directions: (i) insulin secretion by the beta cells and (ii) metabolic clearance rate of insulin. Thus, the curvi-linear relationship between ([ΔI/ΔG] ÷ IR) and (2-h PG) during the OGTT is lost if ΔC-peptide is substituted for ΔI (DeFronzo et al. 2014). This indicates that the linear relationship between the log of these two variables holds only if one uses the incremental plasma insulin response during the OGTT. Stated otherwise, the body is capable of reading the severity of insulin resistance and adjusting insulin secretion, insulin clearance, or the composite of the two to achieve a plasma insulin concentration that offsets the underlying insulin resistance (DeFronzo et al. 2014). This is consistent with the well-established observation that the metabolic clearance rate of insulin is reduced in insulin resistant states (Jones et al. 1997; Flier et al. 1982).

Beta Cell Glucose Sensitivity and Rate Sensitivity

A characteristic defect in the patient with T2DM is the "blindness" of the beta cell to a rise in plasma glucose concentration. When beta cell function is evaluated using glucose sensitivity (i.e., slope of the insulin secretion/plasma glucose dose response during the OGTT or hyperglycemic clamp), the slope is markedly reduced (Fig. 3)

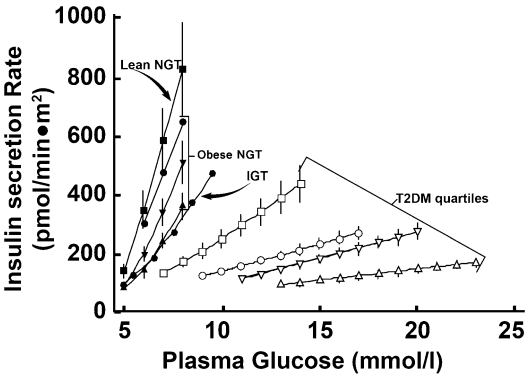


Fig. 3 Plot of insulin secretion rate against the concomitant plasma glucose concentration in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (T2D) by quartile of fasting hyperglycemia. The mean slope of the fitting functions measures β-cell glucose sensitivity. (Source: Ferrannini et al., *J Clin Endocrinol Metab* 90:493–500, 2005)

(DeFronzo et al. 2015, 2014; Ferrannini et al. 2005; Marachett and Ferrannini 2015; Mari et al. 2002). The decline in beta cell glucose sensitivity is a continuum, starting within the range of normal glucose tolerance and progressively deteriorating as subjects move to IGT and then to T2DM (Fig. 3). Reduced glucose sensitivity is a powerful predictor of the development of diabetes, independent of insulin resistance and other classic phenotype predictors (Mari et al. 2002). Although decreased glucose sensitivity and reduced insulin secretion/insulin resistance index are uniformly observed in subjects with T2DM and IGT, they are numerically independent of each other across the entire range of glucose tolerance (DeFronzo et al. 2014; Mari et al. 2002; Ferrannini and Mari 2014, 2004). In animal models of diabetes, decreased glucose transport and glucokinase activity have been shown to explain the impairment in beta cell glucose sensitivity (Drucker 2006). In addition to the defect in glucose sensitivity, the beta cell response to the rate of rise in plasma glucose concentration also is impaired in T2DM patients, although this defect occurs later in the natural history of diabetes and is not observed in IGT (Ferrannini et al. 2005).

Beta Cell Function in IGT and IFG

IGT and IFG are "prediabetic" states with a similar and high rate of progression to T2DM (reviewed in reference (DeFronzo and Abdul-Ghani 2011) and (Ferrannini and Mari 2014)). However, the pathophysiologic disturbances present in these two prediabetic states are quite distinct (DeFronzo and Abdul-Ghani 2011; Abdul-Ghani et al. 2006a, b, c, 2009a; Daniele et al. 2014; Kanat et al. 2012). IFG subjects manifest a defect in the early insulin response (0–30 min) during the OGTT (and 1st

phase [0–10 min] insulin response during IV glucose administration) and hepatic insulin resistance. This results in an excessive early rise in plasma glucose during the OGTT. However, since the late insulin response (60–120 min during the OGTT) is intact and muscle insulin sensitivity is not impaired, the plasma glucose concentration at 2 h returns to its basal, albeit elevated level. Individuals with IGT have defects in both the early (0–30 min) and late (60–120 min) plasma insulin response during the OGTT (and 1st [0–10 min] and 2nd [10–120 min] phase insulin response during IV glucose administration) and muscle insulin resistance. Thus, although the FPG concentration is not increased in IGT subjects, following glucose ingestion the plasma glucose concentration rises progressively and remains elevated after 2 h. Both IGT and IFG individuals manifest impaired beta cell sensitivity to glucose while rate sensitivity is intact (Ferrannini et al. 2005; DeFronzo et al. 2014).

In summary, beta cell function is severely impaired long before the onset of T2DM and even before the development of IGT. Individuals in the upper tertile of IGT (Gastaldelli et al. 2004; Ferrannini et al. 2005, 2011; Abdul-Ghani et al. 2006a, b) have lost over 80% of their β-cell function, while subjects in the upper tertile of NGT have lost over 50% of their β-cell function. Even more ominous are studies demonstrating a significant reduction in β-cell mass in prediabetic (IFG/IGT) individuals (Butler et al. 2003; Henquin and Rahier 2011; Stefan et al. 1982) with a further decrease in β-cell mass with progression to overt diabetes (Butler et al. 2003; Henquin and Rahier 2011; Stefan et al. 1982; Westermark and Wilander 1978; Sakuraba et al. 2002). This presents a major problem, since no therapeutic intervention has been shown to increase β-cell mass in humans.

Type 2 Diabetes with Hypoinsulinemia

In typical T2DM individuals, hyperinsulinemia and insulin resistance precede the onset of diabetes. However, severe insulin deficiency, with or without impaired tissue insulin sensitivity, can lead to the type 2 diabetic phenotype, and this is best exemplified by patients with maturity onset diabetes of youth (MODY) (Polonsky 1995; McCarthy and Froguel 2002; Steck and Winter 2011), which is characterized by early age of onset, autosomal dominant inheritance with high penetrance, mild-to-moderate fasting hyperglycemia, and impaired insulin secretion.

MODY-1 originally was described by Fajans and shown to result from a nonsense mutation in exon 7 of the hepatic nuclear factor (HNF4α) gene, resulting in impaired glycolysis in the beta cell (Bell et al. 1991). Subsequently, it was demonstrated that MODY in French families resulted from mutations in the glucokinase gene on chromosome 7p (MODY-2) (Vaxillaire and Froguel 2008). More than eight specific mutations in different genes have been shown to cause MODY including glucokinase and seven transcription factors (Polonsky 1995; McCarthy and Froguel 2002; Steck and Winter 2011; Bell et al. 1991; Vaxillaire and Froguel 2008): MODY-1 = HNF4α; MODY-2 = glucokinase; MODY-3 = HNF1α; MODY-4 = insulin promoter factor 1; MODY-5 = HNF1β; MODY-6 = neurogenic differentiation 1/β-cell E-box transactivator 2; MODY-7 = KLF11 or Kruppel-like factor 11 that

regulates Pdx1 transcription in β cells; MODY-8 = carboxyl-ester lipase gene. HNF1α, HNF1β, and HNF4α constitute a network of transcription factors that function collectively during embryonic development and during adulthood to regulate insulin gene expression. The hallmark defect in MODY individuals is impaired insulin secretion in response to glucose and other secretagogues. However, peripheral tissue resistance to insulin and abnormalities in hepatic glucose metabolism also has been shown to play a role in the development of impaired glucose homeostasis (Beck-Nielsen et al. 1988; Mohan et al. 1988). Although glucokinase mutations are characteristic of MODY-2, genetic studies in typical older-onset type 2 diabetic individuals have shown that glucokinase mutations account for less than 1% of the common form of T2DM (Elbein et al. 1994). The characteristic phenotype of glucokinase MODY is mild fasting hyperglycemia that is present at birth with little deterioration with age and usually does not require treatment with antidiabetic medications. Using graded glucose infusions, glucokinase mutations have been shown to be associated with a right-shift in the insulin dose-response curve (Byrne et al. 1994). Diabetes associated with mutations in the two most common transcription factors, HNF1α and HNF1B, is not present at birth, usually develops in adolescents/young adults, is progressive requiring treatment, and is associated with microvascular complications. A number of other transcription factor mutations have been described in the PDX-1, NEUROD1, PAX4, and KLF11 genes, but they are rare.

Mitochondrial gene mutations also have been associated with an insulinopenic type of diabetes (Alcolado et al. 2002). Beta cells contain a mixture of normal and mutated mitochondrial DNA referred to as heteroplasmy. The degree of heteroplasmy differs within tissues and within specific cell types but, if sufficiently severe, can lead to impaired insulin secretion and T2DM. These mitochondrial mutations are inherited maternally and usually associated with sensorineural deafness. The onset of diabetes usually occurs in the third to fourth decade and is progressive, often requiring insulin therapy.

Cerasi, Luft, Hales, and coworkers (Efendic et al. 1988; Davies et al. 1993; Cerasi 1995) have championed the view that insulin deficiency represents the primary defect responsible for glucose intolerance in typical type 2 diabetic patients who do not have glucokinase or other MODY mutations. Accordingly, these investigators have described lean Caucasians with mild fasting hyperglycemia (<140 mg/dl, 7.8 mmol/L) who demonstrate a major defect in the early insulin response (0–30 min) and insulin deficiency at all time points during an OGTT. A low plasma insulin response has also been described in oriental populations including Japanese (Abdul-Ghani et al. 2007a) and Chinese (Yang and Weng 2014; Li et al. 2004), who present with typical T2DM. Unfortunately, few of these studies have provided information about insulin sensitivity. However, the great majority of these individuals have a low BMI (18–22 kg/m^2), making it likely that they are insulin sensitive. As discussed earlier, these individuals would require a major loss of beta cell function and/or mass in order to develop overt T2DM. This does not mean that they have more beta cell failure (as opposed to more insulin deficiency) than the more typical obese T2DM patient encountered in Western societies. Thus, if one

were to calculate the insulin secretion/insulin resistance index ([ΔI/ΔG] ÷ IR), beta cell function is likely to be similarly reduced to levels observed in typical obese T2DM patients seen in the USA and Europe (Abdul-Ghani et al. 2007a).

Normal insulin sensitivity with severely impaired insulin secretion has been demonstrated in a minority of T2DM individuals (Arner et al. 1991; Ferrannini et al. 1997) and in some African American T2DM patients (Banjeri and Lebovitz 1992; Mbanya et al. 2000). Thus, impaired insulin secretion, in the absence of or in the presence of only mild/moderate insulin resistance, can lead to the development of typical T2DM. However, in non-Asian populations, a pure β-cell defect resulting in typical T2DM is uncommon.

First-Phase Insulin Secretion

When glucose is administered intravenously, insulin secretion is biphasic with an early burst of insulin release within the first 10 min followed by a progressively increasing phase of insulin secretion that persists as long as the hyperglycemic stimulus is present (DeFronzo et al. 1979b). The beta cell response is determined by: (i) glucose sensitivity, (ii) rate sensitivity, (iii) potentiation which includes multiple factors: time related effect of hyperglycemia; enzymatic/molecular changes within the beta cell, neurohormonal factors, i.e. GLP-1/GIP, autocrine effect of insulin to stimulate its own release, change in plasma FFA/beta cell lipid levels, etc. (Ferrannini et al. 2005; Mari et al. 2002; Ferrannini and Mari 2014, 2004). Because of the more gradual rate of rise in plasma glucose concentration when glucose is ingested, the 1st phase insulin response observed with IV glucose is not observed (DeFronzo et al. 1979b). Although the early (0–30 min) insulin response during the OGTT has been assumed to reflect the 1st phase insulin response to IV glucose, this is more an assumption than proven fact. Loss of first-phase insulin secretion is a highly characteristic and early abnormality in patients destined to develop T2DM (DeFronzo 1998, 1997, 2009). Most T2DM subjects manifest a reduction in early phase insulin secretion during the OGTT (0–30 min) and IVGTT (0–10 min). This early defect in insulin secretion becomes evident when the FPG concentration exceeds >110–120 mg/dl (6.1–6.7 mmol/L) (DeFronzo 1998, 1997, 2009; Bergman et al. 2002; Kahn 2003; Abdul-Ghani et al. 2006a, b; Brunzell et al. 1976). The early defect in insulin secretion during the OGTT is most obvious if the incremental plasma insulin response from 0 to 30 min is expressed relative to the incremental plasma glucose response over the same time interval ($\Delta I_{0-30}/\Delta G_{0-30}$). The defect in 1st phase insulin secretion can be partially restored with tight metabolic control with insulin (Li et al. 2004; Vague and Moulin 1982; Kosaka et al. 1980; Garvey et al. 1985; Weng et al. 2008), and these intensively insulin-treated T2DM patients can maintain good glycemic control for many months, sometimes years, after restoration of normoglycemia with no, or reduced dose of, antidiabetic medication (Yang and Weng 2014; Li et al. 2004; Weng et al. 2008; Hu et al. 2011; Park and Choi 2003). Normalization of plasma glucose levels and marked improvement in beta cell function also have been reported after bariatric surgery (Ferrannini

and Mingrone 2009; Nannipieri et al. 2011). These results indicate that, at least part of, the defect is acquired secondary to metabolic decompensation (see subsequent discussion on glucotoxicity and lipotoxicity). Loss of the first phase of insulin secretion has important pathogenic consequences, since this early burst of insulin primes insulin target tissues, especially the liver, that are responsible for the maintenance of normal glucose homeostasis (Luzi and DeFronzo 1989). If first phase insulin secretion is abolished experimentally in humans, hepatic glucose production fails to suppress normally and there is an excessive early rise in plasma glucose following glucose ingestion.

Pathogenesis of β-Cell Failure (Fig. 4)

Age. Numerous studies (Muller et al. 1996; Rosenthal et al. 1982) have demonstrated that aging is associated with a modest decline in β-cell function, as well as decrease in tissue sensitivity to insulin (DeFronzo 1979). This is consistent with the well-established observation that the incidence of diabetes increases progressively with advancing age. However, factors other than age play a more prominent role in the progressive deterioration in β-cell function observed in T2DM.

Genes. T2DM and β-cell failure clusters in families, and studies in first-degree relatives of T2DM parents and in twins have provided strong evidence for the genetic basis of the β-cell dysfunction (Gautier et al. 2001; Vauhkonen et al. 1997; Vaag et al. 1995). A number of genes, most notably transcription factors, have been associated with β-cell dysfunction and T2DM in multiple ethnic populations (Groop and Lyssenko 2008; DeFronzo et al. 2015; Fuchsberger et al. 2016; Morris et al. 2012; Grant et al. 2006; Lyssenko et al. 2007; Flannick et al. 2014; Sladek et al. 2007; Helgason et al. 2007; Steinthorsdottir et al. 2007; Ahlqvist et al. 2011; Imamura and Maeda 2011; Kahn et al. 2012; Teo et al. 2015). In Finnish families with T2DM impaired insulin secretion is an inherited trait with evidence for a susceptibility locus on chromosome 12 (Watanabe et al. 1999). Of the genes associated with beta cell failure, the transcription factor TCF7L2 is best established (Grant et al. 2006; Helgason et al. 2007). Lyssenko et al. (Lyssenko et al. 2007) have

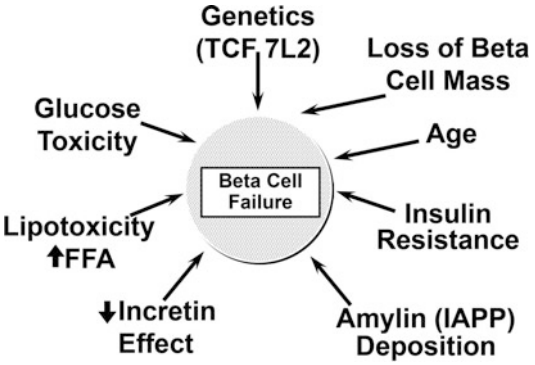


Fig. 4 Pathogenetic factors implicated in the progressive impairment in insulin secretion in T2DM

shown that the T-allele of single nucleotide polymorphism rs7903146 of the TCF7L2 gene is associated with impaired insulin secretion in vivo and reduced responsiveness to glucagon-like peptide 1 (GLP-1). Further, both the CT and TT genotypes predict T2DM in multiple ethnic groups (Cauchi et al. 2006). In the Malmö and Botnia studies, both the CT and TT genotypes were associated with a decrease in diabetes-free survival time (Lyssenko et al. 2007). TCF7L2 encodes for a transcription factor involved in Wnt signaling, which plays a central role in the regulation of β-cell proliferation and insulin secretion and is essential for Wnt signaling (Welters and Kulkarni 2008). This has important clinical implications since the stimulatory effect of GLP-1 receptor agonists is mediated via the Wnt signaling pathway. A number of other transcription factors also have been associated with impaired insulin secretion in T2DM including GCK, a gene responsible for MODY-2; SLC30A8, a zinc transporter involved in maintaining the appropriate amount of zinc in β-cell secretion granules; KCNJ11 and ABCC8 which encode the subunits of the ATP-sensitive potassium channel; and others (Kahn et al. 2012). A variant in the MTNR1B gene (which encodes the melatonin receptor) also has been shown to be associated with T2DM, and cultured human islets carrying the risk allele have reduced β-cell function and survival (Lyssenko et al. 2009). Impaired β-cell function in T2DM also has been associated with epigenetic modifications (De Jesus and Kulkarni 2014) and microRNA patterns (Ozcan 2014).

At present no known therapeutic interventions have been shown to reverse genetic-related factors responsible for impaired insulin secretion. However, a recent study suggests that this may be achievable. In the diabetic GK rat, impaired insulin secretion is explained by a variant of the ADRA2 gene, which results in over-expression of the alpha 2A-adrenergic receptor in islets (Rosengren et al. 2010). When human islets carrying the ADRA2A variant were treated with yohimbine, an inhibitor of the receptor, insulin secretion was normalized (Tang et al. 2014). Treatment of human carriers with yohimbine also improved insulin secretion (Tang et al. 2014).

Insulin resistance. Insulin resistance is present in the great majority of T2DM patients and places an increased demand on the β cells to hypersecrete insulin, thereby contributing to the progressive β-cell failure in T2DM (DeFronzo 1998, 1997, 2009; Kahn et al. 2014; Diamond et al. 1995; Bergman et al. 2002; Kahn 2003). The precise mechanism(s) via which insulin resistance causes β-cell failure remain(s) unknown. It commonly is stated that the β cell, by being forced to continuously hypersecrete insulin, eventually wears out. Although simplistic in nature, this explanation lacks a mechanistic cause. Nonetheless, β cell "unloading" with thiazolidinediones in IGT subjects markedly enhances β-cell function and reduces the conversion of IGT to T2DM (Xiang et al. 2006; DeFronzo et al. 2011). An alternate hypothesis is that the basic etiology of the insulin resistance also is responsible for the β-cell failure. Thus, excess deposition of toxic lipid metabolites (long chain-fatty acyl CoAs, diacylglycerol, and ceramides) in liver and muscle impairs insulin signaling, causing insulin resistance in these organs. This is referred to as lipotoxicity (DeFronzo 1998, 1997, 2009; Bays et al. 2004, 2008). Increased fat deposition in the pancreas of humans with T2DM has been

demonstrated using magnetic resonance imaging and associated with beta cell failure (Lim et al. 2011; Tushuizen et al. 2007). Although it cannot be documented that the pancreatic fat is localized to the beta cell because of spatial resolution, beta cell fat accumulation has been demonstrated in rodent models of diabetes and linked to beta cell dysfunction (Lee et al. 2010). Physiologic elevated of the plasma FFA concentration in NGT humans for as little as 48–72 h has been shown to markedly inhibit insulin secretion (Kashyap et al. 2003). Insulin is secreted in a one-to-one ratio with islet amyloid polypeptide (IAPP), and in insulin resistant states such as T2DM, IAPP secretion, along with insulin secretion, is increased and has been associated with β-cell failure (Guardado-Mendoza et al. 2009; Montane et al. 2012; Westermark et al. 2011). IAPP is especially toxic to the β cell in the presence of elevated intracellular fat content (Clark et al. 1988). Further, as IAPP ammulates it coalesces and encroaches upon the β cell, leading to β cell destruction (Guardado-Mendoza et al. 2009; Westermark and Wilander 1978; Westermark et al. 2011; Ritzel et al. 2007). Lastly, studies in the β-cell insulin receptor knock out (BIRKO) mouse (Kulkarni et al. 1999) and in humans with gly → arg substitution of codon 972 of IRS-1 (Sigal et al. 1996; Marchetti et al. 2002; Goldfine and Kulkarni 2012) have demonstrated that defects in insulin signaling in the β cell are associated with impaired insulin secretion. Thus, the insulin receptor on the beta cell plays a key role in modulating insulin secretion and its inhibition, not only impairs insulin action in liver and muscle, but also impedes insulin secretion.

Lipotoxicity. Lipid accumulation in the β cell (Bays et al. 2004, 2008; Lim et al. 2011; Tushuizen et al. 2007; Lee et al. 2010) and chronic elevation of the plasma FFA concentration (Kashyap et al. 2003) impair insulin secretion, and this has been referred to as lipotoxicity. A physiologic increase in the plasma FFA concentration for as little as 48–72 h markedly impairs insulin secretion in genetically predisposed individuals (Kashyap et al. 2003). In vivo studies in rodents (Higa et al. 1999; Matsui et al. 2004) and in vitro studies (Igoillo-Esteve et al. 2010; Lupi et al. 2002) have shown that beta cell fat accumulation, both directly and indirectly via activation of inflammatory pathways, inhibits insulin secretion. Incubation of human pancreatic islets for 48 h with FFA (oleate-to-palmitate ratio 2:1) impairs both the acute and late insulin response, inhibits insulin mRNA expression, reduces islet insulin content, and activates apoptotic pathways (Lupi et al. 2002). Peroxisome proliferator–activated receptor (PPAR) γ agonists have been shown to prevent all of these deleterious effects of FFA (Higa et al. 1999; Matsui et al. 2004; Lupi et al. 2004; Gastaldelli et al. 2007a; DeFronzo et al. 2013a). Consistent with these in vitro observations, both rosiglitazone and pioglitazone markedly improve the insulin secretion/insulin resistance index in vivo in type 2 diabetic humans (Gastaldelli et al. 2007a; DeFronzo et al. 2013a). Weight loss, which mobilizes fat out of the β cell, also reverses lipotoxicity and preserves β-cell function (Lim et al. 2011). Elevated plasma FFA levels and accumulation of toxic lipid metabolites can activate inflammatory pathways, including NFkB/IkB, toll-like receptor-4, and others, and increase reactive oxygen species (ROS) (Teo et al. 2015; Igoillo-Esteve et al. 2010; Lupi et al. 2002; Sriwijitkamol et al. 2006; Abdul-Ghani et al. 2008, 2009b; Reyna et al. 2008; Eizirik et al. 2008), thereby impairing insulin secretion and activating apoptotic pathways.

Glucotoxicity. Chronically elevated plasma glucose levels impair β-cell function both in vivo and in vitro in animals and humans, and this glucotoxic effect of hyperglycemia on beta cell function has been referred to as glucotoxicity (Rossetti et al. 1990; Yki-Jarvinen and DA 2015; Zhang et al. 2013). Chronic exposure of isolated human islets in vitro to elevated plasma glucose levels impairs insulin secretion (Patane et al. 2002; Andreozzi et al. 2004), while in rats, elevation of the mean day-long plasma glucose concentration in vivo by as little as 16 mg/dl leads to a marked inhibition of glucose-stimulated insulin secretion (Leahy et al. 1987). Similar observations have been made in subtotal pancreatectomized dogs (Imamura et al. 1988). Studies by Rossetti et al. (Rossetti et al. 1987a) have provided definitive proof of the glucotoxicity concept. Partially pancreatectomized diabetic rats have severe defects in both first- and second-phase insulin secretion compared with control rats. Phlorizin, an inhibitor of SGLT2/SGLT1 transport in the kidney, normalizes the plasma glucose profile by inducing glucosuria without change in any other circulating metabolites and restores to normal both the first and second phases of insulin secretion. In humans both dapagliflozin (Merovci et al. 2015, 2016) and empagliflozin (Ferrannini et al. 2014) inhibit renal glucose transport, induce glucosuria, lower the plasma glucose concentration, and markedly improve beta cell function in T2DM patients. These studies with SGLT2 inhibitors provide definitive proof for the glucotoxic effect of hyperglycemia on beta cell function. In humans correction of hyperglycemia with insulin (Li et al. 2004; Vague and Moulin 1982; Garvey et al. 1985; Weng et al. 2008; Andrews et al. 1984; Bunck et al. 2011) improves beta cell function, but these studies are difficult to interpret since insulin therapy also reverses lipotoxicity. Chronic hyperglycemia also causes muscle insulin resistance (Yki-Jarvinen et al. 1996; Copeland et al. 2008; McClain et al. 2002; Rossetti et al. 1987b), which can be ameliorated by inducing glucosuria and reducing the plasma glucose concentration with an inhibitor of renal glucose transport (Merovci et al. 2014). Glucotoxicity also has been implicated in the development of hyperglucagonemia in diabetic dogs (Starke et al. 1985) and rodents (Jamison et al. 2011). Two mechanisms have been implicated in hyperglycemia-induced beta cell dysfunction: (i) O-linked glycosylation of serine and threonine residues of nuclear and cystolic proteins secondary to increased hexosamine flux and (ii) oxidative stress (reviewed in references (Rossetti et al. 1990) and (Yki-Jarvinen and DA 2015)). Beta cells are especially sensitive to oxidative stress because they contain low levels of antioxidant enzymes (Tiedge et al. 1997).

IAPP. Excessive IAPP secretion resulting in amyloid deposition within the pancreas also contributes to progressive β-cell failure in T2DM (Hansen and Bodkin 1986; Haataja et al. 2008; Guardado-Mendoza et al. 2009; Montane et al. 2012; Westermark et al. 2011; Clark et al. 1988). Convincing evidence for a pathogenic role of IAPP has been generated in rodents (Bretherton-Watt et al. 1989; Ohsawa et al. 1989), baboons (Guardado-Mendoza et al. 2009; Howard 1986; Cox et al. 2006), and humans (Westermark and Wilander 1978; Clark et al. 1988; Ritzel et al. 2007; Chavez et al. 2008; Huang et al. 2007). The natural history of pancreatic amylin deposition in humans parallels that in rodents and primates (Howard 1986). In baboons, as the amyloid area of the pancreatic islets increases from $<5\%$ to

>51%, there is a progressive decline in the log of HOMA-β, which correlates strongly with the increase in FPG concentration (Hansen and Bodkin 1986). It follows that insulin sensitizing interventions (i.e., thiazolidinediones and weight loss), by reducing insulin secretion and therefore IAPP secretion (insulin and IAPP are co-secreted in a one-to-one molar ratio), should preserve β-cell function. Consistent with this, rosiglitazone has been shown to protect human islets against IAPP toxicity by a PI-3 kinase-dependent pathway (Lin et al. 2005).

Incretins. The insulin response following glucose ingestion is ~2.5-fold greater than a similar level of hyperglycemia created by intravenous glucose (Nauck et al. 1986a), and this has been referred to as the incretin effect. Two hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide, account for 90% of the incretin effect and, following oral glucose, they account for 60–70% of the insulin that is secreted (Nauck et al. 1986b). In T2DM and NGT obese subjects, the incretin effect is characteristically lost (Nauck et al. 1986a; Michaliszyn et al. 2014; Holst et al. 2011; Muscelli et al. 2008); this could be explained by a decrease in GLP-1/GIP secretion or resistance to GLP-1/GIP. A small decline in GLP-1 secretion or a delayed GLP-1 response has been reported in some studies (Drucker 2013; Nauck and Meier 2016; Nauck et al. 2011), while GIP secretion generally has been normal (Drucker 2013; Nauck and Meier 2016; Holst and Gromada 2004). In contrast, there is severe resistance to the beta cell stimulatory effect of both GLP-1 and GIP (Drucker 2013; Nauck and Meier 2016; Michaliszyn et al. 2014; Hojberg et al. 2009; Vilsboll et al. 2002; Tura et al. 2014). The resistance to GLP-1 is observed in individuals with IGT and worsens with progression to T2DM (Meier et al. 2001). GLP-1 resistance can be overcome by infusing high doses of GLP-1 (Zander et al. 2002) or administration of GLP-1 receptor antagonists (RA) (Bunck et al. 2011; Chang et al. 2003; Degn et al. 2004) to generate pharmacologic plasma levels (70–90 pM) of the incretin. This explains why GLP-1 RAs, but not DPP-4 inhibitors, overcome the incretin defect and cause a normalization/near normalization of beta cell function (Bunck et al. 2011; Chang et al. 2003; Kapitza et al. 2016). Tight glycemic control for as little as 4 weeks can improve the insulin secretory response to both GLP-1 and GIP (Hojberg et al. 2009). Studies in patients with diabetes secondary to chronic pancreatitis and typical T2DM also suggest that the incretin defect in T2DM is, in part, an acquired disturbance related to poor metabolic control (Knop et al. 2007). Thus, β-cell resistance to GLP-1 and GIP is another manifestation of glucotoxicity.

In Utero Fetal Malnutrition

Low birth weight in humans and primates is associated with the development of IGT and T2DM later in life (Eriksson 1996; Martin-Gronert and Ozanne 2012). Poor nutrition and impaired fetal growth (small babies at birth) are associated with impaired insulin secretion and/or reduced β-cell mass, as well as insulin resistance (Phillips 1996). Thus, an environmental influence, i.e., impaired fetal nutrition leading to an acquired defect in insulin secretion or reduced β-cell mass, when

superimposed on insulin resistance, could eventuate in T2DM later in life. During normal aging, with the onset of obesity or with a worsening of the genetic component of the insulin resistance, the β cell would be called upon to augment its secretion of insulin to offset the defect in insulin action. If β-cell mass (or function) is reduced (or impaired) by an environmental insult during fetal life, this could lead to the development of IGT and eventually overt T2DM. Over nutrition during fetal development also has been associated with the onset of obesity and T2DM later in life (Godfrey et al. 2017; Cox et al. 2013). Thus, both the fetal and maternal environment during gestation can have a profound effect on the development of obesity and diabetes in adulthood.

Beta Cell Mass

With a few exceptions, the majority of pancreatic autopsy studies have demonstrated a reduction in beta cell mass, volume, ranging from 20% to 50% (Henquin and Rahier 2011; Yoon et al. 2003; Rahier et al. 2008; Hanley et al. 2010; Marselli et al. 2014). A decrease in beta cell mass volume also has been described in prediabetic individuals with IFG (Butler et al. 2003; Yoneda et al. 2013). In a well-controlled study involving 57 T2DM and 52 nondiabetic subjects of European descent, beta cell mass was reduced by ~35% (Henquin and Rahier 2011) (Fig. 5). This study makes two additional points: (i) within the T2DM and nondiabetic groups, there was considerable overlap so that a clear separation between the two groups is difficult to discern; and (ii) the alpha cell mass is similar between the two groups, indicating that the hyperglucagonemia in T2DM patients results from a reduced paracrine effect of insulin and/or decreased circulating levels of insulin to inhibit glucagon secretion.

The mechanism(s) responsible for the decreased beta cell mass in T2DM remain controversial. Increased apoptosis consistently has been observed in pancreatic samples obtained at autopsy (Hanley et al. 2010; Yoneda et al. 2013; Wang et al. 2013), but accelerated autophagy also has been described (Masini et al. 2009). Evidence to support defects in beta cell replication (Gianani 2011; Desgraz et al. 2011), neogenesis (Bonner-Weir et al. 2008; Halban et al. 2010), and transdifferentiation of mature beta cells (Bonner-Weir et al. 2008) also has been generated. Whatever are the mechanisms, reduced beta cell mass alone cannot explain the reduction in insulin secretion, especially in the early developmental stages of T2DM for the following reasons: (i) at the time of diagnosis of T2DM over 80% of beta cell function has been lost (Fig. 2), whereas beta cell mass is, at most, reduced by 20–40%; (ii) estimates of beta cell mass in T2DM subjects overlap considerably with those in nondiabetic individuals (Fig. 5); (iii) following bariatric surgery full recovery of beta cell function with resolution of hyperglycemia is observed (Ferrannini and Mingrone 2009); (iv) treatment with GLP-1 RAs and thiazolidinediones markedly increase or even restore normal beta cell function (Gastaldelli et al. 2007a; DeFronzo et al. 2013a; Bunck et al. 2011; Chang et al. 2003; Kapitza et al. 2016); and (v) studies with isolated islets from T2DM individuals consistently have demonstrated a severe defect in insulin secretion (Marchetti et al. 2004; Del Guerra

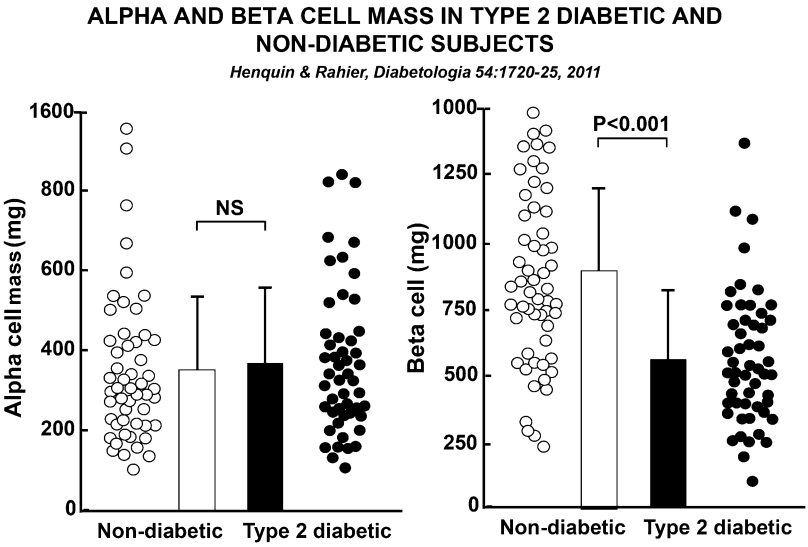


Fig. 5 Beta cell and alpha cell mass in 57 type 2 diabetic and 52 nondiabetic individuals. On mean, beta cell mass was decreased by ~35% in diabetic subjects. There was no difference in alpha cell mass. (From Henquin et al., *Diabetologia* 54:1720–1725, 2011)

et al. 2005). It is likely that light and EM studies have greatly underestimated the number of living, but functionally incompetent beta cells, due to the marked reduced number/absence of insulin granules.

Summary. Although insulin resistance in liver and muscle are well established early in the natural history of the disease, overt T2DM does not occur in the absence of progressive β-cell failure.

Insulin Resistance and Type 2 Diabetes Mellitus

Cross-sectional and long-term, prospective longitudinal studies have demonstrated that hyperinsulinemia precedes the onset of T2DM in ethnic populations with a high incidence of T2DM (DeFronzo 1998, 1997, 2009; Saad et al. 1989; Haffner et al. 1995; Weyer et al. 2001, 2000; Gulli et al. 1992; Reaven et al. 1989; Godfrey et al. 2017; Cox et al. 2013; Yoon et al. 2003; Rahier et al. 2008; Hanley et al. 2010; Sicree et al. 1987; Lillioja et al. 1991). The euglycemic insulin clamp, minimal model, and insulin suppression techniques have provided direct quantitative evidence that the progression from normal to impaired glucose tolerance is associated with the development of severe insulin resistance, whereas the fasting and glucose-stimulated plasma insulin concentrations (Fig. 1) are increased in absolute terms (see earlier discussion about insulin secretion). The major exception to this is the development of T2DM in Asian populations, where insulin deficiency is the predominant pathophysiologic abnormality (Abdul-Ghani et al. 2007a; Yang and Weng 2014; Ma et al. 2014). As discussed earlier,

this most likely is explained by the low BMI (18–20 kg/m^2) which renders them insulin sensitive and requires a major reduction (≥80%, i.e., similar to that in T1DM) in insulin secretion before diabetes becomes manifest.

From the historical prospective, Himsworth and Kerr, using a combined oral glucose and IV insulin tolerance test, were the first to demonstrate that tissue sensitivity to insulin was diminished in T2DM patients (Himsworth and Kerr 1939). Subsequently, Reaven et al. using the insulin suppression test, provided further evidence that T2DM individuals were resistant to insulin (Reaven et al. 1989; Ginsberg et al. 1975). Muscle insulin resistance in T2DM also was demonstrated with direct infusion of insulin into the brachial (forearm muscle) and femoral (leg muscle) arteries, with radioisotope turnover studies, with the frequently sampled IV glucose tolerance test, and with the minimal model technique (DeFronzo 1998, 1997, 2009; Ferrannini et al. 1988; Bergman 1989; Butterfield and Whichelow 1965; Katz et al. 1994).

Definitive evidence of insulin resistance in lean, as well as obese T2DM individuals, was provided by DeFronzo et al., with the more physiologic euglycemic insulin clamp technique (Fig. 6) (DeFronzo 1998, 1997, 2009; Groop et al. 1989; Eriksson et al. 1989; Pendergrass et al. 2007; DeFronzo et al. 1979a, 1978b; Bajaj and DeFronzo 2003). Because diabetic patients with severe fasting hyperglycemia (>180–200 mg/dl, 10.0–11.1 mmol/l) are insulinopenic (Fig. 1) and because insulin deficiency is associated with the emergence of intracellular defects in insulin action, initial studies focused on diabetic subjects with mild to modest elevations in the fasting plasma glucose concentration (mean = 150 ± 8 mg/dl, 8.3 ± 0.4 mmol/l). Insulin-mediated whole-body glucose disposal in these lean T2DM subjects was reduced by ~40–50%, providing conclusive proof of the presence of moderate–severe insulin resistance. Six additional points are noteworthy: (i) lean

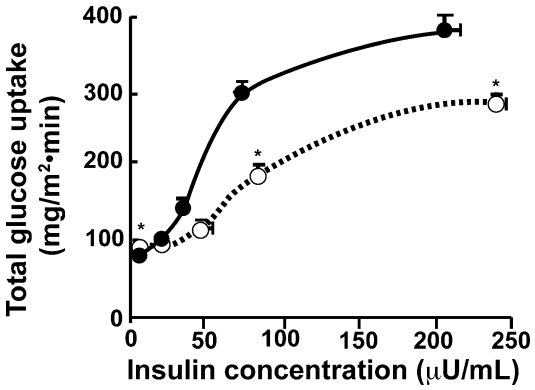


Fig. 6 Dose-response curve relating the plasma insulin concentration to the rate of insulin-mediated whole-body glucose uptake in control (solid circles, solid line) and type 2 diabetic (open circles, dashed line) subjects. *$p < 0.01$ vs. control subjects. (Source: Groop L, et al. *Journal of Clinical Investigation* 1989;**84**:205–215. Reproduced with permission of American Society for Clinical Investigation)

T2DM individuals with marked fasting hyperglycemia (198 ± 10 mg/dl) have a severity of insulin resistance that is only slightly (10–20%) greater than in diabetics with mild fasting hyperglycemia; (ii) the defect in insulin action is observed at all plasma insulin concentrations, spanning the physiologic and pharmacologic range (Fig. 6); (iii) maximally stimulating plasma insulin concentrations cannot elicit a normal glucose metabolic response in diabetic patients with overt fasting hyperglycemia; (iv) individuals with IGT are nearly as insulin resistance as individuals with T2DM; (v) obese NGT individuals are as insulin resistant as lean T2DM subjects; and (vi) insulin resistance in obese T2DM individuals is only slightly greater than that in obese NGT or lean T2DM subjects. Virtually all investigators have demonstrated that lean T2DM subjects are resistant to the action of insulin (DeFronzo 1998, 1997, 2009; Eriksson et al. 1989; Bergman et al. 2002; Kahn 2003; Cox et al. 2013; Yoon et al. 2003; Rahier et al. 2008; Hanley et al. 2010; Firth et al. 1987; Campbell et al. 1988; Bogardus et al. 1984).

Glucose-Mediated Glucose Uptake

Glucose (hyperglycemia) exerts its own effect to stimulate glucose uptake. In T2DM patients, the mass action effect of hyperglycemia also is impaired in T2DM (Del Prato et al. 1997).

Site of Insulin Resistance in Type 2 Diabetes

Both the liver and muscle, the two tissues primarily responsible for the maintenance of normal glucose homeostasis following ingestion of an oral glucose load, are severely resistant to insulin in T2DM individuals (reviewed in references (DeFronzo 1998; DeFronzo 1997; DeFronzo 2009)). However, adipose tissue (Groop et al. 1989; Guilherme et al. 2008), kidney (Meyer et al. 1998a; Gerich et al. 2001), gastrointestinal tract (Honka et al. 2013), brain (Blazquez et al. 2014; Kleinridders et al. 2014), and pancreatic beta cells (Kulkarni et al. 1999; Oliveira et al. 2014) also are resistant to insulin. When discussing insulin resistance, it is important to distinguish what tissues are responsible for the insulin resistance in the basal (fasting) state and what tissues are responsible for insulin resistance in the insulin-stimulated (prandial) state.

Liver. The brain and all neuronal tissues have an obligate need for glucose and are responsible for ~50% of glucose utilization under basal or fasting conditions (DeFronzo and Ferrannini 2010; Grill 1990). This glucose demand is met by glucose production by the liver (80–90%) and kidneys (10–20%) (DeFronzo and Ferrannini 2010). In nondiabetic individuals, endogenous (liver plus kidneys) glucose production (EGP) following an overnight fast occurs at the rate of ~2.0 mg/kg per min (DeFronzo 1998, 1997, 2009; DeFronzo et al. 1989) (Fig. 7). In T2DM individuals, the basal rate of EGP is increased, averaging ~2.5 mg/kg^{-1} per min (DeFronzo 1998, 1997, 2009; Lyssenko et al. 2008) (Fig. 7). This amounts to the addition of an extra

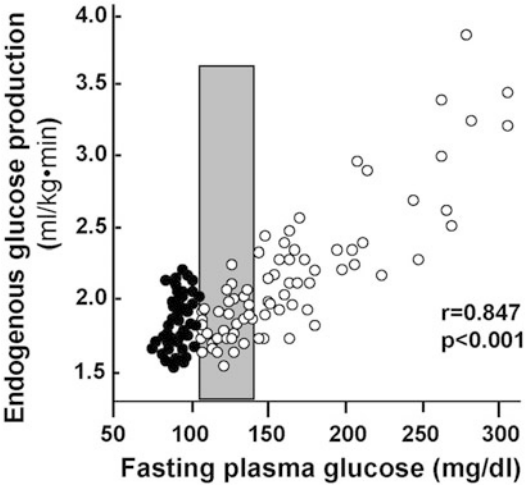


Fig. 7 Summary of HGP in 77 normal-weight type 2 diabetic subjects (open circles) with fasting plasma glucose concentrations ranging from 105 to >300 mg/dl; 72 control subjects matched for age and weight are shown by solid circles. In the 33 diabetic subjects with fasting plasma glucose levels <140 mg/dl (shaded area), the mean rate of HGP was identical to that of control subjects. In diabetic subjects with fasting plasma glucose concentrations >140 mg/dl, there was a progressive rise in HGP that correlated closely ($r = 0.847$, $p < 0.001$) with the fasting plasma glucose concentration. (Source: DeFronzo RA, et al. *Metabolism* 1989;**38**:387–395. Reproduced with permission of Elsevier)

25–30 g of glucose to the systemic circulation every night in an 80-kg person. In NGT subjects with FPG = 85–90 mg/dl, the basal rate of EGP averages ~2 mg/kg per min. In T2DM subjects, EGP is increased and the FPG concentration rises in direct proportion to the increase in the basal rate of EGP ($r = 0.847, p < 0.001$). The excessive glucose production by the liver and kidney occurs despite fasting plasma insulin levels that are increased 2.5- to 3-fold, indicating resistance to the suppressive effect of insulin on EGP. Similar observations consistently have been made by others (Groop et al. 1989; Ferrannini et al. 1988; Firth et al. 1987; Campbell et al. 1988; Shulman et al. 1985; Chen et al. 1988; Henry et al. 1986). The increase in basal EGP is explained entirely by an increase in hepatic and renal (the kidney contains little glycogen) gluconeogenesis (DeFronzo and Ferrannini 1987; Magnusson et al. 1992; Consoli et al. 1990). In addition to insulin resistance, multiple other factors contribute to the accelerated rate of basal HGP including: (i) increased circulating glucagon levels and enhanced hepatic sensitivity to glucagon (Baron et al. 1987; Matsuda et al. 2002; Unger et al. 1970); (ii) increased substrate (fatty acids, lactate, amino acids, glycerol) delivery (DeFronzo and Ferrannini 1987; Magnusson et al. 1992; Consoli et al. 1990; Gastaldelli et al. 2000; Samuel and Shulman 2016); (iii) lipotoxicity leading to increased expression and activity of phosphoenolpyruvate carboxykinase and pyruvate carboxylase (Tordjman et al. 2004), the rate-limiting enzymes for gluconeogenesis; (iv) increased expression and activity of glucose-6-phosphatase, the rate-limiting enzyme for glucose escape

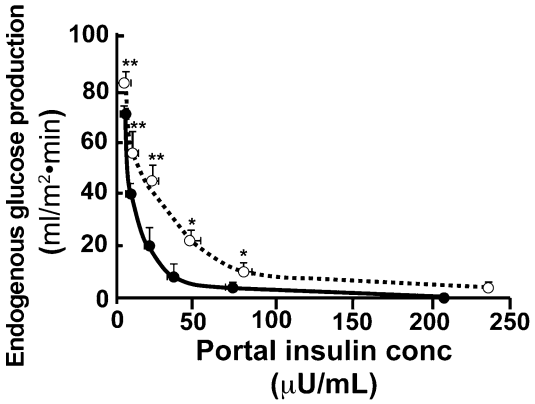


Fig. 8 Dose-response curve relating the plasma insulin concentration to the suppression of HGP in control (solid circles, solid line) and type 2 diabetic (open circles, dashed line) subjects with moderately severe fasting hyperglycemia. *$p < 0.05$, **$p < 0.01$ vs. control subjects. (Source: Groop L, et al. *Journal of Clinical Investigation* 1989;**84**:205–215. Reproduced with permission of American Society for Clinical Investigation)

from the liver; in rodents increased G6Pase activity results from glucotoxicity (Clore et al. 2000); and (v) resistance to the suppressive effect of GLP-1 on glucagon secretion and stimulatory effect of GLP-1 on insulin secretion, resulting in an increase in the portal glucagon/insulin ratio.

It is noteworthy that an increase in EGP does not occur until the FPG exceeds ~140 mg/dl (DeFronzo 1998, 2009; DeFronzo et al. 1989) (Fig. 7). Thus, in subjects with IFG and T2DM individuals with mild fasting hyperglycemia, reduced glucose clearance accounts for the increase in FPG concentration. Further, the decrease in glucose clearance occurs in noninsulin-dependent tissues and resides, at least in part, in the splanchnic (gastrointestinal and liver) bed (Alatrach et al. 2017). Following glucose or mixed meal ingestion, both the liver and kidney of T2DM patients are resistant to the suppressive effect of insulin on glucose production (Gerich et al. 2001; DeFronzo et al. 1978a; Ferrannini et al. 1988). Using the euglycemic insulin clamp in combination with isotopic glucose, the dose-response relationship between endogenous (hepatic plus renal) glucose production and the plasma insulin concentration has been examined (Groop et al. 1989) (Fig. 8). The following points deserve emphasis: (i) the dose-response curve relating inhibition of EGP to the plasma insulin concentration is very steep, with a half-maximal insulin concentration (ED50) of ~30–40 μU/ml; (ii) in T2DM subjects the dose-response curve is shifted rightward, indicating resistance to the inhibitory effect of insulin on EGP; however, high physiologic plasma insulin concentrations (~100 uU/ml) can overcome the insulin resistance and cause a normal/near normal suppression of EGP; (iii) the severity of hepatic/renal insulin resistance is related to the level of glycemic control. In T2DM patients with mild fasting hyperglycemia, a rise in plasma insulin concentration of 100 μU/ml causes a complete suppression of EPG. However, in diabetic subjects with more severe fasting hyperglycemia, the ability of the same plasma insulin concentration to suppress EGP is

impaired, indicating that there is an acquired component of hepatic/renal insulin resistance that becomes progressively worse with deteriorating glycemic control; (iv) in NGT subjects the kidney contributes ~10–20% of total EGP under fasting conditions. The kidney contains little glycogen but possesses all of the gluconeogenic enzymes required to produce glucose (Gerich et al. 2001; Ekberg et al. 1999; Moller et al. 2001). Renal gluconeogenesis is inhibited by insulin (Meyer et al. 1998a; Gustavson et al. 2004) and stimulated by epinephrine (Stumvoll et al. 1995) but not glucagon (Stumvoll et al. 1998; Gustavson et al. 2004). In T2DM subjects, the basal rate of renal glucose production is increased (Meyer et al. 1998b) despite the presence of fasting hyperinsulinemia, indicating resistance to the suppressive effect of insulin on renal glucose output.

Muscle. Following ingestion of glucose or intravenous glucose administration, muscle is the major site of insulin-mediated glucose disposal in humans (DeFronzo 1998, 1997, 2009; DeFronzo et al. 1985; Ferrannini et al. 1985). Using the euglycemic insulin clamp technique in combination with radiolabeled glucose to measure total body glucose disposal (DeFronzo 1998, 1997, 2009; DeFronzo et al. 1985, 1978a, 1979a; Groop et al. 1989; Lillioja et al. 1993; Weyer et al. 2001; Eriksson et al. 1989; Pendergrass et al. 2007; Bajaj and DeFronzo 2003; Abdul-Ghani et al. 2006a; Kahn et al. 2012; Firth et al. 1987; Campbell et al. 1988; Bogardus et al. 1984; Shulman et al. 1985; Chen et al. 1988; Henry et al. 1986; Reaven 1988; Kolterman et al. 1981), it conclusively has been demonstrated that lean type 2 diabetic individuals are severely resistant to insulin compared with age-, weight-, and sex-matched controls. By combining femoral arterial/venous catheterization with the insulin clamp, muscle insulin resistance has been documented to account for 85–90% of the defect in total body glucose disposal in T2DM subjects (DeFronzo et al. 1985; Pendergrass et al. 2007) (Fig. 9). Following the start of insulin infusion, there is a 20–30 min delay in muscle glucose uptake and the rate of insulin-stimulated glucose disposal remains 50% less than in control subjects even if the insulin infusion is continued for an additional 60 min to compensate for the delayed onset of insulin action. Impaired insulin-stimulated muscle glucose uptake in T2DM subjects also has been demonstrated using the limb catheterization technique (Pendergrass et al. 2007; Butterfield and Whichelow 1965; Cline et al. 1999; Zierler and Rabinowitz 1963; Bonadonna et al. 1996). It is noteworthy that NGT obese subjects are as insulin resistant as lean T2DM individuals and that muscle is the major tissue responsible for the insulin resistance (DeFronzo 1998, 2009; Groop et al. 1989; Jallut et al. 1990; Pendergrass et al. 2007; DeFronzo et al. 1978b; Reaven et al. 1989; Himsworth and Kerr 1939; Bogardus et al. 1984; Reaven 1988). Obese T2DM individuals are only modestly more insulin resistant than lean T2DM or obese NGT subjects.

In T2DM subjects multiple intramyocellular defects in insulin action have been demonstrated (reviewed in references (DeFronzo 1998; DeFronzo 1997; DeFronzo 2009; Bajaj and DeFronzo 2003)), including impaired insulin signal transduction (DeFronzo 2009, 2010; Cusi et al. 2000), reduced glucose transport and phosphorylation (Pendergrass et al. 2007; Cline et al. 1999; Bonadonna et al. 1996; Rothman et al. 1992; Mandarino et al. 1995, 1987), decreased glycogen synthesis (DeFronzo 1997; Groop et al. 1989; Mandarino et al. 1987; Shulman et al. 1990), and impaired

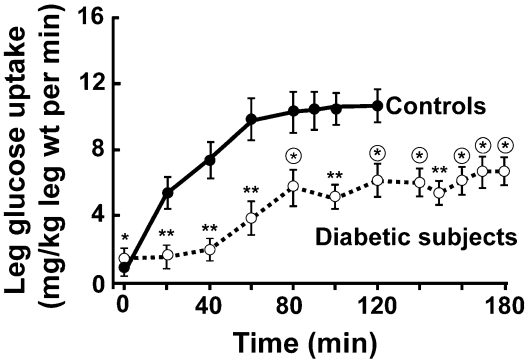


Fig. 9 Time course of change in leg glucose uptake in type 2 diabetic (open circles, dashed line) and control (solid circles, solid line) subjects. In the postabsorptive state, glucose uptake in the diabetic group was significantly greater than that in control subjects. However, the ability of insulin (euglycemic insulin clamp) to stimulate leg glucose uptake was reduced by 50% in the diabetic subjects. (Source: DeFronzo RA, et al. *Journal of Clinical Investigation* 1985;**76**:149–155. Reproduced with permission of American Society for Clinical Investigation)

glucose oxidation (DeFronzo 1997; Groop et al. 1989, 1991; Jallut et al. 1990; Shulman et al. 1990).

Insulin signal transduction. The first step in insulin action involves its binding to and the activation of the insulin receptor by phosphorylating 3 key tyrosine residues on the β chain of the receptor (DeFronzo 1997, 2010; Bajaj and DeFronzo 2003; Cusi et al. 2000; Tanijuchi et al. 2006; Saltiel and Kahn 2001; Musi and Goodyear 2006) (Fig. 10). This causes the translocation of insulin receptor substrate-1 (IRS)-1 to the plasma membrane, where it interacts with the insulin receptor and also undergoes tyrosine phosphorylation on contiguous tyrosine residues. This results in activation of PI-3 kinase and Akt, leading to (i) glucose transport into the cell, (ii) activation of nitric oxide synthase, increased nitric oxide generation, and arterial vasodilation (Kashyap and DeFronzo 2007; Kashyap et al. 2005; Montagnani et al. 2001), and (iii) stimulation of multiple intracellular metabolic processes involved in glucose, protein, and lipid metabolism.

DeFronzo and colleagues were the first to demonstrate in humans that tyrosine phosphorylation of IRS-1 by insulin was severely impaired in muscle of lean T2DM individuals (DeFronzo 2009, 2010; Cusi et al. 2000; Hundal et al. 2002), in obese NGT individuals (Cusi et al. 2000), and in the insulin-resistant NGT offspring of two T2DM parents (Pratipanawatr et al. 2001) (Fig. 11). A similar defect has been demonstrated by others in human muscle from T2DM individuals (Bajaj and DeFronzo 2003; Krook et al. 2000; Kim et al. 2002; Hundal et al. 2002; Bouzakri et al. 2003). Impaired insulin signaling leads to (i) decreased glucose transport, (ii) impaired nitric oxide generation causing endothelial dysfunction, and (iii) multiple defects in intramyocellular glucose metabolism.

In contrast to the severe defect in IRS-1 activation, the mitogen-activated protein (MAP) kinase pathway, which alternatively can be activated by Shc, is normally

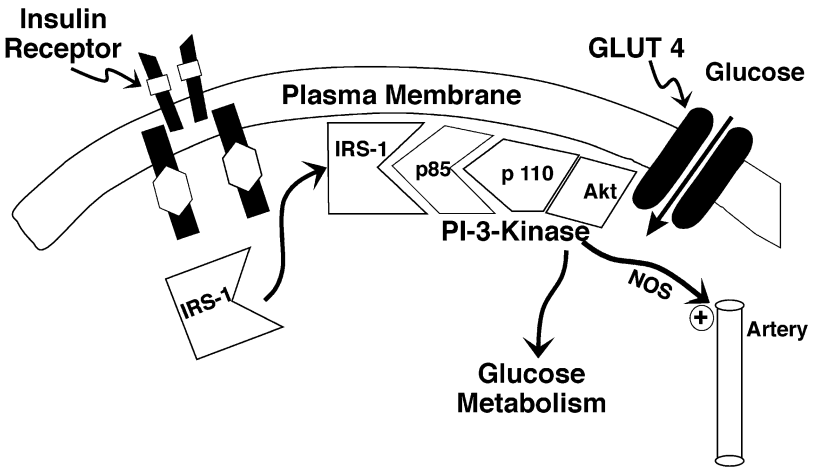


Fig. 10 Insulin signaling pathway in healthy subjects

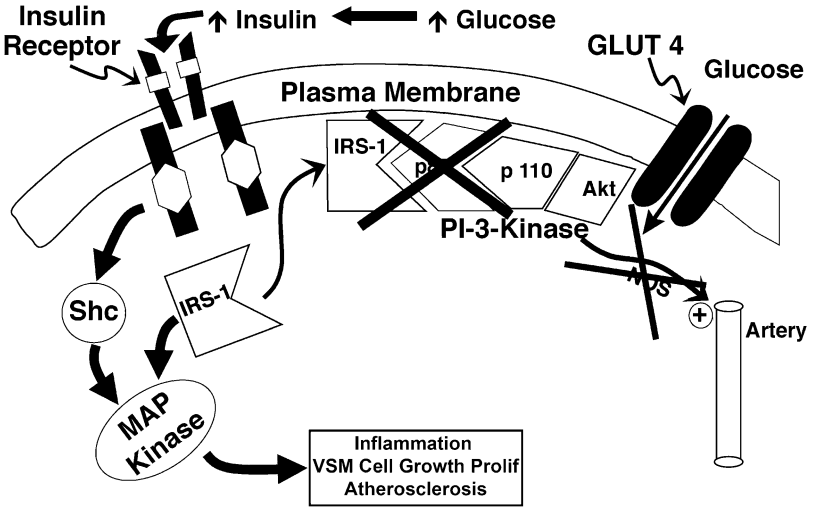


Fig. 11 Consequences of impaired insulin signaling in individuals with type 2 diabetes mellitus. See text for more detailed description

responsive to insulin (Cusi et al. 2000) (Fig. 11). Activation of the MAP kinase pathway stimulates multiple intracellular pathways involved in inflammation, cellular proliferation, and atherosclerosis (DeFronzo 2010; Wang et al. 2004; Draznin 2006; Hsueh and Law 1999). Inhibition of the insulin signaling pathway at the level of IRS-1 impairs glucose transport, leading to glucose intolerance and hyperglycemia which stimulates insulin secretion. Because the MAP kinase pathway is normally sensitive to

insulin (Cusi et al. 2000; DeFronzo 2010; Krook et al. 2000; Hsueh and Law 1999), the hyperinsulinemia, which results as a compensatory response to insulin resistance, leads to excessive stimulation of the MAP kinase pathway with activation of multiple intracellular pathways involved in inflammation and atherogenesis. This, in part, can explain the strong association between insulin resistance and atherosclerotic cardiovascular disease in nondiabetic, as well as in type 2 diabetic, individuals (DeFronzo 2010, 2006; Hanley et al. 2002; Isomaa et al. 2001; Rutter et al. 2005; Bonora et al. 2007; Howard et al. 1998). Further, in the insulin resistant, hyperinsulinemic NGT offspring of two T2DM parents, the MAP kinase pathway is overactive (Pratipanawatr et al. 2001) despite the defect in the PI-3 kinase pathway. This may explain why many newly diagnosed T2DM patients present with clinically manifest atherosclerotic cardiovascular complications. The only class of oral antidiabetic drugs that simultaneously augment insulin signaling through the IRS-1/PI-3 kinase pathway and inhibit the MAP kinase pathways is the thiazolidinediones (Miyazaki et al. 2003).

Route of glucose administration: oral versus intravenous. The disposal of glucose differs markedly depending upon whether the glucose is ingested or administered intravenously. The euglycemic insulin clamp, by maintaining plasma glucose and insulin levels constant, represents the gold standard for quantitation of insulin sensitivity. However, the normal route of glucose administration in everyday life is via the gastrointestinal tract. Using a double tracer technique (1-^{14}C-glucose orally and 3-^{3}H-glucose intravenously) in combination with hepatic vein catheterization, the disposal of oral versus intravenous glucose has been examined in healthy, normal glucose-tolerant and type 2 diabetic subjects (DeFronzo et al. 1985, 1978a, c, 1983; Ferrannini et al. 1980, 1988). Following an overnight fast with fasting plasma glucose and insulin concentrations of 90 mg/dl and 11 mU/ml, respectively, the splanchnic tissues (primarily reflect the liver) take up glucose at ~0.5 mg/kg per min (Fig. 12), and splanchnic (hepatic) glucose uptake is not augmented by insulin concentrations in excess of 1000 uU/ml. Hyperglycemia increases splanchnic (hepatic) glucose uptake, but only indirect proportion to the rise in plasma glucose concentration. Insulin does not increase splanchnic (hepatic) glucose uptake above that observed with hyperglycemia alone. In contrast, following glucose ingestion splanchnic (hepatic) glucose uptake increases 4.5-fold, despite plasma insulin and glucose concentrations that are much lower than those achieved with intravenous glucose plus insulin (Fig. 12). In type 2 diabetic individuals, hepatic glucose uptake following oral glucose is markedly impaired (by >50%) despite higher plasma glucose and insulin concentrations than in nondiabetic subjects. These results demonstrate that T2DM individuals lack the gut effect responsible for enhancing hepatic glucose uptake following glucose ingestion. Studies in dogs have shown that the gut effect is related to a widening of the portal vein to hepatic artery glucose concentration gradient which, in turn, results in inhibition of the SNS, stimulation of hepatic glucokinase and glycogen synthase, inhibition of hepatic glucose production, and a reduction in glucose uptake by peripheral tissues (Cherrington 1999).

Summary. In summary, multiple pathophysiologic disturbances: impaired insulin secretion, decreased muscle glucose uptake, increased HGP, and decreased hepatic glucose uptake, contribute to the glucose intolerance in type 2 diabetic individuals.

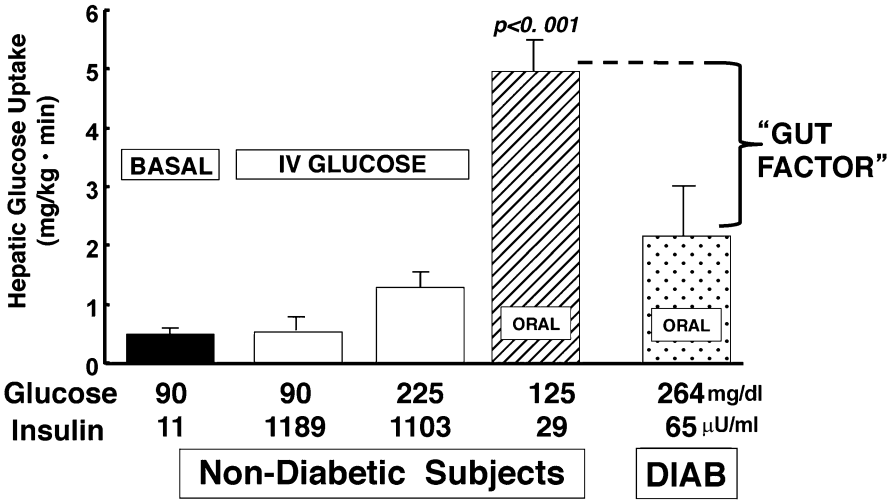


Fig. 12 Hepatic glucose uptake in nondiabetic and diabetic (DIAB) subjects as a function of plasma glucose and insulin concentration and route of glucose administration. Constructed from the results of *PNAS* 75:5173–77, 1978; *Diabetes* 32:35–45, 1983; *Metabolism* 37:79–85, 1988

Cellular Mechanisms of Insulin Resistance

In order for insulin to initiate its stimulatory effect on glucose metabolism, it must first bind to specific receptors that are present on the cell surface of all insulin target tissues (DeFronzo 1998, 1997, 2009, 2010; Cusi et al. 2000; Tanijuchi et al. 2006; Saltiel and Kahn 2001; Musi and Goodyear 2006) (Fig. 10). Following binding to and activation of its receptor, "second messengers" are generated that activate a cascade of phosphorylation-dephosphorylation reactions leading to insulin's multiple actions on glucose, lipid, and protein metabolism. The first step in glucose utilization involves activation of the glucose transport system, leading to glucose influx into insulin target tissues such as muscle and adipocytes (Fig. 13). The intracellular free glucose subsequently is metabolized by a series of enzymatic steps that are under the control of insulin. Of these, the most important are glucose phosphorylation (catalyzed by hexokinase), glycogen synthase and phosphorylase (which control glycogen synthesis), phosphofructokinase (PFK) and PDH (which regulate glycolysis and glucose oxidation, respectively), the Krebs cycle, and the mitochondrial oxidative phosphorylation chain.

Insulin Receptor/Insulin Receptor Tyrosine Kinase

The insulin receptor is a glycoprotein consisting of two α-subunits and two β-subunits linked by disulfide bonds (DeFronzo 1998, 1997, 2009, 2010; Cusi et al. 2000; Tanijuchi et al. 2006; Saltiel and Kahn 2001; Musi and Goodyear 2006) (Fig. 10).

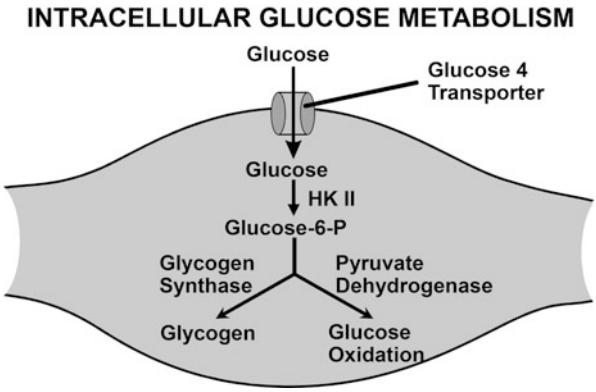


Fig. 13 Intramyocellular glucose metabolism. Type 2 diabetic individuals have defects in insulin-stimulated glucose transport, glucose phosphorylation, glucose oxidation, and glycogen synthesis. See text for detailed description

The α-subunits are entirely extracellular and contain the insulin-binding domain. The β-subunits have an extracellular domain, a transmembrane domain, and an intracellular domain that expresses insulin-stimulated kinase activity directed towards its own tyrosine residues. Phosphorylation of the β-subunit, with subsequent activation of insulin receptor tyrosine kinase, represents the first step in the action of insulin on glucose metabolism. Three tyrosine moieties on the B-subunit are essential for the action of insulin. Mutagenesis of any of these three major phosphorylation sites (at residues 1158, 1163, and 1162) impairs insulin receptor (IR) kinase activity and inhibits the metabolic and growth promoting effects of insulin (Chou et al. 1987). Serine phosphorylation of the insulin receptor and/or IRS-1 inhibits tyrosine phosphorylation of the IR/IRS-1 and causes insulin resistance. Multiple intracellular disturbances have been shown to increase serine phosphorylation of the insulin receptor and IRS-1, including ectopic lipid deposition (Belfort et al. 2005; DeFronzo 2010; Bajaj et al. 2010; Adams 2nd et al. 2004; Krssak et al. 1999; Petersen et al. 2005, 2002; Lara-Castro and Garvey 2008), mitochondrial dysfunction (Rains and Jain 2011), inflammation (Rains and Jain 2011; Romeo et al. 2012; Arkan et al. 2005; de Alvaro et al. 2004; Lebrun and Van Obberghen 2008; Shi et al. 2006), endoplasmic reticulum (ER) stress (Herschkovitz et al. 2007; Boden 2009; Sengupta et al. 2010; Shah et al. 2004), and increased hexosamine flux (Yki-Jarvinen and DA 2015; Zhang et al. 2013; Liu et al. 2010) (reviewed in reference (DeFronzo et al. 2015)).

Insulin Receptor Signal Transduction

Following its activation, insulin receptor tyrosine kinase phosphorylates specific intracellular proteins, of which at least nine have been identified (Saltiel and Kahn 2001; Musi and Goodyear 2006; Virkamaki et al. 1999). In muscle insulin-receptor substrate-1 (IRS-1) is the major docking protein that interacts with the insulin receptor tyrosine kinase and undergoes tyrosine phosphorylation in regions containing specific amino acid sequence motifs that, when phosphorylated, serve

as recognition sites for proteins containing *src*-homology 2 (SH2) domains. Mutation of these specific tyrosines impairs the ability of insulin to stimulate muscle glycogen synthesis, glucose oxidation, and other acute metabolic and growth promoting effects of insulin (Chou et al. 1987). In liver, IRS-2 serves as the primary docking protein that undergoes tyrosine phosphorylation and mediates insulin's effect on hepatic glucose production, gluconeogenesis, and glycogen formation (Kerouz et al. 1997).

Once phosphorylated, the tyrosine residues of IRS-1 mediate an association with the 85-kDa regulatory subunit of phosphatidylinositol-3 kinase (PI-3 kinase), resulting in activation of the enzyme (DeFronzo 1998, 1997, 2009; Saltiel and Kahn 2001; Musi and Goodyear 2006; Krook et al. 2000; Sun et al. 1992) (Fig. 10). PI-3 kinase is comprised of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The 110-k Da subunit catalyzes the 3-prime phosphorylation of phosphatidylinositol (PI), PI-4-phosphate, and PI-4,5-diphosphate, activating the glucose transport system and stimulating glycogen synthase via a process that involves activation of PKB/Akt and inhibition of kinases, such as glycogen synthase kinase (GSK)-3, and activation of protein phosphatase 1 (PP1). Inhibitors of PI-3 kinase impair glucose transport and block the activation of glycogen synthase and hexokinase (HK)-II expression (DeFronzo 2010; Saltiel and Kahn 2001; Musi and Goodyear 2006; Krook et al. 2000; Sun et al. 1992; Cross et al. 1994; Osawa et al. 1996). The action of insulin to increase protein synthesis and inhibit protein degradation also is mediated by PI-3 kinase.

Other proteins with SH2 domains, including the adapter protein Grb2 and *Shc*, also interact with IRS-1 and become phosphorylated following exposure to insulin (DeFronzo 2010; Saltiel and Kahn 2001; Musi and Goodyear 2006; Krook et al. 2000). Grb2 and *Shc* link IRS-1/IRS-2 to the mitogen-activated protein (MAP) signaling pathway (Fig. 11), which plays an important role in the generation of transcription factors and promotes cell growth, proliferation, and differentiation (Saltiel and Kahn 2001; Krook et al. 2000). Inhibition of the MAP kinase pathway prevents the stimulation of cell growth by insulin but has no effect on the metabolic actions of the hormone (Lazar et al. 1995). In T2DM patients, the MAP kinase pathway retains its sensitivity to insulin despite severe resistance in the PI-3 kinase/Akt pathway and plays a role in the accelerated atherogenesis that is characteristic of people with diabetes (DeFronzo 2010).

Insulin stimulates glycogen synthesis by simultaneously activating glycogen synthase and inhibiting glycogen phosphorylase (Dent et al. 1990; Newgard et al. 2000). This effect is mediated via the PI-3 kinase pathway which inhibits glycogen synthase kinase-3 and activates protein phosphatase 1 (PP1). PP1 is believed to be the primary regulator of glycogen metabolism. In skeletal muscle, PP1 associates with a specific glycogen-binding regulatory subunit, causing dephosphorylation (activation) of glycogen synthase. PP1 also phosphorylates (inactivates) glycogen phosphorylase. Multiple studies have demonstrated that inhibitors of PI-3 kinase abolish glycogen synthase activity and impair glycogen synthesis (Musi and Goodyear 2006; Sheperd et al. 1995).

Insulin Signaling Defects in Type 2 Diabetes

Insulin Receptor Number and Affinity

In type 2 diabetic patients, both insulin receptor and postreceptor defects contribute to the development of insulin resistance. Although some studies have demonstrated a modest 20–30% reduction in insulin binding to monocytes and adipocytes from T2DM patients, this has not been a consistent finding (DeFronzo 1998, 1997, 2009; Freidenberg et al. 1987; Caro et al. 1987, 1986; Trichitta et al. 1989). Decreased insulin binding results from a reduction in the number of insulin receptors without change in insulin receptor affinity. The relevance of these findings in monocytes and adipocytes to muscle and liver is unclear, since insulin binding to solubilized receptors obtained from skeletal muscle and liver is normal in obese and lean diabetic individuals (Caro et al. 1987, 1986; Klein et al. 1995). Further, a decrease in insulin receptor number cannot be demonstrated in more than half of T2DM subjects, and the correlation between reduced insulin binding and the severity of insulin resistance is weak (Kashiwagi et al. 1983; Lonnroth et al. 1983; Olefsky and Reaven 1977). Defects in insulin receptor internalization and processing have been identified in syndromes of severe insulin resistance and diabetes. However, the insulin receptor gene has been sequenced in T2DM individuals from diverse ethnic populations, and with rare exception, physiologically significant mutations have not been observed (Moller et al. 1989; Kusari et al. 1991). This excludes a structural gene abnormality in the insulin receptor as a cause of common type T2DM.

Insulin Receptor Tyrosine Kinase Activity

In skeletal muscle, adipocytes, and hepatocytes from normal weight and obese diabetic subjects, most (DeFronzo 1998, 1997, 2009; Cusi et al. 2000; Caro et al. 1986; Kashiwagi et al. 1983; Lonnroth et al. 1983; Nolan et al. 1994), but not all (Klein et al. 1995), investigators have found a reduction in insulin receptor tyrosine kinase activity (Fig. 11). This defect cannot be explained by reduced insulin receptor number or insulin receptor binding affinity. Restoration of normoglycemia by weight loss can correct the defect in insulin receptor tyrosine kinase activity (Freidenberg et al. 1988), indicating that the defect is acquired secondary to some combination of hyperglycemia, disturbed intracellular glucose metabolism, hyperinsulinemia, and/or ectopic lipid accumulation – all of which improved after weight loss. Of note, when fibroblasts are cultured in a medium containing a high glucose concentration, insulin receptor tyrosine kinase activity is inhibited (Kellerer et al. 1994). In insulin-resistant obese nondiabetic and type 2 diabetic subjects studied with the insulin clamp and muscle biopsies, a significant decrease in insulin receptor tyrosine phosphorylation has been demonstrated (Cusi et al. 2000). However, when examined in normal glucose-tolerant, insulin-resistant individuals (offspring of two diabetic parents who are at high risk of developing T2DM), a normal increase in insulin receptor tyrosine phosphorylation was observed (Pratipanawatr et al. 2001). These findings indicate that impaired insulin

receptor tyrosine kinase activity in T2DM patients is acquired secondary to hyperglycemia or some other metabolic disturbance. Ectopic lipid accumulation in muscle and liver (Belfort et al. 2005; DeFronzo et al. 2015; DeFronzo 2010; Bajaj et al. 2010; Adams 2nd et al. 2004; Krssak et al. 1999; Petersen et al. 2005, 2002; Lara-Castro and Garvey 2008; Yu et al. 2002) causes insulin resistance by increasing tissue levels of diacylglycerol (DAG), fatty acyl CoAs, and ceramides. These toxic lipid metabolites accumulate in obesity and T2DM and activate PKCθ in muscle (Krook et al. 2000; Szendroedi et al. 2014) and PKCδ (Bezy et al. 2011) and PKCε (Samuel et al. 2004, 2007) in liver, leading to serine phosphorylation of IRS proteins and inhibition of insulin signaling. Ceramide levels in plasma (Haus et al. 2009) and muscle (Adams 2nd et al. 2004; Larsen and Tennagels 2014) also are increased in T2DM individuals and are linked to insulin resistance. The length of fatty acid chains (Turpin et al. 2014) and site of cellular compartmentalization (Cantley et al. 2013) play an important role in promoting insulin resistance. Therapies, such as caloric restriction and thiazolidinediones, that reduce ectopic lipid accumulation enhance insulin signaling and improve insulin sensitivity (DeFronzo 2009, 2010).

IRS-1 and PI-3 Kinase Defects

In insulin-resistant obese nondiabetic subjects, the ability of insulin to activate insulin receptor and IRS-1 tyrosine phosphorylation in muscle is modestly reduced, while in T2DM individuals insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation are severely impaired (DeFronzo 2009; Cusi et al. 2000) (Fig. 11). Association of the p85 subunit of PI-3 kinase with IRS-1 and activation of PI-3 kinase also are greatly attenuated in obese nondiabetic and type 2 diabetic subjects compared to lean healthy controls (DeFronzo 2009; Cusi et al. 2000; Krook et al. 2000; Kim et al. 2002) (Fig. 11). The decrease in insulin-stimulated association of the p85 regulatory subunit of PI-3 kinase with IRS-1 is closely correlated with the reduction in insulin-stimulated muscle glycogen synthase activity and in vivo insulin-stimulated glucose disposal (Cusi et al. 2000). Impaired regulation of PI-3 kinase gene expression by insulin also has been demonstrated in skeletal muscle and adipose tissue of type 2 diabetic subjects (Andreelli et al. 1999). In animal models of diabetes, an 80–90% decrease in insulin-stimulated IRS-1 phosphorylation and PI-3 kinase activity has been reported (Folli et al. 1993).

The insulin-resistant, normal glucose-tolerant offspring of two type 2 diabetic parents are at high risk of developing T2DM later in life. In muscle IRS-1 tyrosine phosphorylation and association of p85 protein/PI-3 kinase activity with IRS-1 are markedly decreased despite normal tyrosine phosphorylation of the insulin receptor, and these insulin signaling defects are correlated closely with the severity of insulin resistance, measured with the euglycemic insulin clamp technique (Pratipanawatr et al. 2001). In summary, impaired association of PI-3 kinase with IRS-1 and its subsequent activation are characteristic abnormalities in type 2 diabetic patients, and these defects are correlated closely with in vivo muscle insulin resistance. A common mutation in the IRS-1 gene (Gly 972 Arg) has been associated with T2DM,

insulin resistance, and obesity, but the physiologic significance of this mutation remains to be established (Hitman et al. 1995).

In contrast to the insulin resistance in the PI-3 kinase pathway, activation of the MAP kinase pathway by insulin in insulin-resistant type 2 diabetic and obese non-diabetic individuals is completely intact (DeFronzo 1998, 1997, 2009, 2010; Cusi et al. 2000; Krook et al. 2000; Kim et al. 2002). Insulin normally stimulates MEK1 activity and ERK1/2 phosphorylation and activity in insulin-resistant obese non-diabetic and type 2 diabetic patients. Intact stimulation of the MAP kinase pathway by insulin in the presence of insulin resistance in the PI-3 kinase pathway plays an important role in the development of atherosclerosis (DeFronzo 2010). Since the metabolic (PI-3 kinase) pathway is impaired, plasma glucose levels rise, leading to stimulation of insulin secretion and hyperinsulinemia. Because insulin receptor function is normal or only modestly impaired, this leads to excessive stimulation of the MAP kinase (mitogenic) pathway in vascular tissues, with resultant proliferation of vascular smooth muscle cells, increased collagen formation, and increased production of growth factors and inflammatory cytokines (DeFronzo 2010; Wang et al. 2004; Hsueh and Law 1999; Jiang et al. 1999). Excessive stimulation of the MAP kinase pathway can be demonstrated long before the onset of T2DM (Pratipanawatr et al. 2001) and explains, in part, why 10–20% of T2DM patients at the time of initial diagnosis present with clinical evidence of atherosclerotic cardiovascular disease.

Glucose Transport (GLUT/SLC2A and SGLT/SLC5A Transporters)

Activation of the insulin signal transduction system stimulates glucose transport by promoting translocation of glucose transporters from an intracellular pool (associated with low-density microsomes) to the plasma membrane and their subsequent activation after insertion into the plasma membrane (Shepherd and Kahn 1999; Garvey 1998). There are five major facilitative glucose transporters with distinctive tissue distributions (Bell et al. 1990; Joost et al. 2002) (Table 1). GLUT4 is the insulin regulated transporter and is found in insulin-sensitive tissues, such as muscle and adipocytes. GLUT4 has a

Table 1 Classification of glucose transport and HK activity according to their tissue distribution and functional regulation

Organ	Glucose transporter	HK	Classification
Brain	GLUT1	HK-I	Glucose dependent
Erythrocyte	GLUT1	HK-I	Glucose dependent
Adipocyte	GLUT4	HK-II	Insulin dependent
Muscle	GLUT4	HK-II	Insulin dependent
Liver	GLUT2	HK-IVL	Glucose sensor
GK β cell	GLUT2	HK-IVB (glucokinase)	Glucose sensor
Gut	GLUT3-symporter	–	Sodium dependent
Kidney	GLUT3-symporter	–	Sodium dependent

Source: DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying genes. *Diabetes Reviews* 1997;5:177–269

K_m of ~5 mmol/L, which is close to the plasma glucose concentration, and is associated with hexokinase (HK)-II (Bell et al. 1990; Joost et al. 2002). In adipocytes and muscle of NGT individuals, insulin markedly increases GLUT4 concentration in the plasma membrane in association with a reciprocal decline in the intracellular GLUT4 pool. GLUT1 is the predominant glucose transporter in insulin-independent tissues such as brain and erythrocytes, but also is found in muscle, adipocytes, brain, and kidneys. In the kidney GLUT1 is found in the S2/S3 segment of the proximal tubule, where it participates in glucose reabsorption in concert with SGLT1. GLUT1 is located primarily in the plasma membrane, where its concentration does not change after exposure to insulin. GLUT1 has a low K_m (~1 mmol/L) which is well suited for its function to mediate basal glucose uptake; it is found in association with HKI (Rogers et al. 1975). GLUT2 is the major glucose transporter in liver and pancreatic β cells, where it is found in association with a specific hexokinase, HKIV, or glucokinase (Matchinsky 1996). GLUT2 has a very high K_m (~15–20 mmol L^{-1}), which allows the intracellular glucose concentration to rise in direct proportion to the increase in plasma glucose concentration. This unique characteristic allows these cells to function as glucose sensors. GLUT2 also is found in association with SGLT2 in the S1 segment of the renal proximal tubular cells, where it participates in glucose reabsorption.

Glucose transport activity in muscle and adipocytes of T2DM patients is severely impaired (Krook et al. 2000; Shepherd and Kahn 1999; Garvey 1998; Garvey et al. 1988; Zierath et al. 1996). In adipocytes from human and rodent models of T2DM, GLUT4 mRNA and protein content are markedly reduced, and the ability of insulin to stimulate translocation and activate the GLUT4 transporter is decreased. In contrast to adipocytes, muscle tissue from lean and obese T2DM subjects exhibits normal levels of GLUT4 mRNA and protein, demonstrating that transcriptional and translational regulation of GLUT4 is not impaired (Pedersen et al. 1990; Eriksson et al. 1992). These differences in GLUT4 expression between muscle and adipocytes demonstrate the tissue-specific regulation of this glucose transporter in man. Using a novel triple-tracer technique, the in vivo dose-response curve for the action of insulin on glucose transport in forearm skeletal muscle has been examined in T2DM subjects and has been shown to be severely impaired (Pendergrass et al. 2007; Bonadonna et al. 1996, 1993). Impaired in vivo muscle glucose transport in T2DM also has been demonstrated using MRI (Cline et al. 1999) and PET (Williams et al. 2001). Since the number of GLUT4 transporters in muscle of T2DM subjects is normal, decreased GLUT4 translocation and reduced intrinsic activity of the glucose transporter are responsible for the defect in muscle glucose transport. Large populations of type 2 diabetic individuals have been screened for GLUT4 mutations (Choi et al. 1991). Such mutations are very uncommon and, when detected, have been of questionable physiologic significance.

Glucose Phosphorylation

Glucose phosphorylation and glucose transport are tightly coupled (Perriott et al. 2001). Hexokinase isoenzymes (HK-I–HK-IV) catalyze the intracellular conversion of free glucose to glucose-6-phosphate (G-6-P) (Bell et al. 1990; Joost et al. 2002;

Rogers et al. 1975; Printz et al. 1995) (Table 1). HK-I, HK-II, and HK-III are single-chain peptides that have a very high affinity for glucose and demonstrate product inhibition by G-6-P. HK-IV, also called glucokinase, has a lower affinity for glucose and is not inhibited by G-6-P. Glucokinase (HK-IVB) is the glucose sensor in the β cell, while hepatic HK-IVL plays a central role in regulating hepatic glucose metabolism.

HK-II transcription in human skeletal muscle is regulated by insulin, whereas HK-I mRNA and protein levels are not affected by insulin (Mandarino et al. 1995; Vogt et al. 2000; Pendergrass et al. 1998a). Physiologic hyperinsulinemia for as little as 2–4 h increases HK-II cytosolic activity, protein content, and mRNA levels by 50–200% in healthy nondiabetic subjects, and this is associated with HK-II translocation from the cytosol to the mitochondria. In forearm muscle of lean T2DM individuals, insulin-stimulated glucose transport and glucose phosphorylation (measured with the triple tracer technique) are markedly impaired (Pendergrass et al. 2007; Bonadonna 1996; Bonadonna et al. 1993). However, the defect in glucose phosphorylation exceeds that of glucose transport, leading to an increase in the intracellular free glucose concentration within the space that is accessible to glucose. Thus, while both glucose transport and glucose phosphorylation are severely resistant to insulin in T2DM, impaired glucose phosphorylation (HK-II) appears to be the rate-limiting step for insulin action. Studies using ^{31}P-NMR in combination with 1-^{14}C-glucose also have demonstrated that both muscle glucose transport and glucose phosphorylation are resistant to insulin in T2DM subjects, but results from this study suggest that the glucose transport defect exceeds the defect in glucose phosphorylation (Cline et al. 1999). Because of methodologic differences, the results of the triple tracer (Pendergrass et al. 2007; Bonadonna 1996; Bonadonna et al. 1993) and MRI (Cline et al. 1999) studies cannot be reconciled at present. Nonetheless, both studies clearly demonstrate that both muscle glucose phosphorylation and glucose transport are severely impaired in T2DM patients. Decreased basal muscle HK-II activity and mRNA levels and impaired insulin-stimulated HK-II activity have been reported by other investigators in T2DM patients (Pendergrass et al. 1998a; Ducluzeau et al. 2001) as well as in subjects with IGT (Lehto et al. 1995). Since both defects are present in IGT and in the NGT of offspring of two diabetic parents, they cannot be explained by glucose toxicity.

Although several nucleotide substitutions have been found in the HKII gene in T2DM individuals, none are close to the glucose and ATP binding sites and none have been associated with insulin resistance (Lehto et al. 1995; Laakso et al. 1995; Echwald et al. 1995). Thus, an abnormality in the HKII gene is unlikely to explain the insulin resistance in common variety T2DM.

Glycogen Synthesis

Glucose-6-phosphate either can be converted to glycogen or enter the glycolytic pathway. Of the glucose that enters the glycolytic pathway, ~90% is oxidized and the remaining 10% is released as lactate (anaerobic glycolysis). At physiologic plasma

insulin concentrations, glycogen synthesis and glucose oxidation contribute approximately equally to glucose disposal in muscle. However, with increasing plasma insulin concentrations, glycogen synthesis becomes the dominant pathway (DeFronzo 1998, 1997, 2009; Groop et al. 1989; Thiebaud et al. 1982). Impaired insulin-stimulated glycogen synthesis is a characteristic finding in all insulin-resistant states including obesity, IGT, diabetes, and the metabolic syndrome in all ethnic groups and represents the major defect in insulin-mediated whole body glucose disposal (DeFronzo 1998, 1997, 2009, 2010; Groop et al. 1989; Gulli et al. 1992; Shulman et al. 1990; Golay et al. 1988; Lillioja et al. 1986; Del Prato et al. 1993). Impaired glycogen synthesis occurs early in the natural history of T2DM and can be documented in the insulin-resistant normal glucose-tolerant offspring of two diabetic parents, in the insulin-resistant first-degree relatives of type 2 diabetic individuals, and in the insulin-resistant normoglycemic twin of a monozygotic twin pair in which the other twin has T2DM (Gulli et al. 1992; Pratipanawatr et al. 2001; Rothman et al. 1995; Yki-Jarvinen et al. 1987).

Glycogen synthase is insulin-regulated and controls the rate of muscle glycogen synthesis (Dent et al. 1990; Sheperd et al. 1995; Pendergrass et al. 1998a; Yki-Jarvinen et al. 1987; Frame and Cohen 2001; Cohen 1999). Insulin stimulates glycogen synthase by initiating a cascade of phosphorylation-dephosphorylation reactions, which ultimately lead to the activation of PP1 (also called glycogen synthase phosphatase). The regulatory subunit of PP1 contains two serine phosphorylation sites. Phosphorylation of site 2 by cAMP-dependent kinase (PKA) inactivates PP1, while phosphorylation of site 1 by insulin activates PP1, leading to the stimulation of glycogen synthase. Phosphorylation of site 1 of PP1 by insulin in muscle is catalyzed by insulin-stimulated protein kinase 1 (ISPK-1). Because of their central role in muscle glycogen formation, the three enzymes – glycogen synthase, PP1, ISPK-1 – have been extensively studied in the individuals with T2DM.

Glycogen synthase exists in an active (dephosphorylated) and an inactive (phosphorylated) form (Dent et al. 1990; Newgard et al. 2000; Sheperd et al. 1995). Total glycogen synthase activity in T2DM subjects is reduced, and the ability of insulin to convert glycogen synthase from the inactive to active form is severely impaired (Cusi et al. 2000; Mandarino et al. 1987; Damsbo et al. 1991; Thorburn et al. 1990). The defect in insulin-stimulated glycogen synthase is evident in the normal glucose-tolerant, insulin-resistant relatives of T2DM individuals (Vaag et al. 1992). In insulin-resistant nondiabetic, as well as diabetic, Pima Indians activation of muscle PP1 (glycogen synthase phosphatase) by insulin is severely reduced (Nyomba et al. 1990). Since PP1 dephosphorylates glycogen synthase, leading to its activation, the defect in PP1 plays an important role in the muscle insulin resistance of T2DM.

In vivo studies have demonstrated that insulin does not increase glycogen synthase mRNA or protein expression in human muscle (Mandarino et al. 1995; Pratipanawatr et al. 2002; Vestergaard et al. 1993). However, glycogen synthase mRNA and protein levels are reduced in muscle of type 2 diabetic patients, and these abnormalities in transcription and translation contribute, in part, to the decreased glycogen synthase activity (Vestergaard et al. 1993, 1991). The major abnormality in

T2DM is the inability of insulin to dephosphorylate and activate glycogen synthase as a result of the impairment in insulin receptor signaling (see previous discussion).

Sequencing of the glycogen synthase gene has revealed either no mutations or rare nucleotide substitutions that cannot explain the defect in insulin-stimulated glycogen synthase activity (Majer et al. 1996; Orho et al. 1995; Bjorbaek et al. 1994). Several silent nucleotide substitutions in the PP1 and ISPK-1 genes have been identified in the Danish population, but the mRNA levels of both genes were normal in skeletal muscle (Bjorbaek et al. 1995). No structural gene abnormalities in the catalytic subunit of PP1 were detected in Pima Indians (Procharzka et al. 1995). Thus, neither mutations in the PP1 and ISPK-1 genes nor abnormalities in their translation can explain the impaired enzymatic activities of glycogen synthase and PP1 that have been observed in vivo. Similarly, there is no evidence that glycogen phosphorylase plays a role in the disturbance in glycogen formation in T2DM (Schalin-Jantti et al. 1992).

In summary, although glycogen synthase activity and glycogen synthesis are severely impaired in type 2 diabetic individuals, the basic molecular etiology of the defect remains to be elucidated.

Glycolysis and Glucose Oxidation

Glycolysis accounts for the disposal of approximately half of insulin-stimulated muscle glucose uptake (Groop et al. 1989; Thiebaud et al. 1982; Del Prato et al. 1993). Of the total glycolytic flux, glucose oxidation accounts for ~90% and anaerobic glycolysis (generation of lactate) accounts for the remaining 10%. Phospho-fructokinase (PFK) and pyruvate dehydrogenase (PDH) play pivotal roles in the regulation of glycolysis and glucose oxidation, respectively. In type 2 diabetic individuals, the ability of insulin to stimulate the glycolytic/glucose oxidative pathway is impaired (Groop et al. 1989; Thiebaud et al. 1982; Del Prato et al. 1993). Although one study suggested that PFK activity is modestly reduced in muscle biopsies from type 2 diabetic subjects (Falholt et al. 1988), most evidence indicates that the PFK activity is normal (Mandarino et al. 1987; Vestergaard et al. 1993). Insulin has no effect on muscle PFK activity, mRNA levels, or protein content in either nondiabetic or diabetic individuals (Vestergaard et al. 1993). PDH is a key insulin-regulated enzyme whose activity in muscle is acutely stimulated by insulin (Mandarino et al. 1986). PDH is part of a very large complex of proteins known as the pyruvate dehydrogenase complex (PDC) (Sugden and Holness 2006). Three subunits (E1, E2, E3) catalyze the sequential decarboxylation, acetyl-CoA formation, and reduction of NAD^+ to NADH, respectively. In type 2 diabetic patients, insulin-stimulated PDH activity is decreased in human adipocytes and in skeletal muscle (Mandarino et al. 1986; Kelley et al. 1992) and plays an important role in the muscle insulin resistance.

Obesity and T2DM are insulin-resistant states associated with accelerated FFA turnover and FFA oxidation (DeFronzo 1998, 1997, 2009; Groop et al. 1989, 1991, 1992) which would be expected, according to the Randle cycle (Randle et al. 1963), to inhibit PDH activity and consequently glucose oxidation. The end product of fatty

acid oxidation, AcCoA, is a potent inhibitor of PDH. Further, fatty acid oxidation consumes NAD^+ which is required for the Krebs cycle to turn normally. NAD^+ also is a cofactor in the glycolytic pathway. Thus, the conversion of NAD^+ to NADH during fatty acid oxidation leads to defects in both glycolysis and glucose oxidation. Fatty acyl CoAs, in addition to inhibiting the insulin signaling pathway (see previous discussion), inhibit glycogen synthase and glucose transport and phosphorylation in muscle (Wititsuwannakul and Kim 1977; Johnson et al. 1992; Dresner et al. 1999; Pendergrass et al. 1998b; Kolaczynski et al. 1996). In the liver intracellular fatty acid metabolites stimulate gluconeogenesis and cause hepatic insulin resistance (Bevilacqua et al. 1987; Ferrannini et al. 1983; Bajaj et al. 2002; Roden et al. 1996; Williamson et al. 1966; Chen et al. 1999; Massillon et al. 1997; Kim et al. 2001; Gastaldelli et al. 2006). Reduction in the plasma FFA concentration with acipimox (Bajaj et al. 2005) and pioglitazone (Bajaj et al. 2010) reduce the intramyocellular concentrations of fatty acyl CoAs and diacylglycerol, leading to a marked improvement in insulin sensitivity in T2DM and obese nondiabetic individuals. Since the rates of basal and insulin-stimulated glucose oxidation are not reduced in the normal glucose-tolerant offspring of two diabetic parents or in the first-degree relatives of type 2 diabetic subjects, while they are decreased in overtly diabetic subjects, the FFA-induced defect in glucose oxidation and insulin resistance must be an acquired defect.

Mitochondrial Function

Mitochondrial dysfunction has been described in the muscle, as was as in liver, in experimental animals and humans with type 2 diabetes and obesity (Abdul-Ghani et al. 2008; Patti and Corvera 2010; Ritov et al. 2005; Petersen et al. 2004; Mogensen et al. 2007; Patti et al. 2003; Abdul-Ghani and DeFronzo 2008; Befroy et al. 2007). Reduced mitochondrial density (Morino et al. 2005; Ritov et al. 2005), impaired mitochondrial function secondary to reduced expression of key molecules in the oxidative phosphorylation chain (Petersen et al. 2004; Mogensen et al. 2007), and decreased expression of PGC-1 (the master controller of mitochondrial biogenesis) (Patti et al. 2003) all have been implicated in the disturbed mitochondrial function in T2DM patients. In muscle, impaired mitochondrial function has been proposed to activate redox-sensitive serine kinases which phosphorylate IRS proteins, causing insulin resistance (Rains and Jain 2011). Although it is unclear whether insulin resistance causes mitochondrial dysfunction or vice versa, once established it will aggravate the insulin resistant state.

Summary

In summary, postreceptor defects in insulin action primarily are responsible for the insulin resistance in T2DM. Reduced insulin binding is not a characteristic feature of T2DM patients and, when present, is modest and secondary to down regulation of

the insulin receptor by chronic hyperinsulinemia. In overtly diabetic patients, multiple postbinding abnormalities have been documented: impaired insulin signal transduction, decreased glucose transport and phosphorylation, diminished glycogen synthase activity, reduced PDH activity and glucose oxidation, and mitochondrial dysfunction. Elevated plasma FFA/FFA oxidation and ectopic lipid deposition play a major contributory role in the development of muscle insulin resistance. Importantly, the insulin resistance is present long before the onset of overt diabetes and can be demonstrated in the normal-glucose-tolerant, insulin-resistant offspring of two diabetic parents, in the first degree NGT relatives of individuals with diabetes, and in the "prediabetic" state, i.e., IGT.

Inflammation

Type 2 diabetes is now recognized to be associated with a generalized state of inflammation (Lumeng and Saltiel 2011). Circulating levels of proinflammatory cytokines, e.g., tumor necrosis factor-alpha, interleukin-6, and other inflammatory cytokines, are increased, macrophage infiltration can be found in adipocytes and to a lesser extent in muscle, and there is a shift in anti-inflammatory (M2) to pro-inflammatory (M1) macrophages (Romeo et al. 2012; Lumeng and Saltiel 2011; Feuerer et al. 2009; Bertola et al. 2012; Cai et al. 2005). These inflammatory cytokines can cause insulin resistance by activating down-stream kinases, including IkB-kinase-β, Jun amino-terminal kinase 1 (JNK1), and p38 MAP kinase which phosphorylate serine residues in IRS proteins, thereby rendering them resistant to tyrosine phosphorylation by insulin (Morino et al. 2005; Sriwijitkamol et al. 2006; Abdul-Ghani et al. 2008; Arkan et al. 2005; de Alvaro et al. 2004; Shi et al. 2006). These pro-inflammatory cytokines also stimulate the production of suppressors of cytokine signaling (SOCs) which inhibit the action of IRS proteins (Lebrun and Van Obberghen 2008; Howard and Flier 2006). Macrophage infiltration and inflammation in adipose tissue stimulates lipolysis and inhibits adiponectin, an insulin sensitizing, anti-inflammatory glycoprotein. Of note, treatment of T2DM patients with high dose salicylates, which inhibit the IkB/NFkB pathway, improves glycemic control and reduces the HbA1c by ~0.4% (Goldfine et al. 2010).

Increased plasma FFA levels and intracellular levels of toxic lipid metabolites can activate toll-like receptors (TLR). TLR4 is an integral component of the innate immune system, stimulates the IkB/NFkB system, and causes insulin resistance (Abdul-Ghani et al. 2008; Shi et al. 2006).

ER Stress and Unfolded Protein Response

The endoplasmic reticulum (ER) provides the skeletal backbone for the synthesis and folding of secreted proteins. When the synthesis of proteins exceeds the capacity of the ER to remove the proteins, ER stress results and initiates the unfolded protein response (UPR). To alleviate the stress, three signaling pathways are activated:

IRE1α, PERK, and ATF6α (Ron and Walter 2007). In T2DM, this feedback loop is disrupted by phosphorylation of PERK and IRE1α, leading to the activation of JNK and the development of insulin resistance (Eizirik et al. 2008; Herschkovitz et al. 2007; Ron and Walter 2007; Boden et al. 2008). Elevated plasma fatty acids, which are commonly observed in T2DM and obesity, can elicit ER stress and activate the UPR (Herschkovitz et al. 2007). ER stress also can lead to activation of the mTOR (mammalian target of rapamycin) pathway, leading to inhibition of insulin signaling by blocking insulin-stimulated tyrosine phosphorylation of IRS1 and IRS2 (Shah et al. 2004) and augmenting the degradation of IRS1 (Ozcan et al. 2008).

The Adipocyte, FFA Metabolism, and Lipotoxicity

Deranged adipocyte metabolism and altered fat topography play a central role in the pathogenesis of T2DM (DeFronzo 1998, 1997, 2009, 2010, 2004; Bays et al. 2004, 2008; Groop et al. 1989; Kashyap et al. 2003; Reaven 1988; Bonadonna and DeFronzo 1991): (i) fat cells are resistant to the antilipolytic effect of insulin, leading to day-long elevation in the plasma FFA concentration (DeFronzo 1998, 1997, 2009, 2010; Bays et al. 2004, 2008; Groop et al. 1989; Kashiwagi et al. 1983; Lonnroth et al. 1983; Olefsky and Reaven 1977); (ii) elevated plasma FFA levels stimulate gluconeogenesis (Bevilacqua et al. 1987; Ferrannini et al. 1983; Bajaj et al. 2002; Roden et al. 1996; Williamson et al. 1966; Chen et al. 1999; Massillon et al. 1997; Kim et al. 2001; Gastaldelli et al. 2006), induce hepatic insulin resistance (Bevilacqua et al. 1987; Ferrannini et al. 1983; Bajaj et al. 2002; Roden et al. 1996; Williamson et al. 1966; Chen et al. 1999; Massillon et al. 1997), cause muscle insulin resistance (Sun et al. 1992; Wititsuwannakul and Kim 1977; Johnson et al. 1992; Dresner et al. 1999; Pendergrass et al. 1998b; Ferrannini et al. 1983; Bajaj et al. 2002; Roden et al. 1996; Kim et al. 2001; Thiebaud et al. 1983), and impair insulin secretion (Kashyap et al. 2003; Carpentier et al. 2000); (iii) dysfunctional fat cells produce excessive amounts of insulin resistance–inducing, inflammatory, and atherosclerotic-provoking adipocytokines and fail to secrete normal amounts of insulin-sensitizing adipocytokines such as adiponectin (Bays et al. 2004, 2008); (iv) enlarged fat cells are insulin resistant and have diminished capacity to store fat (Salans et al. 1974; Bray et al. 1977). When the capacity of adipocyte to store fat is exceeded, lipid "overflows" into muscle, liver, and β cells, causing muscle/hepatic insulin resistance and impaired insulin secretion (reviewed in references (Bays et al. 2004) and (Bays et al. 2008)) (Fig. 14). Excess fat deposition in the liver can initiate an inflammatory response resulting in NAFLD/NASH (Yki-Jarvinen 2015; Gaggini et al. 2013), while accumulation of fat in arterial smooth muscle cells promotes atherogenesis (reviewed in reference (DeFronzo 2010)) (Fig. 14). Collectively, these disturbances in adipocyte biology and lipid metabolism are referred to as lipotoxicity (Bays et al. 2004, 2008; DeFronzo 2010) (Table 2). Pioglitazone reduces hepatic fat content and increases hepatic glucose uptake in T2DM patients (Bajaj et al. 2003) and reduces hepatic fat content, inflammation and fibrosis in patients with NASH (Belfort et al. 2006). Another form of lipotoxicity relates to the distribution of fat

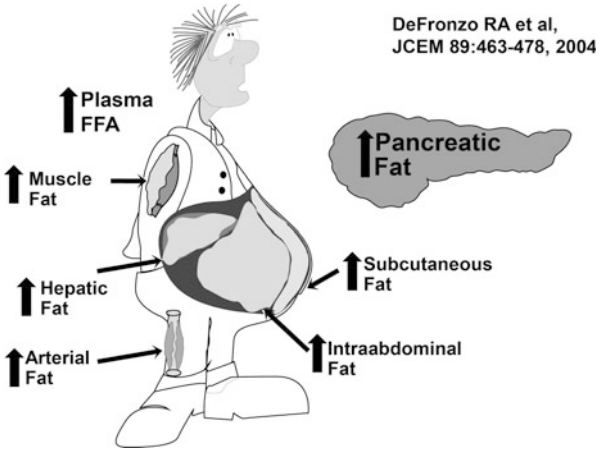


Fig. 14 Altered fat topography (visceral fat excess) is strongly associated with insulin resistance, while ectopic fat deposition in muscle and liver causes insulin resistance in these tissues. Ectopic fat deposition in the beta cell and arteries cause beta cell dysfunction and promotes atherogenesis

Table 2 Lipotoxicity plays a major role in the development of type 2 diabetes and accelerated cardiovascular disease. See text for a detailed discussion

(1) Elevated plasma FFA levels
(2) Increased intracellular levels of toxic lipid metabolites
Fatty acyl CoAs
Diacylglycerol
Ceramides
(3) Altered fat topography
Increased visceral fat
(4) Ectopic fat
Muscle
Liver
Pancreas
Arteries

within the body. Thus, visceral adiposity is strongly associated with both insulin resistance (DeFronzo 2009, 2010; Bays et al. 2004; Gastaldelli et al. 2000, 2007b; Reaven 1988) and accelerated atherosclerosis (Lapidus et al. 1984; Despres et al. 1990). The amount of visceral fat correlates strongly with the amount of liver fat and is closely associated with NAFLD (Gastaldelli et al. 2007b). It is controversial as to whether visceral fat is casually related to hepatic fat content or only correlatively related (Frayn 2000; Seidell and Bouchard 1997). Omental and mesenteric adipocytes are lipolytically more active than and secrete more inflammatory cytokines than subcutaneous adipocytes (Frayn 2000; Seidell and Bouchard 1997; Tchernof and Despres 2013), and this "portal" hypothesis could be the link between visceral adiposity and hepatic steatosis. However, surgical removal of omental fat in humans does not improve insulin sensitivity (Fabbrini et al. 2010). It also has been postulated that the fatty liver produces one or more factors, e.g., fetuin-A, that cause peripheral insulin resistance (Pal et al. 2012). The liver of individuals with NAFLD/NASH also

overproduces a number of factors that are associated with atherosclerotic cardiovascular disease including VLDL triglycerides, C-reactive protein, fibrinogen, coagulation factors (VII-IX, XI, XII), plasminogen activator inhibtor-1, while production of insulin-like growth factor binding protein is reduced.

In type 2 diabetic subjects, both lean and obese, peripheral adipocytes are characterized by marked insulin resistance to the antilipolytic effect of insulin, resulting in elevated fasting plasma FFA levels and impaired suppression of plasma FFA during a meal or insulin clamp (Groop et al. 1989, 1991). Multiple studies have demonstrated that a physiologic elevation in the plasma FFA concentration stimulates HGP and impairs insulin-stimulated glucose uptake in liver and muscle (Randle et al. 1963; Wititsuwannakul and Kim 1977; Johnson et al. 1992; Dresner et al. 1999; Pendergrass et al. 1998b; Kolaczynski et al. 1996; Bevilacqua et al. 1987; Ferrannini et al. 1983; Bajaj et al. 2002; Roden et al. 1996; Williamson et al. 1966; Chen et al. 1999; Massillon et al. 1997; Kim et al. 2001; Gastaldelli et al. 2006; Boden and Shulman 2002; Griffin et al. 1999; Itani et al. 2002; Richardson et al. 2005; Mandarino et al. 1996; Kelley and Mandarino 2000), while chronically elevated plasma FFA levels inhibit insulin secretion (Kashyap et al. 2003; Carpentier et al. 2000), especially in genetically prone individuals. Elevated FFA in muscle impairs glucose oxidation (Thiebaud et al. 1983; Mandarino et al. 1996; Kelley and Mandarino 2000), inhibits glycogen synthase (Randle et al. 1963; Wititsuwannakul and Kim 1977; Johnson et al. 1992), decreases both glucose transport and glucose phosphorylation (Dresner et al. 1999; Pendergrass et al. 1998b), and markedly impairs insulin signaling (Belfort et al. 2005). At the molecular level, increased plasma FFA levels and intramyocellular fatty acylCoA and diacylglycerol levels cause a dose-related inhibition of muscle insulin receptor tyrosine phosphorylation, IRS-1 tyrosine phosphorylation, PI-3 kinase activity, and Akt serine phosphorylation (Belfort et al. 2005) (Fig. 15). Conversely, reduction in the plasma FFA concentration with acipimox or pioglitazone in T2DM individuals enhances insulin sensitivity by ~30% in association with an increase in insulin signaling, glycogen synthesis, and glucose oxidation (Bajaj et al. 2005, 2010; Liang et al. 2013).

Fatty acids can enter the myocyte and hepatocyte via the fatty acid transporter or directly by passing through the plasma membrane lipid bilayer. Once in the cell, fatty acids can be converted to triglycerides, which are inert, or to toxic lipid metabolites such as fatty acyl CoAs, diacylglycerol, and ceramides. Both magnetic resonance spectroscopy and muscle biopsy have demonstrated that the intramyocellular triglyceride content is increased in type 2 diabetic subjects and levels of fatty acyl CoAs, DAG, and ceramides (Bajaj et al. 2005, 2010; Adams 2nd et al. 2004; Krssak et al. 1999; Petersen et al. 2005; Lara-Castro and Garvey 2008; Szendroedi et al. 2014; Samuel et al. 2004, 2007; Ellis et al. 2000; Coletta et al. 2009), all of which inhibit insulin signaling (Belfort et al. 2005, 2006; Yu et al. 2002; Ozcan et al. 2008; Bonadonna and DeFronzo 1991; DeFronzo 2004; Thiebaud et al. 1983; Carpentier et al. 2000; Salans et al. 1974; Bray et al. 1977; Yki-Jarvinen 2015; Gaggini et al. 2013; Bajaj et al. 2003; Gastaldelli et al. 2007b; Lapidus et al. 1984; Liang et al. 2013; Ellis et al. 2000; Montell et al. 2001), are increased in muscle in diabetic subjects.

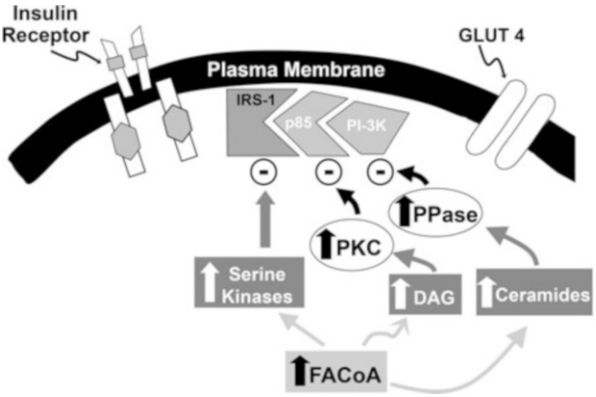


Fig. 15 Elevated intracellular levels of fatty acyl CoAs (FACoA) and diacylglycerol (DAG) inhibit insulin signaling by activating serine kinases and PKC isoforms, causing serine phosphorylation of the insulin receptor and insulin receptor substrate-1. Increased intracellular ceramide levels inhibit insulin signaling by activating tyrosine protein phosphatases, which cause dephosphorylation of tyrosine residues on the insulin receptor and IRS-1

In T2DM individuals and in the normal-glucose-tolerant, insulin-resistant offspring of two diabetic parents, the expression of PGC-1 and multiple other genes involved in oxidative phosphorylation is markedly reduced in muscle and strongly correlated with the defects in glucose oxidation and whole body (muscle) insulin sensitivity (DeFronzo 2010; Patti and Corvera 2010; Patti et al. 2003; Coletta et al. 2009). Treatment of diabetic patients with thiazolidinediones activates peroxisome proliferation-activated γ coactivator (PGC-1) and multiple mitochondrial genes leading to a reduction in intramyocellular lipid, fatty acyl CoA, and DAG concentrations and enhanced insulin sensitivity in muscle and liver (Coletta et al. 2009). The decrement in muscle fatty acyl CoA content is closely related to the improvement in insulin-stimulated muscle glucose disposal (Bajaj et al. 2010; Coletta et al. 2009). Acipimox, a potent inhibitor of lipolysis, also reduces intramyocellular fatty acyl CoA content and improves insulin-mediated glucose disposal (Bajaj et al. 2005, 2004; Liang et al. 2013). Intramyocellular levels of diacylglycerol (Yu et al. 2002; Szendroedi et al. 2014; Boden and Shulman 2002; Griffin et al. 1999; Itani et al. 2002; Montell et al. 2001) and ceramides (Adams 2nd et al. 2004; Larsen and Tennagels 2014; Turpin et al. 2014; Cantley et al. 2013; Folli et al. 1993) also have been shown to be elevated in type 2 diabetic and obese nondiabetic subjects and to contribute to the insulin resistance and impaired insulin signaling in muscle.

In obese nondiabetic and obese T2DM subjects, the increase in vascular supply fails to match the increase in adipocyte mass. This results in hypoxia, necrosis of fat cells, infiltration with M1 inflammatory macrophages surrounding dead adipocytes, increased expression of proinflammatory cytokines and chemokines (Trayhurn 2013; Weisberg et al. 2003), fibrosis, and impaired release of adiponectin (Turer and Scherer 2012). Inflamed adipose tissue renders the fat cell resistant to the antilipolytic effect of insulin and inhibits insulin-stimulated glucose uptake (Kotronen et al. 2008).

Alpha Cell and Glucagon

In T2DM individuals the basal plasma glucagon concentration is increased and fails to suppress normally after a meal (Cherrington 1999; Baron et al. 1987; Matsuda et al. 2002; Unger et al. 1970; Reaven et al. 1987; Boden et al. 1983). The important contribution of the elevated fasting plasma glucagon concentration to the accelerated basal rate of hepatic glucose production (HGP) in type 2 diabetic individuals was provided by Baron et al. (Baron et al. 1987) who demonstrated that the elevated basal rate of HGP correlated closely with the increase in fasting plasma glucagon concentration. Reduction in the plasma glucagon concentration by 44% with somatostatin resulted in a 58% decrease in basal HGP (Fig. 16). These results conclusively demonstrate the pivotal role of hyperglucagonemia in the pathogenesis of fasting hyperglycemia in T2DM. There also is evidence that the liver is hypersensitive to the stimulatory effect of glucagon on hepatic gluconeogenesis (Matsuda et al. 2002). The increase in plasma glucagon is related to four factors: (i) reduced local paracrine effect of insulin due to reduced beta cell mass (Henquin and Rahier 2011), (ii) resistance to GLP-1 (Sandoval and D'Alessio 2015), (iii) glucotoxicity (Jamison et al. 2011; Abdul-Ghani and DeFronzo 2007), and (iv) increased proglucagon conversion to glucagon in gastrointestinal cells (Sandoval and D'Alessio 2015). Alpha cell mass is not increased in T2DM individuals (Henquin and Rahier 2011).

Amino acids are potent glucagon secretagogues. Nonetheless, plasma glucagon levels decline following a meal in normal glucose-tolerant subjects, due to the release of insulin and GLP-1, and the decrease in portal vein glucagon concentration contributes to the suppression of HGP (Cherrington 1999). In contrast, following ingestion of a mixed meal in T2DM patients there is a paradoxical rise in plasma glucagon concentration which

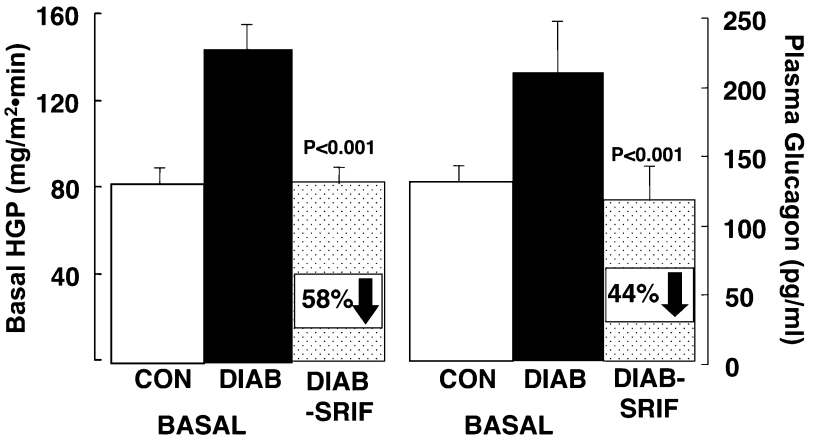


Fig. 16 Effect of somatostatin (SRIF) infusion with basal insulin replacement on basal (fasting) hepatic glucose production (HGP) (left) and plasma glucagon concentration (right) in normal glucose-tolerant control (CON) and type 2 diabetic (DIAB) subjects. Normalization of the plasma glucagon concentration reduced HGP by 58% to values observed in CON subjects. (Source: Baron AD, et al. *Diabetes* 1987;**36**:274–283)

antagonizes the decline in HGP, resulting in postprandial hyperglycemia (Wahren et al. 1976; Mitrakou et al. 1992). Further, the diabetic liver is hypersensitive to glucagon (Matsuda et al. 2002). Thus, deranged glucagon secretion by the pancreatic α cell contributes to both fasting and postprandial hyperglycemia in T2DM patients.

The Kidney: Increased Glucose Reabsorption

With a glomerular filtration rate of ~180 L/day and a mean day-long plasma glucose concentration of ~100 mg/dl, the kidney filters ~180 grams of glucose every day (Abdul-Ghani et al. 2015, 2011; DeFronzo et al. 2017). About 90% of the filtered glucose is reabsorbed by the SGLT2 transporter in the S1 segment of the proximal convoluted, and the remaining 10% of the filtered glucose is reabsorbed by the SGLT1 transporter in the S2/S3 segment of the proximal tubule (Abdul-Ghani et al. 2015, 2011; DeFronzo et al. 2017; Wright et al. 2011). The result is that no glucose appears in the urine. Although the SGLT1 transporter has been referred to as a low capacity transporter, under conditions of SGLT2 blockade the SGLT1 transporter can reabsorb up to 30–40% of the filter glucose load (Abdul-Ghani et al. 2013).

In T1DM and T2DM animal models, the maximal renal tubular reabsorptive capacity for glucose (Tm_G) is increased (Noonan et al. 2001; Dominguez et al. 1994; Kamran et al. 1997). In humans with T1DM (Mogensen 1971) and T2DM (Farber et al. 1951), the Tm for glucose is increased. In one study, SGLT2 mRNA and protein levels were found to be increased in cultured human proximal renal tubular cells from T2DM patients (Rahmoune et al. 2005). However, a more recent study has found down regulation of SGLT2 and a marked up regulation of SGLT1 in kidney biopsies from type 2 diabetic individuals (Norton et al. 2017). However, the increase in TmG is not the major pathophysiologic abnormality responsible for the increase in renal glucose reabsorption. More importantly, the renal threshold for glucose spillage in the urine is markedly increased and this abnormality occurs early in the natural history of T2DM (DeFronzo et al. 2013b). Thus, in T2DM patients with a HbA1c of 6.5%, the renal threshold has increased from ~180 mg/d to ~205 mg/dl and continues to rise progressively with worsening glycemic control (DeFronzo et al. 2013b). Thus, in individuals with a HbA1c of 8%, the renal threshold is ~220–230 mg/dl. Thus, during the evolution of man, an adaptive response by the kidney to conserve glucose, which is essential to meet the energy demands of the body (especially the brain and other neural tissues which have an obligate need for glucose), becomes maladaptive in the diabetic patient. Instead of excreting glucose in the urine to correct the hyperglycemia, the kidney augments it reabsorption of glucose and this provides the rationale for development of the SGLT2 inhibitor class of drugs for the treatment of T2DM.

The Brain

The brain, along with the beta cell, alpha cell, muscle, liver, kidney, adipocyte, and gastrointestinal tract, forms the eighth component of the Ominous Octet (Fig. 17).

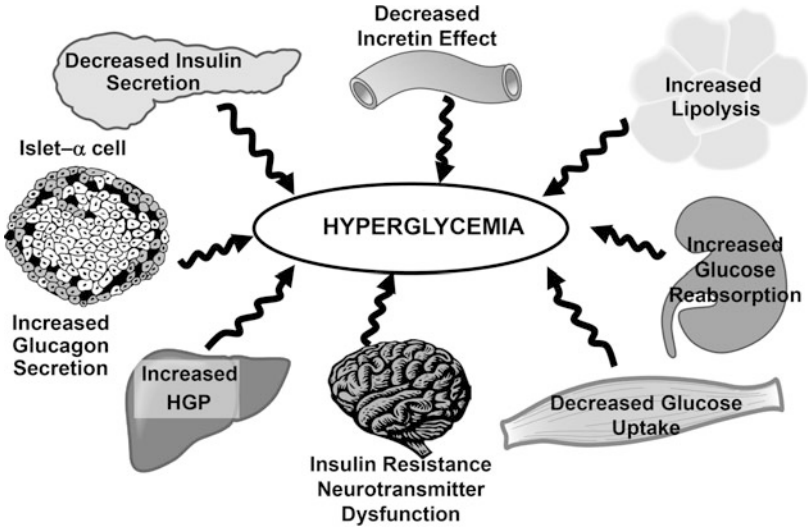


Fig. 17 The Ominous Octet describing the major pathophysiologic defects which involve multiple organs in type 2 diabetes. See text for a more detailed explanation. (Source: DeFronzo RA. *Diabetes* 2009;**58**:773–795)

The current epidemic of diabetes, which has enveloped westernized countries over the last 50 years, is being driven by the epidemic of obesity (Hedley et al. 2004). Porte and colleagues (Porte 2006; Schwartz et al. 2000; Plum et al. 2006) were among the first to demonstrate that, in rodents, insulin was a powerful appetite suppressant. Injection of insulin into the third ventricle of baboons inhibits appetite (Woods et al. 1979), and this appetite suppressant effect of insulin has been documented across a variety of different species (reviewed in reference (Kullmann et al. 2016)). Obese individuals, both diabetic and nondiabetic, are resistant to insulin and manifest compensatory hyperinsulinemia. Nonetheless, despite the presence of hyperinsulinemia food intake is increased in obese subjects and obese individuals tend to progressively gain weight. Thus, the insulin resistance in peripheral tissues and liver extends to the brain.

Using functional magnetic resonance imaging (MRI), the cerebral response to an ingested glucose load has been studied (Matsuda et al. 1999; ten Kulve et al. 2015). After glucose ingestion, two hypothalamic areas consistently show inhibition in NGT individuals: the lower posterior hypothalamus, which contains the ventromedial nuclei, and the upper posterior hypothalamus, which contains the paraventricular nuclei. Both of these hypothalamic areas are key centers for appetite regulation. Following glucose ingestion, the magnitude of the inhibitory response is reduced in obese, insulin-resistant, and normal glucose-tolerant subjects, and there is a delay in the time taken to reach the maximum inhibitory response, even though the plasma insulin response was markedly increased in the obese group (Matsuda et al. 1999). Similar results have been reported by others in obese nondiabetic, as well as in obese and lean T2DM individuals (ten Kulve et al. 2015; van Bloemendaal et al. 2014).

Table 3 Ominous octet for obesity. Hypothalamic resistance to appetite suppressing hormones and altered neurotransmitter levels contribute increase energy intake

(1) GLP-1 resistance
(2) PYY resistance
(3) Amylin resistance
(4) Insulin resistance
(5) Leptin resistance
(6) Elevated CNS serotonin levels
(7) Decreased CNS dopamine levels
(8) Altered CNS catecholamine levels

Whether the impaired functional MRI response in obese subjects contributes to or is a consequence of the insulin resistance and weight gain remains to be determined. Nonetheless, these results suggest that the brain, like other organs (liver, muscle, and fat) in the body, is resistant to insulin. In rodents, there is considerable evidence that the brain directly contributes to insulin resistance via enhanced neural output to peripheral tissue, including muscle and adipocytes, as well as the liver (Obici et al. 2002, 2001; Jastreboff et al. 2013). However, it is unclear whether similar disturbances are present in higher vertebrates, including man (Edgerton and Cherrington 2015).

In addition to insulin resistance, there are at least 7 other pathophysiologic disturbances that contribute to the dysregulation of appetite in the brain and one can create an Ominous Octet for obesity which includes resistance to the appetite suppressant effect of GLP-1, PYY, amylin, and leptin, as well as reduced neuronal dopamine levels, increased neuronal serotonin levels, and altered neuronal catecholamine levels in the hypothalamus and other CNS centers involved with appetite regulation (Table 3). This constellation of physiologic disturbances explains why weight loss is refractory to lifestyle intervention with pharmacologic therapy (Dansinger et al. 2007; Knowler et al. 2002; Gregg et al. 2012). Because obesity is an insulin resistant state, this places stress on the beta cell to enhance its secretion of insulin and, thus, contributes to the progressive decline in beta cell function that characterizes T2DM.

Gut Microbiota

The gut microbiota harbor trillions of microorganisms and comprise 1–2 kg of an individual's body weight. The neonatal intestinal tract is colonized by the bacteria from the mother and surrounding environment after birth and by age 3–4 the gut microbiota composition closely resembles that of the adult (Patterson et al. 2016; Bauer and Duca 2016; Blandino et al. 2016). Evidence is starting to accumulate that an individuals' microbial signature could be an important risk factor for the development of obesity and diabetes. Both obesity and diabetes are characterized by a state of low grade inflammation. In mice, genetic models of obesity and diabetes are associated with "metabolic endotaxemia" and increased levels of lipopolysaccharide (LPS) (Turnbaugh et al. 2006;

Cani et al. 2009). Further, the gut microbiota can produce a variety of metabolites (i.e., short-chain fatty acids, conjugated fatty acids and neuroactive metabolites such as GABA and serotonin) which can be absorbed and influence the metabolism of the host. Of the short chain fatty acids, butyrate appears to be particularly important by its ability to enhance insulin secretion (Tilg and Moschen 2014). In female twins discordant for obesity, transplantation of human gut microbiota from each twin was able to reproduce the obese or lean phenotype in germ free mice (Ridaura et al. 2013). In patients with T2DM butyrate-producing bacteria have been reported to be reduced compared to nondiabetic healthy control subjects (Qin et al. 2012). Following treatment of T2DM patients with metformin, a unique signature of gut microbiome shifts characterized by a depletion of butyrate-producing taxa with a reduction in LPS-triggered local inflammation has been described (Forslund et al. 2015). Although the role of the gut microbiota in the development of T2DM is in its infancy, it seems clear that certain bacterial strains and the pharmabiotics they produce can have positive or negative effects on systemic glucose metabolism. The importance of these effects will require additional study to define their role in the pathogenesis of T2DM.

Implications for Therapy

Identification of the pathophysiologic abnormalities responsible for T2DM has important therapeutic implications (DeFronzo 2009) (Fig. 16). First, effective control of glycemia in T2DM patients will require the use of multiple drugs used in combination to correct the multiple pathophysiologic disturbances. Second, the selection of antidiabetic medications should be based upon their ability to correct known pathogenic abnormalities and NOT simply on their ability to reduce HbA1c levels. Third, therapy must be started early in the natural history of T2DM to prevent the progressive β-cell failure and loss of beta cell mass. Fourth, since T2DM is a disease that affects both the microvasculature (retinopathy, nephropathy, neuropathy) and macrovascular (MI, stroke, PVD), preference should be given to antidiabetic agents that reduce both microvascular and macrovascular complications. The treatment of T2DM is discussed in detail in Pathogenesis of Type 2 Diabetes Mellitus.

References

Abdul-Ghani M, DeFronzo RA. Fasting hyperglycemia impairs glucose- but not insulin-mediated suppression of glucagon secretion. J Clin Endocrinol Metab. 2007;92(5):1778–84.

Abdul-Ghani MA, DeFronzo RA. Mitochondrial dysfunction, insulin resistance, and type 2 diabetes mellitus. Curr Diab Rep. 2008;8(3):173–8.

Abdul-Ghani MA, DeFronzo RA. Plasma glucose concentration and prediction of future risk of type 2 diabetes. Diabetes Care. 2009;32:S194–S8.

Abdul-Ghani M, Jenkinson C, Richardson D, et al. Insulin secretion and insulin action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study (VAGES). Diabetes. 2006a;55:1430–5.

Abdul-Ghani M, Tripathy D, DeFronzo RA. Contribution of beta cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care. 2006b;29:1130–9.

Abdul-Ghani MA, Williams K, DeFronzo R, Stern M. Risk of progression to type 2 diabetes based on relationship between postload plasma glucose and fasting plasma glucose. Diabetes Care. 2006c;29(7):1613–8.

Abdul-Ghani MA, Matsuda M, Sabbah M, et al. The relative contribution of insulin resistance and beta cell failure to the transition from normal to impaired glucose tolerance varies in different ethnic groups. Diabetol Metab Syndr. 2007a;1:105–12.

Abdul-Ghani MA, Williams K, DeFronzo RA, Stern M. What is the best predictor of future type 2 diabetes? Diabetes Care. 2007b;30(6):1544–8.

Abdul-Ghani MA, Muller FL, Liu Y, et al. Deleterious action of FA metabolites on ATP synthesis: possible link between lipotoxicity, mitochondrial dysfunction, and insulin resistance. Am J Physiol Endocrinol Metab. 2008;295(3):E678–85.

Abdul-Ghani MA, Lyssenko V, Tuomi T, DeFronzo RA, Groop L. Fasting versus postload plasma glucose concentration and the risk for future type 2 diabetes: results from the Botnia study. Diabetes Care. 2009a;32(2):281–6.

Abdul-Ghani MA, Jani R, Chavez A, Molina-Carrion M, Tripathy D, DeFronzo RA. Mitochondrial reactive oxygen species generation in obese non-diabetic and type 2 diabetic participants. Diabetologia. 2009b;52(4):574–82.

Abdul-Ghani MA, Norton L, DeFronzo RA. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. Endocr Rev. 2011;32:515–31.

Abdul-Ghani MA, DeFronzo RA, Norton L. Novel hypothesis to explain why SGLT2 inhibitors inhibit only 30–50% of filtered glucose load in humans. Diabetes. 2013;62(10):3324–8.

Abdul-Ghani MA, Norton L, DeFronzo RA. Renal sodium-glucose cotransporter inhibition in the management of type 2 diabetes mellitus. Am J Physiol Renal Physiol. 2015;309(11):F889–900.

Adams JM 2nd, Pratipanawatr T, Berria R, et al. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes. 2004;53(1):25–31.

Ahlqvist E, Ahluwalia TS, Groop L. Genetics of type 2 diabetes. Clin Chem. 2011;57:241–54.

Ahrén B, Taborsky GJ. Beta-cell function and insulin secretion. In: Porte D, Sherin RS, Baron A, editors. Ellenberg and Rifkin's diabetes mellitus. New York: McGraw Hill; 2003. p. 43–65.

Alatrach M, Agyin C, Adams J, DeFronzo RA, Abdul-Ghani A. Decreased basal heaptic glucose uptake in subjects with impaired fasting glucose. Diabetologia. 2017;60(7):1325–32.

Alcolado JC, Laji K, Gill-Randall R. Maternal transmission of diabetes. Diabet Med. 2002;19 (2):89–98.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2008;31(Suppl 1):S65–0.

Andreelli F, Laville M, Ducluzeau P-H, et al. Defective regulation of phosphatidylinositol-3-kinase gene expression in skeletal muscle and adipose tissue of non-insulin-dependent diabetes mellitus patients. Diabetologia. 1999;42:358–64.

Andreozzi F, D'Alessandris C, Federici M, et al. Activation of the hexosamine pathway leads to phosphorylation of insulin receptor substrate-1 on Ser307 and Ser612 and impairs the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin insulin biosynthetic pathway in RIN pancreatic beta-cells. Endocrinology. 2004;145:2845–57.

Andrews WJ, Vasquez B, Nagulesparan M, et al. Insulin therapy in obese, non-insulin-dependent diabetes induces improvements in insulin action and secretion that are maintained for two weeks after insulin withdrawal. Diabetes. 1984;33:634–42.

Arkan MC, Hevener AL, Greten FR, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med. 2005;11(2):191–8.

Arner P, Pollare T, Lithell H. Different etiologies of type 2 (non-insulin-dependent) diabetes mellitus in obese and non-obese subjects. Diabetologia. 1991;34:483–7.

Bajaj M, DeFronzo RA. Metabolic and molecular basis of insulin resistance. J Nucl Cardiol. 2003;10:311–23.

Bajaj M, Pratipanawatr T, Berria R, et al. Free fatty acids reduce splanchnic and peripheral glucose uptake in patients with type 2 diabetes. Diabetes. 2002;51(10):3043–8.
Bajaj M, Suraamornkul S, Pratipanawatr T, et al. Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. Diabetes. 2003;52 (6):1364–70.
Bajaj M, Suraamornkul S, Kashyap S, Cusi K, Mandarino L, DeFronzo RA. Sustained reduction in plasma free fatty acid concentration improves insulin action without altering plasma adipocytokine levels in subjects with strong family history of type 2 diabetes. J Clin Endocrinol Metab. 2004;89(9):4649–55.
Bajaj M, Suraamornkul S, Romanelli A, et al. Effect of a sustained reduction in plasma free fatty acid concentration on intramuscular long-chain fatty acyl-CoAs and insulin action in type 2 diabetic patients. Diabetes. 2005;54(11):3148–53.
Bajaj M, Baig R, Suraamornkul S, et al. Effects of pioglitazone on intramyocellular fat metabolism in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010;95(4):1916–23.
Banjeri MA, Lebovitz HE. Insulin action in black Americans with NIDDM. Diabetes Care. 1992;15:1295–302.
Baron AD, Schaeffer L, Shragg P, Kolterman OG. Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes. 1987;36:274–83.
Bauer PV, Duca FA. Targeting the gastrointestinal tract to treat type 2 diabetes. J Endocrinol. 2016;230(3):R95–R113.
Bays H, Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonsits provide a rational therapeutic approach. J Clin Endocrinol Metab. 2004;89:463–78.
Bays HE, Gonzalez-Campoy JM, Bray GA, et al. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert Rev Cardiovasc Ther. 2008;6:343–68.
Beck-Nielsen H, Nielsen OH, Pedersen O, et al. Insulin action and insulin secretion in identical twins with MODY: evidence for defects in both insulin action and insulin secretion. Diabetes. 1988;37:730–5.
Befroy DE, Petersen KF, Dufour S, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes. 2007;56(5):1376–81.
Belfort R, Mandarino L, Kashyap S, et al. Dose-response effect of elevated plasma free fatty acid on insulin signaling. Diabetes. 2005;54(6):1640–8.
Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355(22):2297–307.
Bell G, Kayano T, Buse JB, et al. Molecular biology of mammalian glucose transporters. Diabetes Care. 1990;13:198–200.
Bell GI, Zian K, Newman M, et al. Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. PNAS. 1991;88:1484–8.
Benninger RK, Piston DW. Cellular communication and heterogeneity in pancreatic islet insulin secretion dynamics. Trends Endocrinol Metab. 2014;25(8):399–406.
Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes. 1989;38:1512–27.
Bergman RN. Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein? Diabetologia. 2000;43:946–52.
Bergman RN, Finegood DT, Kahn SE. The evolution of beta-cell dysfunction and insulin resistance in type 2 diabetes. Eur J Clin Investig. 2002;32:35–45.
Bertola A, Ciucci T, Rousseau D, et al. Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. Diabetes. 2012;61(9):2238–47.
Bevilacqua S, Bonadonna R, Buzzigoli G, et al. Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. Metabolism. 1987;36(5):502–6.

Bezy O, Tran TT, Pihlajamaki J, et al. PKCdelta regulates hepatic insulin sensitivity and hepatosteatosis in mice and humans. J Clin Invest. 2011;121(6):2504–17.

Bjorbaek C, Echward SM, Hubricht P, et al. Genetic variants in promoters and coding regions of the muscle glycogen synthase and the insulin-responsive GLUT4 genes in NIDDM. Diabetes. 1994;43:976–83.

Bjorbaek C, Fik TA, Echward SM, et al. Cloning of human insulin-stimulated protein kinase (ISPK-1) gene and analysis of coding regions and mRNA levels of the ISPK-1 and the protein phosphatase-1 genes in muscle from NIDDM patients. Diabetes. 1995;44:90–7.

Blandino G, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. Diabetes Metab. 2016;42(5):303–15.

Blazquez E, Velazquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. Front Endocrinol. 2014;5:161.

Boden G. Endoplasmic reticulum stress: another link between obesity and insulin resistance/inflammation? Diabetes. 2009;58(3):518–9.

Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Investig. 2002;32(Suppl 3):14–23.

Boden G, Soriano M, Hoeldtke RD, Owen OE. Counterregulatory hormone release and glucose recovery after hypoglycemia in non-insulin-dependent diabetic patients. Diabetes. 1983;32:1055–9.

Boden G, Duan X, Homko C, et al. Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. Diabetes. 2008;57(9):2438–44.

Bogardus C, Lillioja S, Howard BV, et al. Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in non-diabetic and noninsulin-dependent subjects. J Clin Investig. 1984;74:1238–46.

Bonadonna RC, DeFronzo RA. Glucose metabolism in obesity and type 2 diabetes. Diabetes Metab. 1991;17:112–35.

Bonadonna RC, Del Prato S, Saccomani MP, et al. Transmembrane glucose transport in skeletal muscle of patients with non-insulin-dependent diabetes. J Clin Investig. 1993;92:486–94.

Bonadonna RC, Del Prato S, Bonora E, et al. Roles of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. Diabetes. 1996;45:915–25.

Bongaerts BW, Rathmann W, Kowall B, et al. Postchallenge hyperglycemia is positively associated with diabetic polyneuropathy: the KORA F4 study. Diabetes Care. 2012;35(9):1891–3.

Bonner-Weir S, Inada A, Yatoh S, et al. Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. Biochem Soc Trans. 2008;36(Pt 3):353–6.

Bonora E, Kiechl S, Willeit J, et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the Bruneck study. Diabetes Care. 2007;30:318–24.

Bosco D, Armanet M, Morel P, et al. Unique arrangement of alpha- and beta-cells in human islets of Langerhans. Diabetes. 2010;59(5):1202–10.

Bouzakri K, Roques M, Gual P, et al. Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. Diabetes. 2003;52:1319–25.

Bray GA, Glennon JA, Salans LB, et al. Spontaneous and experimental human obesity: effects of diet and adipose cell size on lipolysis and lipogenesis. Metabolism. 1977;26:739–47.

Bretherton-Watt D, Ghatei MA, Bloom SR, et al. Altered islet amyloid polypeptide (amylin) gene expression in rat models of diabetes. Diabetologia. 1989;32:881–3.

Brunzell JD, Robertson RP, Lerner RL, et al. Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. J Clin Endocrinol. 1976;46:222–9.

Bunck MC, Corner A, Eliasson B, et al. Effects of exenatide on measures of beta-cell function after 3 years in metformin-treated patients with type 2 diabetes. Diabetes Care. 2011;34(9):2041–7.

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;52(1):102–10.

Butterfield WJH, Whichelow MJ. Peripheral glucose metabolism in control subjects and diabetic patients during glucose, glucose-insulin, and insulin sensitivity tests. Diabetologia. 1965;1:43–53.

Byrne MM, Sturis J, Clement K, et al. Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations. J Clin Invest. 1994;93(3):1120–30.

Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med. 2005;11(2):183–90.

Campbell PJ, Mandarino LJ, Gerich JE. Quantification of the relative impairment in actions of insulin on hepatic glucose production and peripheral glucose uptake in non-insulin dependent diabetes mellitus. Metabolism. 1988;37:15–21.

Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091–103.

Cantley JL, Yoshimura T, Camporez JP, et al. CGI-58 knockdown sequesters diacylglycerols in lipid droplets/ER-preventing diacylglycerol-mediated hepatic insulin resistance. Proc Natl Acad Sci U S A. 2013;110(5):1869–74.

Caro JF, Ittoop O, Pories WJ, et al. Studies on the mechanism of insulin resistance in the liver from humans with non-insulin-dependent diabetes. Insulin action and binding in isolated hepatocytes, insulin receptor structure, and kinase activity. J Clin Investig. 1986;78:249–58.

Caro JF, Sinha MK, Raju SM, et al. Insulin receptor kinase in human skeletal muscle from obese subjects with and without non-insulin dependent diabetes. J Clin Investig. 1987;79:1330–7.

Carpentier A, Mittelman SD, Bergman RN, et al. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. Diabetes. 2000;49:399–408.

Cauchi S, Meyre D, Dina C, et al. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes. 2006;55:2903–8.

Cerasi E. Insulin deficiency and insulin resistance in the pathogenesis of NIDDM: is a divorce possible? Diabetologia. 1995;38:992–7.

Chang AM, Jakobsen G, Sturis J, et al. The GLP-1 derivative NN2211 restores beta-cell sensitivity to glucose in type 2 diabetic patients after a single dose. Diabetes. 2003;52:1786–91.

Chavez AO, Lopez-Alvarenga JC, Triplitt C, et al. Physiological and molecular determinants of insulin action in the baboon. Diabetes. 2008;57:899–908.

Chen YD, Jeng CY, Hollenbeck CB, et al. Relationship between plasma glucose and insulin concentration, glucose production, and glucose disposal in normal subjects and patients with non-insulin-dependent diabetes. J Clin Investig. 1988;82:21–5.

Chen X, Iqbal N, Boden G. The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. J Clin Invest. 1999;103(3):365–72.

Cherrington AD. Control of glucose uptake and release by the liver in vivo. Diabetes. 1999;48:1198–214.

Choi WH, O'Rahilly S, Rees A, et al. Molecular scanning of the insulin-responsive glucose transporter (GLUT 4) gene in patients with non-insulin dependent diabetes mellitus. Diabetes. 1991;40:1712–8.

Chou DK, Dull TJ, Russell DS, et al. Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. J Biol Chem. 1987;262:1842–7.

Clark A, Wells CA, Buley ID, et al. Islet amyloid, increased α-cells, reduced β-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res. 1988;9:151–9.

Cline GW, Petersen KF, Krssak M, et al. Impaired glucose transport as a cause of decreased insulin stimulated muscle glycogen synthesis in type 2 diabetes. N Engl J Med. 1999;341:240–6.

Clore JN, Stillman J, Sugerman H. Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. Diabetes. 2000;49:969–74.

Cohen P. The Croonian lecture 1999. Identification of a protein kinase cascade of major importance in insulin signal transduction. Philos Trans R Soc Lond Ser B Biol Sci. 1999;354:485–95.
Coletta DK, Sriwijitkamol A, Wajcberg E, et al. Pioglitazone stimulates AMPK signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo. Diabetologia. 2009;52:723–32.
Consoli A, Nurjhan N, Reilly JJ Jr, et al. Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. J Clin Investig. 1990;86:2038–45.
Copeland RJ, Bullen JW, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity. Am J Physiol Endocrinol Metab. 2008;295(1): E17–28.
Cox LA, Mahaney MC, Vandeberg JL, Rogers J. A second-generation genetic linkage map of the baboon (Papio hamadryas) genome. Genomics. 2006;88:274–81.
Cox LA, Comuzzie AG, Havill LM, Karere GM, Spradling KD, Mahaney MC, Nathanielsz PW, Nicolella DP, Shade RE, Voruganti S, VandeBerg JL. Baboons as a model to study genetics and epigenetics of human disease. ILAR J. 2013;54:106–21.
Cross D, Alessi D, Vandenheed J, et al. The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal muscle cell line L6 is blocked by wortmannin but not rapamycin. Biochem J. 1994;303:21–6.
Cusi K, Maezono K, Osman A, et al. Insulin resistance differentially affects the PI 3-kinase and MAP kinase-mediated signaling in human muscle. J Clin Investig. 2000;105:311–20.
Damsbo P, Vaag A, Hother-Nielsen O, et al. Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 1991;34:239–45.
Daniele G, Abdul-Ghani M, DeFronzo RA. What are the pharmacotherapy options for treating prediabetes? Expert Opin Pharmacother. 2014;15(14):2003–18.
Dansinger ML, Tatsioni A, Wong JB, Chung M, Balk EM. Meta-analysis: the effect of dietary counseling for weight loss. Ann Intern Med. 2007;147(1):41–50.
Davies MJ, Metcalfe J, Gray IP, et al. Insulin deficiency rather than hyperinsulinaemia in newly diagnosed type 2 diabetes mellitus. Diabet Med. 1993;10:305–12.
de Alvaro C, Teruel T, Hernandez R, Lorenzo M. Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappaB kinase in a p38 MAPK-dependent manner. J Biol Chem. 2004;279(17):17070–8.
De Jesus DF, Kulkarni RN. Epigenetic modifiers of islet function and mass. Trends Endocrinol Metab. 2014;25(12):628–36.
Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998;20(3):284–7.
DeFronzo RA. Glucose intolerance and aging: evidence for tissue insensitivity to insulin. Diabetes. 1979;28(12):1095–101.
DeFronzo RA. Pathogenesis of type 2 diabetes mellitus: metabolic and molecular implications for identifying diabetes genes. Diabetes. 1997;5:117–269.
DeFronzo RA. Lilly lecture. The triumvirate: beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1998;37:667–87.
DeFronzo RA. Dysfunctional fat cells, lipotoxicity, and type 2 diabetes. Int J Clin Pract. 2004;143 (Suppl):9–21.
DeFronzo RA. Is insulin resistance atherogenic? Possible mechanisms. Atheroscler Suppl. 2006;7:11–5.
DeFronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58:773–95.
DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard lecture 2009. Diabetologia. 2010;53:1270–87.
DeFronzo RA, Abdul-Ghani MA. Preservation of beta-cell function: the key to diabetes prevention. J Clin Endocrinol Metab. 2011;96(8):2354–66.

DeFronzo RA, Ferrannini E. Regulation of hepatic glucose metabolism in humans. Diabetes Metab Rev. 1987;3:415–60.
DeFronzo RA, Ferrannini E. Regulation of intermediatory metabolism during fasting and feeding. In: Jameson JL, DeGroot LJ, editors. Endocrinology. Philadelphia: Saunders Elsevier; 2010. p. 673–98.
DeFronzo RA, Ferrannini E, Hendler R, et al. Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. PNAS. 1978a;75:5173–7.
DeFronzo RA, Soman V, Sherwin RS, et al. Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. J Clin Investig. 1978b;62:204–13.
DeFronzo RA, Ferrannini E, Wahren J, Felig P. Lack of gastrointestinal mediator of insulin action in maturity onset diabetes. Lancet. 1978c;2:1077–9.
DeFronzo RA, Diebert D, Hendler R, Felig P. Insulin sensitivity and insulin binding in maturity onset diabetes. J Clin Investig. 1979a;63:939–46.
DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979b;6:E214–23.
DeFronzo RA, Jacot E, Jequier E, et al. The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry. Diabetes. 1981;30:1000–7.
DeFronzo RA, Ferrannini E, Hendler R, et al. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia. Diabetes. 1983;32:35–45.
DeFronzo RA, Gunnarsson R, Bjorkman O, et al. Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent diabetes mellitus. J Clin Investig. 1985;76:149–55.
DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism. 1989;38:387–95.
DeFronzo RA, Tripathy D, Schwenke DC, et al. Pioglitazone for diabetes prevention in impaired glucose tolerance. N Engl J Med. 2011;364:1104–15.
DeFronzo RA, Tripathy D, Schwenke DC, et al. Prevention of diabetes with pioglitazone in ACT NOW: physiologic correlates. Diabetes. 2013a;62(11):3920–6.
DeFronzo RA, Hompesch M, Kasichayanula S, et al. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. Diabetes Care. 2013b;36(10):3169–76.
DeFronzo RA, Tripathy D, Abdul-Ghani M, Musi N, Gastaldelli A. The disposition index does not reflect beta-cell function in IGT subjects treated with pioglitazone. J Clin Endocrinol Metab. 2014;99(10):3774–81.
DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1:15019.
DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. Nat Rev Nephrol. 2017;13:11–26.
Degn KB, Juhl CB, Sturis J, et al. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes. 2004;53:1187–94.
Del Guerra S, Lupi R, Marselli L, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes. 2005;54(3):727–35.
Del Prato S, Bonadonna RC, Bonora E, et al. Characterization of cellular defects of insulin action in type 2 (non-insulin-dependent) diabetes mellitus. J Clin Investig. 1993;91:484–94.
Del Prato S, Simonson DC, Sheehan P, et al. Studies on the mass effect of glucose in diabetes. Evidence for glucose resistance. Diabetologia. 1997;40:687–97.
Dent P, Lavoinne A, Nakielny S, et al. The molecular mechanisms by which insulin stimulates glycogen synthesis in mammalian skeletal muscle. Nature. 1990;348:302–7.
Desgraz R, Bonal C, Herrera PL. beta-cell regeneration: the pancreatic intrinsic faculty. Trends Endocrinol Metab. 2011;22(1):34–43.

Despres JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis. 1990;10 (4):497–511.
Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, Novartis Institutes of BioMedical Research, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316(5829):1331–6.
Diamond MP, Thornton K, Connolly-Diamond M, et al. Reciprocal variation in insulin-stimulated glucose uptake and pancreatic insulin secretion in women with normal glucose tolerance. J Soc Gynecol Investig. 1995;2:708–15.
Dominguez JH, Camp K, Maianu L, et al. Molecular adaptations of GLUT1 and GLUT2 in renal proximal tubules of diabetic rats. Am J Physiol. 1994;266:F283–90.
Dowse GK, Zimmet PZ, Collins VR. Insulin levels and the natural history of glucose intolerance in Nauruans. Diabetes. 1996;45:1367–72.
Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin. Diabetes. 2006;55:2392–7.
Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest. 1999;103(2):253–9.
Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153–65.
Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. Diabetes. 2013;62(10):3316–23.
Ducluzeau P-H, Perretti N, Laville M, et al. Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. Diabetes. 2001;50:1134–42.
Echwald SM, Bjorbaek C, Hansen T, et al. Identification of four amino acid substitutions in hexokinase II and studies of relationships to NIDDM, glucose effectiveness, and insulin sensitivity. Diabetes. 1995;44:347–53.
Edgerton DS, Cherrington AD. Is brain insulin action relevant to the control of plasma glucose in humans? Diabetes Educ. 2015;64:696–9.
Efendic S, Grill V, Luft R, Wajngot A. Low insulin response: a marker of pre-diabetes. Adv Exp Med Biol. 1988;246:167–74.
Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev. 2008;29(1):42–61.
Ekberg K, Landau BR, Wajngot A, et al. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes. 1999;48:292–8.
Elbein SC, Hoffman M, Qin H, et al. Molecular screening of the glucokinase gene in familial type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 1994;37:182–7.
Ellis BA, Poynten A, Lowy AJ, et al. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. Am J Physiol Endocrinol Metab. 2000;279: E554–60.
Eriksson UJ. Lifelong consequences of metabolic adaptations in utero? Diabetologia. 1996;39:1123–5.
Eriksson J, Franssila-Kallunki A, Ekstrand A, et al. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med. 1989;321:337–43.
Eriksson J, Koranyi L, Bourey R, et al. Insulin resistance in type 2 (non-insulin-dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT-4) gene in human skeletal muscle. Diabetologia. 1992;35:143–7.
Fabbrini E, Tamboli RA, Magkos F, et al. Surgical removal of omental fat does not improve insulin sensitivity and cardiovascular risk factors in obese adults. Gastroenterology. 2010;139 (2):448–55.
Falholt K, Jensen I, Lindkaer Jensen S, et al. Carbohydrate and lipid metabolism of skeletal muscle in type 2 diabetic patients. Diabet Med. 1988;5:27–31.

Farber SJ, Berger EY, Earle DP. Effect of diabetes and insulin of the maximum capacity of the renal tubules to reabsorb glucose. J Clin Investig. 1951;30:125–9.
Ferrannini E, DeFronzo RA. Insulin actions in vivo: glucose metabolism. In: Zimmet P, Alberti KGMM, editors. International textbook of diabetes mellitus. Chichester: Wiley; 2015. p. 211–33.
Ferrannini E, Mari A. Beta cell function and its relation to insulin action in humans: a critical appraisal. Diabetologia. 2004;47(5):943–56.
Ferrannini E, Mari A. beta-Cell function in type 2 diabetes. Metabolism. 2014;63(10):1217–27.
Ferrannini E, Mingrone G. Impact of different bariatric surgical procedures on insulin action and beta-cell function in type 2 diabetes. Diabetes Care. 2009;32(3):514–20.
Ferrannini E, Wahren J, Felig P, DeFronzo RA. Role of fractional glucose extraction in the regulation of splanchnic glucose metabolism in normal and diabetic man. Metabolism. 1980;29:28–35.
Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA. Effect of fatty acids on glucose production and utilization in man. J Clin Invest. 1983;72(5):1737–47.
Ferrannini E, Bjorkman O, Reichard GA Jr, et al. The disposal of an oral glucose load in healthy subjects. A quantitative study. Diabetes. 1985;34:580–8.
Ferrannini E, Simonson DC, Katz LD, et al. The disposal of an oral glucose load in patients with non-insulin dependent diabetes. Metabolism. 1988;37:79–85.
Ferrannini E, Natali A, Bell P, et al. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Investig. 1997;100:1166–73.
Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab. 2005;90(1):493–500.
Ferrannini E, Natali A, Muscelli E, et al. Natural history and physiological determinants of changes in glucose tolerance in a non-diabetic population: the RISC study. Diabetologia. 2011;54:1507–16.
Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest. 2014;124(2):499–508.
Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009;15(8):930–9.
Firth R, Bell P, Rizza R. Insulin action in non-insulin-dependent diabetes mellitus: the relationship between hepatic and extrahepatic insulin resistance and obesity. Metabolism. 1987;36:1091–5.
Flannick J, Thorleifsson G, Beer NL, et al. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nat Genet. 2014;46(4):357–63.
Flier JS, Minaker KL, Landsberg L, Young JB, Pallotta J, Rowe JW. Impaired in vivo insulin clearance in patients with severe target-cell resistance to insulin. Diabetes. 1982;31(2):132–5.
Folli F, Saad JA, Backer JM, Kahn CR. Regulation of phosphatidylinositol 3-kinase activity in liver and muscle of animal models of insulin-resistant and insulin-deficient diabetes mellitus. J Clin Investig. 1993;92:1787–94.
Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015;528(7581):262–6.
Frame S, Cohen P. GSK3 takes centre stage more than 20 years after its discovery. Biochem J. 2001;359(Pt 1):1–16.
Frayn KN. Visceral fat and insulin resistance–causative or correlative? Br J Nutr. 2000;83(Suppl 1): S71–7.
Freidenberg GR, Henry RR, Klein HH, et al. Decreased kinase activity of insulin receptors from adipocytes of non-insulin-dependent diabetic studies. J Clin Investig. 1987;79:240–50.
Freidenberg GR, Reichart D, Olefsky JM, Henry RR. Reversibility of defective adipocyte insulin receptor kinase activity in non-insulin dependent diabetes mellitus. Effect of weight loss. J Clin Investig. 1988;82:1398–406.
Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. Nature. 2016;536(7614):41–7.

Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Forum Nutr. 2013;5(5):1544–60.

Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. Am J Med. 1998;105:331–45.

Garvey WT, Olefsky JM, Griffin J, et al. The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. Diabetes. 1985;34:222–34.

Garvey WT, Hueckstead TP, Mattaei S, Olefsky JM. Role of glucose transporters in the cellular insulin resistance of type II non-insulin dependent diabetes mellitus. J Clin Investig. 1988;81:1528–36.

Gastaldelli A, Baldi S, Pettiti M, et al. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. Diabetes. 2000;49:1367–73.

Gastaldelli A, Ferrannini E, Miyazaki Y, et al. Beta cell dysfunction and glucose intolerance: results from the San Antonio Metabolism (SAM) study. Diabetologia. 2004;47:31–9.

Gastaldelli A, Miyazaki Y, Mahankali A, et al. The effect of pioglitazone on the liver: role of adiponectin. Diabetes Care. 2006;29(10):2275–81.

Gastaldelli A, Ferrannini E, Miyazaki Y, et al. Thiazolidinediones improve beta-cell function in type 2 diabetic patients. Am J Physiol Endocrinol Metab. 2007a;292:E871–83.

Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology. 2007b;133 (2):496–506.

Gautier JF, Wilson C, Weyer C, et al. Low acute insulin secretory responses in adult offspring of people with early onset type 2 diabetes. Diabetes. 2001;50:1828–33.

Gerich JE, Meyer C, Woerle HJ, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. Diabetes Care. 2001;24(2):382–91.10.

Gianani R. Beta cell regeneration in human pancreas. Semin Immunopathol. 2011;33(1):23–7.

Ginsberg H, Kimmerling G, Olefsky JM, Reaven GM. Demonstration of insulin resistance in untreated adult-onset diabetic subjects with fasting hyperglycemia. J Clin Investig. 1975;55:454–61.

Godfrey KM, Reynolds RM, Prescott SL, et al. Influence of maternal obesity on the long-term health of offspring. Lancet Diabetes Endocrinol. 2017;5(1):53–64.

Golay A, DeFronzo RA, Ferrannini E, et al. Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin dependent) diabetic patients. Diabetologia. 1988;31:585–91.

Goldfine AB, Kulkarni RN. Modulation of β-cell function: a translational journal from the bench to the bedside. Diabetes Obes Metab. 2012;14(Suppl 3):152–60.

Goldfine AB, Fonseca V, Jablonski KA, et al. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med. 2010;152(6):346–57.

Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006;38:320–3.

Gregg EW, Chen H, Wagenknecht LE, et al. Association of an intensive lifestyle intervention with remission of type 2 diabetes. JAMA. 2012;308(23):2489–96.

Griffin ME, Marcucci MJ, Cline GW, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes. 1999;48:1270–4.

Grill V. A comparison of brain glucose metabolism in diabetes as measured by positron emission tomography or by arteriovenous techniques. Ann Med. 1990;22:171–5.

Groop L, Lyssenko V. Genes and type 2 diabetes mellitus. Curr Diab Rep. 2008;8:192–7.

Groop LC, Bonadonna RC, Del Prato S, et al. Glucose and free fatty acid metabolism in non-insulin dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Investig. 1989;84:205–15.

Groop LC, Saloranta C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA. The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1991;72(1):96–107.

Groop L, Bonadonna R, Simonson DC, et al. Effect of insulin on oxidative and non-oxidative pathways of glucose and free fatty acid metabolism in human obesity. Am J Physiol. 1992;263: E79–84.
Group DPPR. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. Diabet Med. 2007;24:137–44.
Guardado-Mendoza R, Davalli AM, Chavez AO, et al. Pancreatic islet amyloidosis, beta-cell apoptosis, and alpha-cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. PNAS. 2009;106:13992–7.
Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(5):367–77.
Gulli G, Ferrannini E, Stern M, et al. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. Diabetes. 1992;41:1575–86.
Gustavson SM, Chu CA, Nishizawa M, et al. Effects of hyperglycemia, glucagon, and epinephrine on renal glucose release in the conscious dog. Metabolism. 2004;53(7):933–41.
Haataja L, Gurlo T, Huang CJ, Butler PC. Islet amyloid in type 2 diabetes and the toxic oligomer hypothesis. Endocr Rev. 2008;29:303–16.
Haffner SM, Miettinen H, Gaskill SP, Stern MP. Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. Diabetes. 1995;44:1386–91.
Halban PA, German MS, Kahn SE, Weir GC. Current status of islet cell replacement and regeneration therapy. J Clin Endocrinol Metab. 2010;95(3):1034–43.
Hanley AJ, Williams K, Stern MP, Haffner SM. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio heart study. Diabetes Care. 2002;25:1177–84.
Hanley SC, Austin E, Assouline-Thomas B, et al. {beta}-Cell mass dynamics and islet cell plasticity in human type 2 diabetes. Endocrinology. 2010;151(4):1462–72.
Hansen BC, Bodkin NH. Heterogeneity of insulin responses: phases leading to type 2 (noninsulin-dependent) diabetes mellitus in the rhesus monkey. Diabetologia. 1986;29:713–9.
Haus JM, Kashyap SR, Kasumov T, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes. 2009;58(2):337–43.
Hedley AA, Ogden CL, Johnson CL, et al. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. JAMA. 2004;291:2847–50.
Helgason A, Palsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet. 2007;39:218–25.
Henquin JC, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. Diabetologia. 2011;54(7):1720–5.
Henry RR, Wallace P, Olefsky JM. Effects of weight loss on mechanisms of hyperglycemia in obese non-insulin-dependent diabetes mellitus. Diabetes. 1986;35:990–8.
Herschkovitz A, Liu YF, Ilan E, Ronen D, Boura-Halfon S, Zick Y. Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. J Biol Chem. 2007;282(25):18018–27.
Higa M, Zhou YT, Ravazzola M, et al. Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. PNAS. 1999;96:11513–8.
Himsworth HP, Kerr RB. Insulin-sensitive and insulin-insensitive types of diabetes mellitus. Clin Sci. 1939;4:120–52.
Hitman GA, Hawrammi K, McCarthy MI, et al. Insulin receptor substrate-1 gene mutations in NIDDM: implication for the study of polygenic disease. Diabetologia. 1995;38:481–6.
Hojberg PV, Vilsboll T, Rabol R, et al. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia. 2009;52:199–207.
Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409–39.
Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab. 2004;287(2):E199–206.

Holst JJ, Knop FK, Vilsboll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. Diabetes Care. 2011;34(Suppl 2):S251–7.
Honka H, Makinen J, Hannukainen JC, et al. Validation of [^{18}F]fluorodeoxyglucose and positron emission tomography (PET) for the measurement of intestinal metabolism in pigs, and evidence of intestinal insulin resistance in patients with morbid obesity. Diabetologia. 2013;56(4):893–900.
Howard CF. Longitudinal studies on the development of diabetes in individual macaca nigra. Diabetologia. 1986;29:301–6.
Howard JK, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. Trends Endocrinol Metab. 2006;17(9):365–71.
Howard G, Bergman R, Wagenknecht LE, et al. Ability of alternative indices of insulin sensitivity to predict cardiovascular risk: comparison with the "minimal model". Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Ann Epidemiol. 1998;8:358–69.
Hsueh WA, Law RE. Insulin signaling in the arterial wall. Am J Cardiol. 1999;84:21J–4J.
Hu Y, Li L, Xu Y, et al. Short-term intensive therapy in newly diagnosed type 2 diabetes partially restores both insulin sensitivity and beta-cell function in subjects with long-term remission. Diabetes Care. 2011;34(8):1848–53.
Huang CJ, Lin CY, Haataja L, et al. High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. Diabetes. 2007;56:2016–27.
Hundal RS, Petersen KF, Mayerson AB, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Investig. 2002;109:1321–6.
Igoillo-Esteve M, Marselli L, Cunha DA, et al. Palmitate induces a pro-inflammatory response in human pancreatic islets that mimics CCL2 expression by beta cells in type 2 diabetes. Diabetologia. 2010;53:1395–405.
Imamura M, Maeda S. Genetics of type 2 diabetes: the GWAS era and future perspectives [Review]. Endocr J. 2011;58:723–39.
Imamura T, Koffler M, Helderman JH, et al. Severe diabetes induced in subtotally depancreatized dogs by sustained hyperglycemia. Diabetes. 1988;37(5):600–9.
Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24:683–9.
Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes. 2002;51:2005–11.
Jain R, Lammert E. Cell-cell interactions in the endocrine pancreas. Diabetes Obes Metab. 2009;11 (Suppl 4):159–67.
Jallut D, Golay A, Munger R, et al. Impaired glucose tolerance and diabetes in obesity: a 6 year follow-up study of glucose metabolism. Metabolism. 1990;39:1068–75.
James WP. The fundamental drivers of the obesity epidemic. Obes Rev. 2008;9(Suppl 1):6–13.
Jamison RA, Stark R, Dong J, et al. Hyperglucagonemia precedes a decline in insulin secretion and causes hyperglycemia in chronically glucose-infused rats. Am J Physiol Endocrinol Metab. 2011;301(6):E1174–83.
Jastreboff AM, Sinha R, Lacadie C, et al. Neural correlates of stress- and food- cue-induced food craving in obesity: association with insulin levels. Diabetes Care. 2013;36(2):394–402.
Jiang ZY, Lin YW, Clemont A, et al. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Investig. 1999;104:447–57.
Johnson AB, Argyraki M, Thow JC, Cooper BG, Fulcher G, Taylor R. Effect of increased free fatty acid supply on glucose metabolism and skeletal muscle glycogen synthase activity in normal man. Clin Sci (Lond). 1992;82(2):219–26.
Jones CN, Pei D, Staris P, Polonsky KS, Chen YD, Reaven GM. Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals. J Clin Endocrinol Metab. 1997;82(6):1834–8.
Joost H-G, Bell GI, Best JD, et al. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endocrinol Metab. 2002;282:E974–6.

Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia. 2003;46:3–19.
Kahn SE, Suvag S, Wright LA, Utzschneider KM. Interactions between genetic background, insulin resistance and β-cell function. Diabetes Obes Metab. 2012;14(Suppl 3):46–56.
Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet. 2014;383(9922):1068–83.
Kamran M, Peterson RG, Dominguez JH. Overexpression of GLUT2 gene in renal proximal tubules of diabetic Zucker rats. J Am Soc Nephrol. 1997;8:943–8.
Kanat M, Mari A, Norton L, et al. Distinct beta-cell defects in impaired fasting glucose and impaired glucose tolerance. Diabetes. 2012;61(2):447–53.
Kanat M, DeFronzo RA, Abdul-Ghani MA. Treatment of prediabetes. World J Diabetes. 2015;6 (12):1207–22.
Kapitza C, Dahl K, Jacobsen JB, Axelsen MB, Flint A. The effects of semaglutide on β-cell function in subjects with type 2 diabetes. Diabetes. 2016;(Suppl 1):A262.
Kashiwagi A, Verso MA, Andrews J, et al. In vitro insulin resistance of human adipocytes isolated from subjects with non-insulin-dependent diabetes mellitus. J Clin Investig. 1983;72:1246–54.
Kashyap SR, DeFronzo RA. The insulin resistance syndrome: physiological considerations. Diab Vasc Dis Res. 2007;4:13–9.
Kashyap S, Belfort R, Gastaldelli A, et al. A sustained increase in plasma free fatty acids impairs insulin secretion in non-diabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes. 2003;52:2461–74.
Kashyap SR, Roman LJ, McLain J, et al. Insulin resistance is associated with impaired nitric oxide synthase (NOS) activity in skeletal muscle of type 2 diabetic subjects. J Clin Endocrinol Metab. 2005;90:1100–5.
Katz H, Homan M, Jensen M, et al. Assessment of insulin action in NIDDM in the presence of dynamic changes in insulin and glucose concentration. Diabetes. 1994;43:289–96.
Kellerer M, Kroder G, Tippmer S, et al. Troglitazone prevents glucose-induced insulin resistance of insulin receptor in rat-1 fibroblasts. Diabetes. 1994;43:447–53.
Kelley D, Mandarino L. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes. 2000;49:677–83.
Kelley D, Mokan M, Mandarino L. Intracellular defects in glucose metabolism in obese patients with noninsulin-dependent diabetes mellitus. Diabetes. 1992;41:698–706.
Kerouz NJ, Horsch D, Pons S, Kahn CR. Differential regulation of insulin receptor substrates-1 and −2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. J Clin Investig. 1997;100:3164–72.
Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A. 2001;98(13):7522–7.
Kim YB, Ciaraldi TP, Kong A, et al. Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110 beta protein levels in skeletal muscle of type 2 diabetic subjects. Diabetes. 2002;51:443–8.
Klein HH, Vestergaard H, Kotzke G, Pedersen O. Elevation of serum insulin concentration during euglycemic hyperinsulinemic clamp studies leads to similar activation of insulin receptor kinase in skeletal muscle of subjects with and without NIDDM. Diabetes. 1995;344:1310–7.
Kleinridders A, Ferris HA, Cai W, Kahn CR. Insulin action in brain regulates systemic metabolism and brain function. Diabetes. 2014;63(7):2232–43.
Knop FK, Vilsboll T, Hojberg PV, et al. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes. 2007;56:1951–9.
Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.
Koivisto VA, DeFronzo RA. Physical training and insulin sensitivity. Diabetes Metab Rev. 1986;1:445–81.
Kolaczynski JW, Nyce MR, Considine RV, et al. Acute and chronic effects of insulin on leptin production in humans: studies in vivo and in vitro. Diabetes. 1996;45(5):699–701.

Kolterman OG, Gray RS, Griffin J, et al. Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. J Clin Investig. 1981;68:957–69.
Kosaka K, Kuzuya T, Akanuma Y, Hagura R. Increase in insulin response after treatment of overt maturity onset diabetes mellitus is independent of the mode of treatment. Diabetologia. 1980;18:23–8.
Kotronen A, Seppala-Lindroos A, Bergholm R, Yki-Jarvinen H. Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. Diabetologia. 2008;51(1):130–8.
Krook A, Bjornholm M, Galuska D, et al. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. Diabetes. 2000;49(2):284–92.
Krssak M, Falk Petersen K, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. Diabetologia. 1999;42(1):113–6.
Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell. 1999;96(3):329–39.
Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Haring HU. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. Physiol Rev. 2016;96 (4):1169–209.
Kusari J, Verma US, Buse JB, et al. Analysis of the gene sequences of the insulin receptor and the insulin-sensitive glucose transporter (GLUT4) in patients with common-type non-insulin-dependent diabetes mellitus. J Clin Investig. 1991;88:1323–30.
Laakso M, Malkki M, Kekalainen P, et al. Polymorphisms of the human hexokinase II gene: lack of association with NIDDM and insulin resistance. Diabetologia. 1995;38:617–22.
Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J (Clin Res Ed). 1984;289 (6454):1257–61.
Lara-Castro C, Garvey WT. Intracellular lipid accumulation in liver and muscle and the insulin resistance syndrome. Endocrinol Metab Clin N Am. 2008;37(4):841–56.
Larsen PJ, Tennagels N. On ceramides, other sphingolipids and impaired glucose homeostasis. Mol Metab. 2014;3(3):252–60.
Lazar DF, Wiese RJ, Brady MJ, et al. Mitogen-activated protein kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. J Biol Chem. 1995;270:20801–7.
Leahy JL, Cooper HE, Weir GC. Impaired insulin secretion associated with near normoglycemia. Study in normal rats with 96-h in vivo glucose infusions. Diabetes. 1987;36:459–64.
Lebrun P, Van Obberghen E. SOCS proteins causing trouble in insulin action. Acta Physiol (Oxf). 2008;192(1):29–36.
Lee Y, Lingvay I, Szczepaniak LS, et al. Pancreatic steatosis: harbinger of type 2 diabetes in obese rodents. Int J Obes (Lond). 2010;34:396–400.
Lehto M, Huang X, Davis EM, et al. Human hexokinase II gene: exon-intron organization, mutation screening in NIDDM, and its relationship to muscle hexokinase activity. Diabetologia. 1995;38:1466–74.
Levy J, Atkinson AB, Bell PM, et al. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast diet study. Diabet Med. 1998;15:290–6.
Li Y, Xu W, Liao Z, et al. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients is associated with improvement of beta-cell function. Diabetes Care. 2004;27 (11):2597–602.
Liang H, Tantiwong P, Shanmugasundaram K, et al. Effect of a sustained reduction in plasma free fatty acid concentration on insulin signaling and inflammation in skeletal muscle from human subjects. J Physiol. 2013;591(pt 11):2897–909.
Lillioja A, Mott DM, Zawadzki JK, et al. Glucose storage is a major determinant of in vivo 'insulin resistance' in subjects with normal glucose tolerance. J Clin Endocrinol Metab. 1986;62:922–7.

Lillioja S, Nyomba BL, Saad MF, et al. Exaggerated early insulin release and insulin resistance in a diabetes-prone population: a metabolic comparison of Pima Indians and Caucasians. J Clin Endocrinol Metab. 1991;73:866–76.
Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:1988–92.
Lim EL, Hollingsworth KG, Aribisala BS, et al. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia. 2011;54:2506–14.
Lin CY, Gurlo T, Haataja L, et al. Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3′-kinase-dependent pathway. J Clin Endocrinol Metab. 2005;90:6678–86.
Liu J, Wu X, Franklin JL, et al. Mammalian Tribbles homolog 3 impairs insulin action in skeletal muscle: role in glucose-induced insulin resistance. Am J Physiol Endocrinol Metab. 2010;298 (3):E565–76.
Lonnroth P, Digirolamo M, Krotkiewski M, Smith U. Insulin binding and responsiveness in fat cells from patients with reduced glucose tolerance and type II diabetes. Diabetes. 1983;32:748–54.
Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011;121(6):2111–7.
Lupi R, Dotta F, Marselli L, et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes. 2002;51:1437–42.
Lupi R, Del Guerra S, Marselli L, et al. Rosiglitazone prevents the impairment of human islet function induced by fatty acids: evidence for a role of PPARgamma in the modulation of insulin secretion. Am J Physiol Endocrinol Metab. 2004;286:E560–7.
Luzi L, DeFronzo RA. Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans. Am J Phys. 1989;257(2 Pt 1):E241–6.
Lyssenko V, Almgren P, Anevski D, et al. Botnia Study Group: predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. Diabetes. 2005;54:166–74.
Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest. 2007;117(8):2155–63.
Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med. 2008;359(21):2220–32.
Lyssenko V, Nagorny CL, Erdos MR, et al. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet. 2009;41(1):82–8.
Ma RC, Lin X, Jia W. Causes of type 2 diabetes in China. Lancet Diabetes Endocrinol. 2014;2 (12):980–91.
Magnusson I, Rothman DL, Katz LD, et al. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Investig. 1992;90:1323–7.
Majer M, Mott DM, Mochizuki H, et al. Association of the glycogen synthase locus on 19q13 with NIDDM in Pima Indians. Diabetologia. 1996;39:314–21.
Mandarino LJ, Madar Z, Kolterman OG, et al. Adipocyte glycogen synthase and pyruvate dehydrogenase in obese and type II diabetic patients. Am J Physiol. 1986;251:E489–96.
Mandarino LJ, Wright KS, Verity LS, et al. Effects of insulin infusion on human skeletal muscle pyruvate dehydrogenase, phosphofructokinase, and glycogen synthase. Evidence for their role in oxidative and nonoxidative glucose metabolism. J Clin Investig. 1987;80:655–63.
Mandarino LJ, Printz RL, Cusi KA, et al. Regulation of hexokinase II and glycogen synthase mRNA, protein, and activity in human muscle. Am J Physiol. 1995;269:E701–8.
Mandarino LJ, Consoli A, Jain A, Kelley DE. Interaction of carbohydrate and fat fuels in human skeletal muscle: impact of obesity and NIDDM. Am J Physiol. 1996;270:E463–70.
Mandarino L, Bonadonna R, McGuinness O, Wasserman D. Regulation of muscle glucose uptake in vivo. In: Jefferson LS, Cherrington AD, editors. Handbook of physiology. Section 7: The

endocrine system. The endocrine pancreas and regulation of metabolism. vol. II. New York: Oxford University Press; 2001. p. 803–48.
Marachett P, Ferrannini E. Beta cell mass and function in human type 2 diabetes. In: DeFronzo RA, Ferrannini E, Zimmet P, Alberto KGMM, editors. International textbook of diabetes mellitus. 4th ed. Chichester: Wiley; 2015. p. 413–25.
Marchetti P, Lupi R, Federici M, et al. Insulin secretory function is impaired in isolated human islets carrying the Gly(972)–>Arg IRS-1 polymorphism. Diabetes. 2002;51:1419–24.
Marchetti P, Del Guerra S, Marselli L, et al. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab. 2004;89(11):5535–41.
Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta -cell function: modeling analysis in normal subjects. Am J Physiol Endocrinol Metab. 2002;283(6):E1159–66.
Marselli L, Suleiman M, Masini M, et al. Are we overestimating the loss of beta cells in type 2 diabetes? Diabetologia. 2014;57(2):362–5.
Martin BC, Warren JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet. 1992;340:925–9.
Martin-Gronert MS, Ozanne SE. Metabolic programming of insulin action and secretion. Diabetes Obes Metab. 2012;14(Suppl 3):29–39.
Masini M, Bugliani M, Lupi R, et al. Autophagy in human type 2 diabetes pancreatic beta cells. Diabetologia. 2009;52(6):1083–6.
Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L. Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. Diabetes. 1997;46(1):153–7.
Matchinsky FM. Banting lecture 1995. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes. 1996;45:223–41.
Matsuda M, Liu Y, Mahankali S, et al. Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes. 1999;48:1801–6.
Matsuda M, DeFronzo RA, Glass L, et al. Glucagon dose response curve for hepatic glucose production and glucose disposal in type 2 diabetic patients and normal individuals. Metabolism. 2002;51:1111–9.
Matsui J, Terauchi Y, Kubota N, et al. Pioglitazone reduces islet triglyceride content and restores impaired glucose-stimulated insulin secretion in heterozygous peroxisome proliferator-activated receptor-gamma-deficient mice on a high-fat diet. Diabetes. 2004;53:2844–54.
Mbanya J-CN, Pani LN, Mbanya DNS, et al. Reduced insulin secretion in offspring of African type 2 diabetic patients. Diabetes Care. 2000;23:1761–5.
McCarthy MI, Froguel P. Genetic approaches to the molecular understanding of type 2 diabetes. Am J Physiol. 2002;283:E217–25.
McClain DA, Lubas WA, Cooksey RC, et al. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc Natl Acad Sci U S A. 2002;99(16):10695–9.
Meier JJ, Hucking K, Holst JJ, et al. Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes. 2001;50:2497–504.
Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124(2):509–14.
Merovci A, Mari A, Solis C, et al. Dapagliflozin lowers plasma glucose concentration and improves beta-cell function. J Clin Endocrinol Metab. 2015;100(5):1927–32.
Merovci A, Abdul-Ghani M, Mari A, et al. Effect of dapagliflozin with and without acipimox on insulin sensitivity and insulin secretion in T2DM males. J Clin Endocrinol Metab. 2016;101(3):1249–56.
Meyer C, Dostou J, Nadkarni V, Gerich J. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. Am J Phys. 1998a;275(6 Pt 2):F915–21.
Meyer C, Stumvoll M, Nadkarni V, et al. Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. J Clin Investig. 1998b;102:619–24.

Michaliszyn SF, Mari A, Lee S, et al. beta-Cell function, incretin effect, and incretin hormones in obese youth along the span of glucose tolerance from normal to prediabetes to type 2 diabetes. Diabetes. 2014;63(11):3846–55.
Mitrakou A, Kelley D, Mokan M, et al. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. N Engl J Med. 1992;326:22–9.
Miyazaki Y, He H, Mandarino LJ, DeFronzo RA. Rosiglitazone improves downstream insulin-receptor signaling in type 2 diabetic patients. Diabetes. 2003;52:1943–50.
Mogensen CE. Maximum tubular reabsorpiton capacity for glucose and renal hemodynamics during rapid hypertonic glucose infusion in normal and diabetic subjects. Scand J Clin Lab Investig. 1971;28:101–9.
Mogensen M, Sahlin K, Fernstrom M, et al. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes. 2007;56(6):1592–9.
Mohan V, Sharp PS, Aber VR, et al. Insulin resistance in maturity-onset diabetes of the young. Diabetes Metab. 1988;13:193–7.
Moller DE, Yakota A, Flier JS. Normal insulin receptor cDNA sequence in Pima Indians with non-insulin-dependent diabetes mellitus. Diabetes. 1989;38:1496–500.
Moller N, Rizza RA, Ford GC, Nair KS. Assessment of postabsorptive renal glucose metabolism in humans with multiple glucose tracers. Diabetes. 2001;50:747–51.
Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphorylation by Akt at Ser(1179). J Biol Chem. 2001;276:30392–8.
Montane J, Kimek-Abercrombie A, Potter KJ, et al. Metabolic stress, IAPP and islet amyloid. Diabetes Obes Metab. 2012;14(Suppl 3):68–77.
Montell E, Turini M, Marotta M, et al. DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells. Am J Physiol Endocrinol Metab. 2001;280:E229–37.
Morino K, Petersen KF, Dufour S, et al. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Investig. 2005;115(12):3587–93.
Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012;44(9): 981–90.
Muller DC, Elahi D, Tobin JD, Andres R. Insulin response during the oral glucose tolerance test: the role of age, sex, body fat and the pattern of fat distribution. Aging. 1996;8:13–21.
Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes. 2008;57(5):1340–8.
Musi N, Goodyear LJ. Insulin resistance and improvements in signal transduction. Endocrine. 2006;29:73–80.
Nagi DK, Pettitt DJ, Bennett PH, Klein R, Knowler WC. Diabetic retinopathy assessed by fundus photography in Pima Indians with impaired glucose tolerance and NIDDM. Diabet Med. 1997;14(6):449–56.
Nannipieri M, Mari A, Anselmino M, et al. The role of beta-cell function and insulin sensitivity in the remission of type 2 diabetes after gastric bypass surgery. J Clin Endocrinol Metab. 2011;96 (9):E1372–9.
Nauck MA, Meier JJ. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. Lancet Diabetes Endocrinol. 2016;4(6):525–36.
Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia. 1986a;29(1):46–52.
Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab. 1986b;63(2):492–8.
Nauck MA, Vardarli I, Deacon CF, et al. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? Diabetologia. 2011;54:10–8.
Newgard CB, Brady MJ, O'Doherty RB, Saltiel AR. Organizing glucose disposal. Emerging roles of the glycogen targeting subunits of protein phosphatase-1. Diabetes. 2000;49:1967–77.

Nolan JJ, Friedenberg G, Henry R, et al. Role of human skeletal muscle insulin receptor kinase in the in vivo insulin resistance of noninsulin-dependent diabetes and obesity. J Clin Endocrinol Metab. 1994;78:471–7.
Noonan WT, Shaprio VM, Banks RO. Renal glucose reabsorption during hypertonic glucose infusion in female streptozotocin-induced diabetic rats. Life Sci. 2001;68:2967–77.
Norton L, Shannon C, Fourcaudot M, Hu C, Wang N, Ren W, Song J, Abdul-Ghani M, DeFronzo RA, Ren J, Jia W. Sodium-glucose (SGLT) and glucose (GLUT) transporter expression in the kidney of type 2 diabetic subjects. Diabetes Obes Metab. 2017;19(9):1322–6.
Nyomba BL, Freymond D, Raz I, et al. Skeletal muscle glycogen synthase activity in subjects with non-insulin-dependent diabetes mellitus after glyburide therapy. Metabolism. 1990;39:1204–10.
Obici S, Feng Z, Tan J, et al. Central melanocortin receptors regulate insulin action. J Clin Investig. 2001;108:1079–85.
Obici S, Feng Z, Karkanias G, et al. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci. 2002;5:566–72.
Ohsawa H, Kanatsuka A, Yamaguchi T, et al. Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. Biochem Biophys Res Commun. 1989;160: 961–7.
Olefsky JM, Reaven GM. Insulin binding in diabetes. Relationships with plasma insulin levels and insulin sensitivity. Diabetes. 1977;26:680–8.
Oliveira JM, Rebuffat SA, Gasa R, Gomis R. Targeting type 2 diabetes: lessons from a knockout model of insulin receptor substrate 2. Can J Physiol Pharmacol. 2014;92(8):613–20.
Orci L, Malaisse-Lagae F, Amherdt M, et al. Cell contacts in human islets of Langerhans. J Clin Endocrinol Metab. 1975;41(5):841–4.
Orho M, Nikua-Ijas P, Schalin-Jantti C, et al. Isolatation and characterization of the human muscle glycogen synthase gene. Diabetes. 1995;44:1099–105.
Osawa H, Sutherland C, Robey R, et al. Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. J Biol Chem. 1996;271:16690–4.
Ozcan S. Minireview: microRNA function in pancreatic beta cells. Mol Endocrinol. 2014;28(12): 1922–33.
Ozcan U, Ozcan L, Yilmaz E, et al. Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. Mol Cell. 2008;29(5):541–51.
Pal D, Dasgupta S, Kundu R, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012;18(8):1279–85.
Park S, Choi SB. Induction of long-term normoglycemia without medication in Korean type 2 diabetes patients after continuous subcutaneous insulin infusion therapy. Diabetes Metab Res Rev. 2003;19(2):124–30.
Patane G, Anello M, Piro S, et al. Role of ATP production and uncoupling protein-2 in the insulin secretory defect induced by chronic exposure to high glucose or free fatty acids and effects of peroxisome proliferator-activated receptor-gamma inhibition. Diabetes. 2002;51:2749–56.
Patterson E, Ryan PM, Cryan JF, et al. Gut microbiota, obesity and diabetes. Postgrad Med J. 2016;92(1087):286–300.
Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev. 2010;31(3):364–95.
Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A. 2003;100(14):8466–71.
Pedersen O, Bak J, Andersen P, et al. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. Diabetes. 1990;39:865–70.
Pendergrass M, Koval J, Vogt C, et al. Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. Diabetes. 1998a;47:387–94.
Pendergrass M, Nucci G, DeFronzo R. In vivo glucose transport (GT) and phosphorylation (GP) in skeletal muscle are impared by elevation of plasma FFA (Abstract). Diabetes. 1998b;47 (Suppl 1):A65.

Pendergrass M, Bertoldo A, Bonadonna R, et al. Muscle glucose transport and phosphorylation in type 2 diabetic, obese non-diabetic, and genetically predisposed individuals. Am J Physiol Endocrinol Metab. 2007;292:E92–100.
Perriott LM, Kono T, Whitesell RR, et al. Glucose uptake and metabolism by cultured human skeletal muscle cells: rate-limiting steps. Am J Physiol Endocrinol Metab. 2001;281:E72–80.
Petersen KF, Oral EA, Dufour S, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109(10):1345–50.
Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med. 2004;350(7):664–71.
Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes. 2005;54(3):603–8.
Phillips DIW. Insulin resistance as a programmed response to fetal undernutrition. Diabetologia. 1996;39:1119–22.
Plantinga LC, Crews DC, Coresh J, et al. Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. Clin J Am Soc Nephrol. 2010;5(4):673–82.
Plum L, Belgardt BF, Bruning JC. Central insulin action in energy and glucose homeostasis. J Clin Investig. 2006;116:1761–6.
Polonsky KS. Lilly lecture 1994. The beta cell in diabetes: from molecular genetics to clinical research. Diabetes. 1995;44:705–17.
Porte D. Central regulation of energy homeostasis. Diabetes. 2006;55(Suppl 2):S155–60.
Pratipanawatr W, Pratipanawatr T, Cusi K, et al. Skeletal muscle insulin resistance in normoglycemic subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated IRS-1 tyrosine phosphorylation. Diabetes. 2001;50:2572–8.
Pratipanawatr T, Cusi K, Ngo P, et al. Normalization of plasma glucose concentration by insulin therapy improves insulin-stimulated glycogen synthesis in type 2 diabetes. Diabetes. 2002;51:462–8.
Printz RL, Ardehali H, Koch S, Granner DK. Human hexokinase II mRNA and gene structure. Diabetes. 1995;44:290–4.
Procharzka M, Michizuki H, Baier LJ, et al. Molecular and linkage analysis of type-1 protein phosphatase catalytic beta-subunit gene: lack of evidence for its mjaor role in insulin resistance in Pima Indians. Diabetologia. 1995;38:461–6.
Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490(7418):55–60.
Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab. 2008;10(Suppl 4):32–42.
Rahmoune H, Thompson PW, Ward JM, et al. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. Diabetes. 2005;54:3427–34.
Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med. 2011;50 (5):567–75.
Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;1:785–9.
Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37:1595–607.
Reaven GM, Chen YD, Golay A, et al. Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1987;64:106–10.
Reaven GM, Hollenbeck CB, Chen YD. Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. Diabetologia. 1989;32:52–5.
Reyna SM, Ghosh S, Tantiwong P, et al. Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. Diabetes. 2008;57(10):2595–602.

Richardson DK, Kashyap S, Bajaj M, et al. Lipid infusion induces an inflammatory/fibrotic response and decreases expression of nuclear encoded mitochondrial genes in human skeletal muscle. J Biol Chem. 2005;280:10290–7.
Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes. 2005;54(1):8–14.
Ritzel RA, Meier JJ, Lin CY, Veldhuis JD, Butler PC. Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islets. Diabetes. 2007;56(1):65–71.
Roden M, Price TB, Perseghin G, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 1996;97(12):2859–65.
Rogers PA, Fisher RA, Harris H. An electrophoretic study of the distribution and properties of human hexokinases. Biochem Genet. 1975;13:857–66.
Romeo GR, Lee J, Shoelson SE. Metabolic syndrome, insulin resistance, and roles of inflammation–mechanisms and therapeutic targets. Arterioscler Thromb Vasc Biol. 2012;32(8):1771–6.
Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8(7):519–29.
Rosengren AH, Jokubka R, Tojjar D, et al. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science. 2010;327(5962):217–20.
Rosenthal M, Doberne L, Greenfield M, et al. Effect of age on glucose tolerance, insulin secretion, and in vivo insulin action. J Am Geriatr Soc. 1982;30:562–7.
Rossetti L, Shulman GI, Zawalich W, DeFronzo RA. Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. J Clin Investig. 1987a;80:1037–44.
Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest. 1987b;79(5):1510–5.
Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity [Review]. Diabetes Care. 1990;13:610–30.
Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Investig. 1992;89:1069–75.
Rothman DL, Magnusson I, Cline G, et al. Decreased muscle glucose transport/phosphorylation is an early defect in the pathogenesis of non-insulin-dependent diabetes mellitus. PNAS. 1995;92:983–7.
Rutter MK, Meigs JB, Sullivan LM, et al. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham offspring study. Diabetes. 2005;54:3252–7.
Saad MF, Knowler WC, Pettitt DJ, et al. The natural history of impaired glucose tolerance in the Pima Indians. N Engl J Med. 1988;319:1500–5.
Saad MF, Knowler WC, Pettitt DJ, et al. Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. Lancet. 1989;1:1356–9.
Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA, Bennett PH. A two-step model for development of non-insulin-dependent diabetes. Am J Med. 1991;90(2):229–35.
Sakuraba H, Mizukami H, Yagihashi N, et al. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese type II diabetic patients. Diabetologia. 2002;45:85–96.
Salans LB, Bray GA, Cushman SW, et al. Glucose metabolism and the response to insulin by human adipose tissue in spontaneous and experimental obesity. Effects of dietary composition and adipose cell size. J Clin Investig. 1974;53:848–56.
Saltiel AR, Kahn CR. Insulin signaling and the regulation of glucose and lipid metabolism. Nature. 2001;414:799–806.
Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;26:12–22.
Samuel VT, Liu ZX, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem. 2004;279(31):32345–53.

Samuel VT, Liu ZX, Wang A, et al. Inhibition of protein kinase C epsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. J Clin Invest. 2007;117(3):739–45.
Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. Physiol Rev. 2015;95(2):513–48.
Schalin-Jantti C, Harkoenen M, Groop LC. Impaired activation of glycogen synthase in people at increased risk for developing NIDDM. Diabetes. 1992;41:598–604.
Schwartz MW, Woods SC, Porte D, et al. Central nervous system control of food intake. Nature. 2000;404:661–71.
Seidell JC, Bouchard C. Visceral fat in relation to health: is it a major culprit or simply an innocent bystander? Int J Obes Relat Metab Disord. 1997;21(8):626–31.
Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell. 2010;40(2):310–22.
Shah OJ, Wang Z, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol. 2004;14 (18):1650–6.
Sheperd PR, Nave BT, Siddle K. Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3L1 adipocytes: evidence for the involvement of phosphoinositide 3 kinase and p70 ribosomal protein S6 kinase. Biochem J. 1995;305:25–8.
Shepherd PR, Kahn BB. Glucose transporters and insulin action. Implications for insulin resistance and diabetes mellitus. N Engl J Med. 1999;341:248–57.
Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116(11):3015–25.
Shulman GI, Rothman DL, Smith D, et al. Mechanism of liver glycogen repletion in vivo by nuclear magnetic resonance spectroscopy. J Clin Investig. 1985;76:1229–36.
Shulman GI, Rothman DL, Jue T, et al. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. N Engl J Med. 1990;322:223–8.
Sicree RA, Zimmet P, King HO, Coventry JO. Plasma insulin response among Nauruans. Prediction of deterioration in glucose tolerance over 6 years. Diabetes. 1987;36:179–86.
Sigal RJ, Doria A, Warram JH, Krolewski AS. Codon 972 polymorphism in the insulin receptor substrate-1 gene, obesity, and risk of noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1996;81:1657–9.
Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445(7130):881–5.
Sriwijitkamol A, Christ-Roberts C, Berria R, et al. Reduced skeletal muscle inhibitor of kappaB beta content is associated with insulin resistance in subjects with type 2 diabetes: reversal by exercise training. Diabetes. 2006;55(3):760–7.
Starke A, Grundy S, McGarry JD, Unger RH. Correction of hyperglycemia with phloridzin restores the glucagon response to glucose in insulin-deficient dogs: implications for human diabetes. Proc Natl Acad Sci U S A. 1985;82(5):1544–6.
Steck AK, Winter WE. Review on monogenic diabetes. Curr Opin Endocrinol Diabetes Obes. 2011;18:252–8.
Stefan Y, Orci L, Malaisse-Lagae F, et al. Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. Diabetes. 1982;31:694–700.
Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet. 2007;39:770–5.
Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J. Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. J Clin Invest. 1995;96(5):2528–33.
Stumvoll M, Meyer C, Kreider M, Perriello G, Gerich J. Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. Metabolism. 1998;47(10): 1227–32.

Sugden MC, Holness MJ. Mechanisms underlying regulation of the expression and activities of the mammalian pyruvate dehydrogenase kinases. Arch Physiol Biochem. 2006;112(3):139–49.
Sun XJ, Miralpeix M, Myers MG Jr, et al. Expression and function of IRS-1 in insulin signal transmission. J Biol Chem. 1992;267(31):22662–72.
Szendroedi J, Yoshimura T, Phielix E, et al. Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A. 2014;111(26):9597–602.
Tang Y, Axelsson AS, Spegel P, et al. Genotype-based treatment of type 2 diabetes with an alpha2A-adrenergic receptor antagonist. Sci Transl Med. 2014;6(257):257ra139.
Tanijuchi CM, Emanuelli B, Kahn CR. Critical nodes in signaling pathways: insight into insulin action. Nat Rev Mol Cell Biol. 2006;7:85–96.
Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359–404.
ten Kulve JS, Veltman DJ, van Bloemendaal L, et al. Endogenous GLP-1 mediates postprandial reductions in activation in central reward and satiety areas in patients with type 2 diabetes. Diabetologia. 2015;58(12):2688–98.
Teo AK, Gupta MK, Doria A, Kulkarni RN. Dissecting diabetes/metabolic disease mechanisms using pluripotent stem cells and genome editing tools. Mol Metab. 2015;4(9):593–604.
Thiebaud D, Jacot E, DeFronzo RA, et al. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. Diabetes. 1982;31:957–63.
Thiebaud D, DeFronzo RA, Jacot E, et al. Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism. 1983;31:1128–36.
Thorburn AW, Gumbiner B, Bulacan F, et al. Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. J Clin Investig. 1990;85:522–9.
Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes. 1997;46(11):1733–42.
Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. Gut. 2014;63(9):1513–21.
Tordjman J, Khazen W, Antoine B, Chauvet G, Quette J, Fouque F, Beale EG, Benelli C, Forest C. Regulation of glyceroneogenesis and phosphoenolpyruvate carboxykinase by fatty acids, retinoic acids and thiazolidinediones: potential relevance to type 2 diabetes. Biochimie. 2004;85:1213–8.
Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev. 2013;93(1):1–21.
Trichitta V, Brunetti A, Chiavetta A, et al. Defects in insulin-receptor internalization and processing in monocytes of obese subjects and obese NIDDM patients. Diabetes. 1989;38:1579–84.
Tura A, Muscelli E, Gastaldelli A, Ferrannini E, Mari A. Altered pattern of the incretin effect as assessed by modelling in individuals with glucose tolerance ranging from normal to diabetic. Diabetologia. 2014;57(6):1199–203.
Turer AT, Scherer PE. Adiponectin: mechanistic insights and clinical implications. Diabetologia. 2012;55(9):2319–26.
Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
Turpin SM, Nicholls HT, Willmes DM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. Cell Metab. 2014;20(4):678–86.
Tushuizen ME, Bunck MC, Pouwels PJ, et al. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. Diabetes Care. 2007;30:2916–21.
UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.
Unger RH, Aguilar-Parada E, Muller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Investig. 1970;49:837–48.
Vaag A, Henriksen JE, Beck-Nielsen H. Decreased insulin activation of glycogen synthase in skeletal muscles in young non-obese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Investig. 1992;89:782–8.

Vaag A, Henriksen JE, Madsbad S, et al. Insulin secretion, insulin action, and hepatic glucose production in identical twins discordant for non-insulin-dependent diabetes mellitus. J Clin Investig. 1995;95:690–8.
Vague P, Moulin J-P. The defective glucose sensitivity of the B cell in insulin dependent diabetes. Improvement after twenty hours of normoglycaemia. Metabolism. 1982;31:139–42.
van Bloemendaal L, RG IJ, Ten Kulve JS, et al. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. Diabetes. 2014;63(12):4186–96.
Vauhkonen N, Niskanane L, Vanninen E, et al. Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited. Metabolic studies on offspring of diabetic probands. J Clin Investig. 1997;100:86–96.
Vaxillaire M, Froguel P. Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. Endocr Rev. 2008;29:254–64.
Vestergaard H, Bjocbaek C, Andersen PH, et al. Impaired expression of glycogen synthase mRNA in skeletal muscle of NIDDM patients. Diabetes. 1991;40:1740–5.
Vestergaard H, Lund S, Larsen FS, et al. Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. J Clin Investig. 1993;91:2342–50.
Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia. 2002;45:1111–9.
Virkamaki A, Ueki K, Kahn CR. Protein–protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Investig. 1999;103:931–43.
Vogt C, Ardehali H, Iozzo P, et al. Regulation of hexokinase II expression in human skeletal muscle in vivo. Metabolism. 2000;49:814–8.
Wahren J, Felig P, Hagenfeldt L. Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. J Clin Investig. 1976;57:987–99.
Wang CC, Goalstone ML, Draznin B. Molecular mechanisms of insulin resistance that impact cardiovascular biology. Diabetes. 2004;53:2735–40.
Wang X, Misawa R, Zielinski MC, et al. Regional differences in islet distribution in the human pancreas–preferential beta-cell loss in the head region in patients with type 2 diabetes. PLoS One. 2013;8(6):e67454.
Watanabe RM, Valle T, Hauser ER, et al. Familiarity of quantitative metabolic traits in Finnish families with non-insulin-dependent diabetes mellitus. Finland-United States Investigation of NIDDM Genes (FUSION) Study Investigators. Hum Hered. 1999;39:159–68.
Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112 (12):1796–808.
Welters HJ, Kulkarni RN. Wnt signaling: relevance to β-cell biology and diabetes. Trends Endocrinol Metab. 2008;19:359–5.
Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet. 2008;371:1753–60.
Westermark P, Wilander E. The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia. 1978;15:417–21.
Westermark P, Andersson A, Westermark GT. Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev. 2011;91:795–826.
Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest. 1999;104 (6):787–94.
Weyer C, Hanson RL, Tataranni PA, et al. A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance. Evidence for a pathogenic role of relative hyperinsulinemia. Diabetes. 2000;49:2094–101.

Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. Diabetes Care. 2001;24:89–94.

Williams KV, Price JC, Kelley DE. Interactions of impaired glucose transport and phosphorylation in skeletal muscle insulin resistance. A dose-response assessment using positron emission tomography. Diabetes. 2001;50:2069–79.

Williamson JR, Kreisberg RA, Felts PW. Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. Proc Natl Acad Sci U S A. 1966;56(1):247–54.

Wititsuwannakul D, Kim KH. Mechanism of palmityl coenzyme A inhibition of liver glycogen synthase. J Biol Chem. 1977;252(21):7812–7.

Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature. 1979;282(5738):503–5.

Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev. 2011;91(2):733–94.

Xiang AH, Peters RK, Kjos SL, et al. Effect of pioglitazone on pancreatic beta-cell function and diabetes risk in Hispanic women with prior gestational diabetes. Diabetes. 2006;55:517–22.

Yang W, Weng J. Diabetes in Chian 3. Early therapy for type 2 diabetes in China. Lancet Diabetes Endocrinol. 2014;2:992–1002.

Yki-Jarvinen H. Pathogenesis of nonalconolic fatty liver disease. In: DeFronzo RA, Ferrannini E, Zimmet P, Alberti KGMM, editors. International textbook of diabetes mellitus. 4th ed. Chchester: Wiley; 2015. p. 283–91.

Yki-Jarvinen H, DA MC. Glucotoxicity. In: DeFronzo RA, Ferrannini E, Zimmet P, Alberto KGMM, editors. International textbook of diabetes mellitus. 4th ed. Chichester: Wiley; 2015. p. 413–25.

Yki-Jarvinen H, Mott D, Young AA, et al. Regulation of glycogen synthase and phosphorylase activity by glucose and insulin in human skeletal muscle. J Clin Investig. 1987;80:95–100.

Yki-Jarvinen H, Daniels MC, Virkamaki A, Makimattila S, DeFronzo RA, McClain D. Increased glutamine:fructose-6-phosphate amidotransferase activity in skeletal muscle of patients with NIDDM. Diabetes. 1996;45(3):302–7.

Yoneda S, Uno S, Iwahashi H, et al. Predominance of beta-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. J Clin Endocrinol Metab. 2013;98(5):2053–61.

Yoon KH, Ko SH, Cho JH, et al. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab. 2003;88(5):2300–8.

Yu C, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, CushmanSW CGJ, Atcheson B, White MF, Kraegen EW, Shulman GI. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem. 2002;277:50230–6.

Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 2002;359:824–30.

Zhang W, Liu J, Tian L, Liu Q, Fu Y, Garvey WT. TRIB3 mediates glucose-induced insulin resistance via a mechanism that requires the hexosamine biosynthetic pathway. Diabetes. 2013;62(12):4192–200.

Zierath JR, He L, Guma A, et al. Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. Diabetologia. 1996;39:1180–9.

Zierler KL, Rabinowitz D. Roles of insulin and growth hormone, based on studies of forearm metabolism in man. Medicine. 1963;42:385–402.

Zimmet P, Whitehouse S, Alford F, Chisholm D. The relationship of insulin response to a glucose stimulus over a wide range of glucose tolerance. Diabetologia. 1978;15:23–7.

LADA

8

Simona Zampetti and Raffaella Buzzetti

Contents

S. Zampetti · R. Buzzetti (✉)
Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy
e-mail: simona.zampetti@uniroma1.it; raffaella.buzzetti@uniroma1.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_9

Abstract

The term latent autoimmune diabetes of the adult (LADA) has been introduced to define the subgroup of adult type 2 diabetes (T2DM) patients who are initially noninsulin requiring but with immune markers of type 1 diabetes (T1DM).

The prevalence of LADA has been estimated in a number of multicenter studies of both European and non-European populations. Around 4–14% of patients classified with T2DM have diabetes associated autoantibodies. Among these autoantibodies, glutamic acid decarboxylase (GAD) has become the main islet autoantibody for LADA screening and the most sensitive autoimmune marker for LADA diagnosis.

It remains to be clarified whether LADA exists as a distinct disease entity or it just represents the end of a wide spectrum of the heterogeneous immune-mediated diabetes. Uncertainties concern almost all aspects of this disease, including the nomenclature, diagnostic criteria, epidemiology, natural history, and pathogenesis with genetic, metabolic, and immunological aspects.

A number of attractive therapeutic interventions may be envisaged for prevention of beta-cell loss in LADA, including hypoglycemic and immunomodulatory agents. Since the autoimmune process in LADA seems to be slower than in T1DM, there is a wider window of opportunities for intervention.

Keywords

LADA · NIRAD · Islet autoantibodies · GADA · GADA titer · T1DM · T2DM · Insulin · Immunotherapy

Introduction

Distinction between type 1 diabetes (T1DM) and type 2 diabetes (T2DM) is not always straightforward. The disease process in classic T1DM patients is believed to be autoimmune in nature, whereas the disease process in classic T2DM is not autoimmune (Alberti and Zimmet 1998). However, there is increasing clinical evidence that highlights significant overlap between T1DM and T2DM, and the classification of diabetes into two main types has been challenged (Naik et al. 2009). Discovery of islet cell antibodies in 1974 in the sera of subjects with T1DM provided very strong evidence that the beta-cell lesion of T1DM was autoimmune in nature (Bottazzo et al. 1974). Autoimmune beta-cell dysfunction and destruction leads to insulin deficiency and generation of autoantibodies in the circulation, such as

autoantibodies to islet-cell cytoplasm (ICA), and/or to glutamic acid decarboxylase (GAD), and/or to the intracytoplasmatic domain of the tyrosine phosphatase-like protein IA-2 (IA-2) (MacCuish et al. 1974). Since there are no reliable markers for T2DM, absence of markers and/or manifestations of T1DM are often used as indicators for T2DM (Naik et al. 2009). Irvine et al. (1977) showed that about 11% of T2DM subjects were also positive for ICAs. Compared with ICA-negative T2DM, this ICA-positive subset tended to fail sulfonylurea therapy and needed insulin treatment earlier (Irvine et al. 1977). The term latent autoimmune diabetes of the adult (LADA) has been introduced, for the first time in 1993, to define the subgroup of adult phenotypic T2DM patients who are initially noninsulin requiring but with immune markers of T1DM (Tuomi et al. 1993). As expected for an immune attack on the beta-cells, these patients also became insulin dependent more rapidly than "classic" T2DM patients who were negative for islet autoantibodies. It is appropriate to ask if the term LADA is still an accurate descriptor. "Latent" in medical terminology is usually used to describe a dormant or hidden stage of a pathological process (Fourlanos et al. 2005). An individual with "latent autoimmune diabetes," by definition, should therefore have dormant or hidden autoimmune pathology and no clinical manifestations of disease. However, this is not the case; LADA is defined by serological evidence of islet autoimmunity in the setting of reduced and declining insulin secretion (Fourlanos et al. 2005). Evidence for autoimmune pathology is not latent and is a requirement for the diagnosis. Furthermore, diabetes in these patients is not limited to adult. Children can also have slowly progressive or subacute autoimmune diabetes, termed "latent autoimmune diabetes in the young" or "LADY," which is managed with diet and oral hypoglycemic agents for months to years before insulin is required (Lohmann et al. 2008). Several alternatives to the term LADA have been proposed but also appear to be inaccurate (Table 1). "Type 1.5 diabetes" (Juneja and Palmer 1999) implies that individuals always have clinical features of both T1DM (insulin deficiency and islet antibodies) and T2DM (insulin resistance and obesity), but patients with LADA are not necessarily insulin resistant or obese. "Non-insulin-requiring autoimmune diabetes" (NIRAD) does not seem appropriate given that the majority of patients become insulin requiring (Pozzilli and Di Mario 2001). Although LADA patients by definition are not insulin requiring at diagnosis and during the first time after diagnosis of diabetes, within 6 years, beta-cell function is severely impaired, leading to insulin dependency in most LADA patients. "Slowly progressive insulin-dependent type 1 diabetes" does not meet the criterion that patients are insulin independent at diagnosis. Moreover, it is arguable whether progression to insulin dependence after diagnosis is slow, given that insulin treatment may be required within months. All these terms are often considered synonymous based on the fact that this form of diabetes is a type of disease in adults with an autoimmune basis eventually leading to insulin therapy for its treatment (Guglielmi et al. 2012).

The concept of LADA is still strongly debated since many researchers question whether LADA represents a form of T2DM with early or fast destruction of beta-cells, a late manifestation of T1DM, or a distinct entity with its own features (Gale 2005). Lohmann et al. suggested that LADA could be divided into two clinically

Table 1 Different definitions of LADA

Type 1.5 diabetes
Slow progressive insulin dependent type 1 diabetes
Noninsulin requiring autoimmune diabetes
Autoimmune diabetes in adults
Late-onset autoimmune diabetes

distinct types on the basis of autoantibody positivity and titer (Lohmann et al. 2001). LADA patients with multiple autoantibody positivity or high-GADA titer were defined as "LADA-type 1" since they had clinical features that resembled T1DM patients; those subjects with single autoantibody positivity and low GADA titer were described as "LADA – Type 2" as they were phenotypically more similar to T2DM. Also other studies describe a heterogeneous clinical picture within the LADA group. Based on GAD autoantibodies titer, LADA has also been subclassified into two forms: patients with a phenotype closer to T1DM in those LADA with high GADA titer compared to a phenotype closer to T2DM in LADA patients with low GADA titer (Buzzetti et al. 2007).

To date, the World Health Organization (WHO) diabetes classification does not differentiate LADA as a distinct entity, but it has been included in the classification of T1DM (Alberti and Zimmet 1998).

Epidemiology

European Populations

The prevalence of LADA has been estimated in a number of multicenter studies of both European and non-European populations (Table 2). Around 4–14% of patients classified with T2DM have diabetes associated autoantibodies. The frequency of GADA-positive T2DM is higher in studies from northern Europe (7–14%) with decreasing prevalence by increasing patient age (Radtke et al. 2009; Turner et al. 1997). It appears to be lower in southern Europe, Asia, and North America (4–6%) (Buzzetti et al. 2007; Maioli et al. 2010), and within China, lower in the south than in the north (Zhou et al. 2013).

In the United Kingdom Prospective Diabetes Study (UKPDS 25), GADA were positive in 12% of 3672 T2DM patients, whereas ICA were positive in 6% of the patients (Turner et al. 1997). The prevalence was also dependent upon the age at onset, as was higher in younger patients; GADA was positive in 34% of patients aged 25–34 years at diagnosis and 7% of those aged 55–65 years at diagnosis. Among 1122 T2DM patients from the Botnia Study in the western Finland, the prevalence was 9.3% (Tuomi et al. 1999). GAD positivity was considerably higher among diabetes patients with an age at onset of diabetes between 28 and 45 years (19%), whereas it was stable at around 8.2% after 45 years of age. The Botnia Study includes mostly

Table 2 Frequency of LADA in different studies

Study	Country	Study method	Sample size	Age range	Islet autoantibodies	Frequency (%)
UKPDS *Turner R et al.* Lancet (1997)	UK	Clinical based	3672	25–65	GADA, ICA	12
BOTNIA *Tuomi T et al.* Diabetes (1999)	Finland	Registry based	1122	28–83	GADA, IA-2A	9.3
Eihme study *Takeda H et al.* Diabetes Care (2002)	Japan	Clinical based	4980	>20	GADA	3.8
ADOPT *Zinman B et al.* Diabetes Care (2007)	US/ EUR	Clinical based	4357	30–75	GADA, IA-2A	4.2
NIRAD *Buzzetti R et al.* Diabetes Care (2007)	Italy	Clinical based	5330	30–75	GADA, IA-2A	4.5
HUNT *Radtke MA et al.* Diabetes Care (2009)	Norway	Clinical based	1134	≥20	GADA	10
Maioli M et al. Eur J End (2010)	Sardinia	Clinical based	5568	35–70	GADA	4.9
Tianjin *Qi X et al.* Diabetes Care (2011)	China	Population based	8109	≥15	GADA	9.2
ACTION LADA *Hawa M et al.* Diabetes Care (2013)	Europe	Clinical based	6810	30–70	GADA, IA-2A, ZnT8	9.7
LADA China *Zhou Z et al.* Diabetes Care (2013)	China	Clinical based	5324	≥20	GADA	5.9

known T2DM patients from primary care centers. A Diabetes Outcome Progression Trial (ADOPT) was the first study to evaluate LADA prevalence in two different continents (North America and Europe) using a common assay with standardized recruitment criteria (Zinman et al. 2004). In 4134 drug-naïve T2DM patients diagnosed within 3 years, the prevalence of GADA positivity was 4.2%, and there was no difference in the prevalence of these antibodies in North America and Europe (4.7% and 3.7%, respectively). The first large Italian multicenter study to assess the prevalence of LADA patients was the NIRAD Study, in which the prevalence of GADA and/or IA-2 positivity was 4.5% in a cohort of 5330 T2DM patients (Buzzetti et al. 2007). In another Italian study, carried out in Sardinia, GADA positivity was presented in the 4.9% of 5568 T2DM patients (Maioli et al. 2010).

Action LADA 7, the largest European study to date, found that LADA was not rare, as it was reported in 9.7% of a large cohort of 6000 adult-onset diabetic patients diagnosed between 30 and 70 years of age attending primary and secondary care European centers (Hawa et al. 2013). Another study reported that the prevalence of GADA positivity is 3.7% in a Spanish T2DM patients aged 18–65 years (Soriguer-Escofet et al. 2002). There might be a north-south gradient with a lower prevalence in southern than northern Europe.

Non-European Populations

In populations outside Europe, the highest prevalence of LADA was found in Indonesia up to 20% (Sutanegara and Budhiarta 2000) while the lowest rate in Alaska (Mohatt et al. 2002) and Papua Nuova Guinea (Dowse et al. 1994) indicating the low autoimmune component in these ethnic groups. The prevalence of LADA has been shown to vary even within the same country. In Chinese T2DM patients, 16% were found to be GADA positive, and the frequency of positivity was reportedly not associated with the duration of the disease (Thai et al. 1997). Two more recent clinical studies performed in China have shown that the estimated prevalence of LADA in T2DM patients was around 7% (Li et al. 2005a; Zhou et al. 2009). In China as well, GADA positivity was prevalent in hospital-based adult onset diabetic patients from the Hunan province (7.1%) (Li et al. 2005b) and in a local small population-based study in Tianjin (9.2%) (Qi et al. 2011). In a large multicenter study, the LADA China Study, across a great group of centers (25 cities and 46 hospitals) throughout China, that evaluated 4880 subjects with diabetes diagnosed at age >30, LADA was diagnosed in 5.9% of these patients based on GADA positivity lower in the south compared to the north (Zhou et al. 2013). Interestingly, in China, where childhood-onset T1DM is rare, the frequency of LADA was found to be comparable with that in Europe or even surprisingly higher than in some European countries. Moreover, in the UKPDS Study, GADA frequency decreased with age >30 years, while in China GADA frequency showed no such age affect.

Reports from Korea indicated a prevalence of LADA between 5.1% and 5.3% in population based studies (Rho et al. 2013; Lee et al. 2009). The Korea National Diabetes Program (KNDP), which used population-based data, reported that the

frequency of LADA in Korea was approximately 4.4% (Park et al. 2011). In a large Japanese hospital-based study of 4980 diabetic patients with age at onset >20 years, GADA positivity was found in 3.8% (Takeda et al. 2002). In a study performed in Ghana population, the prevalence of LADA was found to be 14% (Adeleye et al. 2012). A Chinese report also noted that the prevalence of LADA slowly increased with age up to 60 years and was high in individuals aged 50–59 years (Qi et al. 2011).

These observations suggest that LADA increases with increasing age decade, confirming reports by Carlsson et al. that older age was an important risk factor for LADA (Carlsson et al. 2007a).

In other racial groups, such as African-Americans and Hispanics, the prevalence of LADA is lower than in white people (Barinas-Mitchell et al. 2004). In northern Indian population GAD antibodies were present in 1.5% of T2DM patients (Sachan et al. 2015); the frequency of either GAD or IA2 antibodies was similar in people with and without diabetes (3.2% vs. 2.1%).

Very recently, a cross-sectional study including 17,000 subjects with adult onset (>30 years) performed in the United Arab Emirates, an affluent Gulf state with one of the highest comparative prevalence figures of diabetes worldwide, showed a prevalence of LADA approximately of 2.6% (Maddaloni et al. 2015).

In the general population, the overall prevalence of LADA is about 0.15–0.25 in all studies, a comparable frequency if not higher than of typical T1DM. Regarding the incidence of LADA, there are only few reports indicating about 10 per year 100,000 people (Szepietowska et al. 2012).

Why There Are Such Differences in the Estimated Prevalence?

Differences in the prevalence of LADA worldwide can be ascribed to study design and inclusion criteria such as: age at diagnosis, gender, mode of ascertainment, and ethnicity. Epidemiological studies have shown conflicting results on the differences in LADA prevalence in terms of gender among people with T2DM. One study showed a male predominance of LADA, whereas other studies found a higher prevalence among women (Falorni and Brozzetti 2005; Genovese et al. 2006).

The low prevalence of LADA reported in some ethnic groups may reflect a reduced genetic predisposition to islet autoimmunity in these populations. A number of other factors contribute to the observed differences in LADA prevalence such as: number of autoantibodies tested, risk of false positivity for autoantibodies, environmental factors, and the numbers of patients progressing to insulin treatment. Although several diabetes associated autoantibodies, GADA, ICA, IA-2, and more recently identified Zinc Transporter 8 (Znt8), can be used for LADA diagnosis, GADA is the most sensitive and prevalent one (Lampasona et al. 2010). Some studies reported that differently from T1DM, in which circulating antibodies often disappear after diagnosis, the prevalence of GADA in LADA patients is similar irrespective of duration of diabetes, which has been taken as evidence for persistence of antibodies. However, these results were not confirmed by the HUNT Study, in

which 41% of LADA patients seroconverted to antibody-negative status during a 10-year follow-up (Sørgjerd et al. 2012).

The differences in estimates prevalence are, also, due to the varying sensitivity and specificity of antibody assays and cut-off point for levels of antibody positivity used. The increasing prevalence of T2DM in some populations could also influence the prevalence of LADA. In the same manner that overall diabetes prevalence by nation remains hard to precisely quantify, in that many rates are based on isolated studies in selected areas of the country using different methodologies, a quite widely differing prevalence of LADA is observed.

Of note, the mode of subject selection is probably one of the most determinants of LADA prevalence estimated in a given study. It would appear that studies based on hospital settings or specialized clinics are more likely to observe a higher prevalence of LADA than are community- or population-based studies. This is probably due to the fact that patients who are likely to need insulin are more frequently referred to secondary care than are those who do not seem to need insulin.

In conclusion, the lack of uniform criteria in the selection of LADA patients contributes to the wide variety in the different estimated prevalence of LADA worldwide.

Pathogenesis

A major question still to be clarified is whether LADA is a distinct form of autoimmune diabetes or just a part of a disease continuum with a similar disease pathogenesis as T1DM.

In the pancreas of T1DM patients, the immune system selectively destroys beta-cells in a process known as insulitis (Campbell-Thompson et al. 2016). Pancreatic biopsy showed that lymphocyte infiltration causes beta-cell destruction in T1DM. Histological analysis of pancreatic biopsy from a Japanese patient diagnosed with T2DM who had GADA and residual beta-cell function showed that T cell insulitis occurs also in LADA (Shimada et al. 1999). An infiltrate of predominantly CD4+ T cells was found in islets which remarkably still contained insulin. The proportion of beta-cells compared to non-beta-cells in this patient's islets was not different to that observed in an age-matched control subject, indicating that beta-cell mass had been preserved. Following this report, another study demonstrated that peripheral blood mononuclear cell (PBMC) responses to GAD65 occurred in patients with LADA (Brooks-Worrell et al. 1999). These findings show that the pathological hallmark of T1DM, insulitis, is also present in LADA, but less pronounced, which protects beta-cells from extensive T cell destruction, at least initially. Thus, both T1DM and LADA are considered to be beta-cell-mediated autoimmune disease direct against the beta-cell. Though both T1DM and LADA are autoimmune, there are apparent similarities and differences regarding antibody and T cell levels.

Recently, pancreatic scintigraphy with interleukin-2 radiolabeled with ^{99m}Tc (99mTc-IL-2) was used to detect in vivo the presence of activated lymphocytes in the pancreas of T1DM and LADA patients (Signore et al. 2015). ^{99m}Tc-IL-2 was

accumulated in the pancreas of approximately 50% of T1DM patients at the time of clinical diagnosis and in 66.6% of patients with LADA (a sign of the presence of activated mononuclear cells), particularly if they are diagnosed within 1 year from the first episode of hyperglycemia. The differences in the percentage of positivity could be related to different stages of the disease. All T1DM patients were insulin dependent, and in some of residual inflammation in pancreatic beta-cells was not present (Kolb 1997). Patients with LADA were all newly diagnosed and three of them were noninsulin dependent at the time of study; therefore, they can be considered as being in a preclinical phase of the disease in which we expect to find insulitis. Different degrees of radiolabeled IL-2 uptake in the pancreas have already been reported in newly diagnosed T1DM patients, concluding that it may reflect a different pattern of infiltration at the time of diagnosis (Signore et al. 1990). Thus, because it is able to identify the degree of lymphocytic infiltration, IL-2 could be used as a marker to highlight the relationship between the autoimmune process and progression of the disease in patients with LADA.

In a healthy individual, immunological tolerance is maintained by multiple central and peripheral mechanisms including the action of a specialized set of regulatory T cells characterized by the expression of CD4 and CD25 (CD4CD25FOXP3 Treg) (Tree et al. 2006). It has been suggested that a defect in this cell population, either numerically or functionally, could contribute to the development of autoimmune diseases, such as T1DM. Yang et al. in their study of lymphocyte subsets showed that CD4 regulatory T cells are reduced and the expression of FOXP3 mRNA in CD4 T cell was decreased in LADA patients (Yang et al. 2007).

Humoral Autoimmunity

The presence of autoantibodies along with islet-reactive T cells in both LADA and classic T1DM provides further evidences that the underlying disease process is autoimmunity. However, differences in autoantibodies between LADA and T1DM suggest potential immune differences. GAD, IA-2, ICA, IAA, and ZnT8 autoantibodies are common in classic autoimmune diabetes; many T1DM patients are also positive for multiple autoantibodies (Wenzlau et al. 2008). Thus, antibody clustering is a characteristic feature of classic childhood T1DM. The UKPDS Study reported that in a large cohort of patients diagnosed with T2DM, GADA at onset was the most prevalent marker, followed by ICA, whereas IA-2A was relatively uncommon (Turner et al. 1997). After diagnosis of LADA, autoantibodies tend to disappear, especially IA–2A and ZnT8A. Instead, GADA appears early and can still be detected in peripheral blood for a long time (until 12 years post diagnosis) (Hawa et al. 2014).

Thus, GADA has become the main islet autoantibody for LADA screening and the most sensitive autoimmune marker for LADA diagnosis. It has been found that the IgG4-subclass of GADA was more prevalent in LADA patients than in adult patients with T1DM, with IgG4 found in about 30% of the former, but being absent in T1DM patients (Hillman et al. 2004). However, when IgG4 was found, it was

always coexpressed with IgG1-subclass. More than 90% of T1DM patients' sera bound to the middle or COOH-terminal portion of GAD65; similar binding was seen in only 65% of sera from LADA patients. In contrast, the NH2-terminal portion of GAD65 was recognized by 20% of LADA patients compared with 5% of T1DM patients (Hampe et al. 2002). In T1DM, GAD65 antibodies are initially generated against the middle and C-terminal regions of GAD65. Dynamic changes in the GAD65Ab epitope recognition have been found in a group of healthy schoolchildren at high risk for the development of T1DM (Schlosser et al. 2005). In genetically predisposed subjects, the autoimmune response may undergo intramolecular epitope spreading toward epitopes on the N-terminus and further epitopes located in the middle. Hampe et al. saw that the GAD65Ab epitope specificities in the LADA prediabetic period change dynamically as in T1DM, with an increase of the number of recognized epitopes. Notwithstanding the epitope spreading did not go toward epitopes on the N-terminus but toward the C-terminus. These differences in intramolecular epitope spreading may suggest different intensities of the underlying autoimmunity in these two populations (Hampe et al. 2007).

In LADA patients, the presence of GAD65Ab directed toward COOH-terminal epitopes of the autoantigen (GAD65-CAb) identifies a subgroup with clinical characteristics similar to those of typical T1DM and at very high risk of progression toward insulin dependency. On the other hand, the exclusive presence of GAD65Ab directed to middle epitopes of the autoantigen characterizes LADA patients with clinical characteristics almost indistinguishable from those of GAD65Ab-negative T2DM patients (Falorni and Calcinaro 2002).

IA-2 is one of the major autoantigens in T1DM, a target of both humoral and T cell reactivity (Bonifacio et al. 1995). IA-2As have also been detected in small percentages of T2DM patients but only in a few cases in addition to GADAs. IA-2A presence, in addition to GADA, increases the risk of LADA patients to require future insulin therapy (Bottazzo et al. 2005). With respect to IA-2 autoantibodies, distinct constructs of the IA-2 were shown indeed to account for different immunoreactivities in LADA (Tiberti et al. 2008). The IA-2IC$_{(605–979)}$ construct showed the highest sensitivity when used to evaluate IA-2 immunoreactivity in patients with newly diagnosed T1DM, whereas this construct was less frequent than the IA-2$_{(256–760)}$ fragment in T2DM patients. IA-2$_{(256–760)}$, an IA-2 construct lacking the COOH terminal portion of the protein, were detected in ~30% of GADA positive patients, and interestingly, in 3.4% of GADA and of IA-2$_{IC}$ antibody-negative patients, thus identifying a subset of T2DM subjects with signs of ongoing islet autoimmunity. This new construct may represent a new sensitive marker and novel diagnostic tool for the detection of islet autoimmunity in T2DM subjects (Buzzetti et al. 2015a).

B Cell Autoimmunity

Although B cells are generally acknowledged for their function in humoral immune response by producing antibodies, they also have been demonstrated to play an immunoregulatory role in the prevention of autoimmune disease.

Some studies have shown that marginal zone B (MZB) cells and follicular B (FoB) cells can activate CD4+ T cells and facilitate their proliferation by serving as important antigen-presenting cells, especially as antigen-specific antigen-presenting cells. In a recent study, T1DM patients and LADA showed an increased frequency of MZB cells but decreased frequency of FoB cells compared to controls and T2DM. These findings suggest that B lymphocytes may be involved in loss of self-tolerance and beta-cell destruction both in T1DM and LADA (Deng et al. 2016).

T Cell Autoimmunity

Brooks-Worrell et al. compared the PBMC reactivity to islet proteins (by cellular immunoblotting) between T1DM and LADA (Brooks-Worrell et al. 1999). The overall magnitude of the cellular response was found to be lower in LADA compared with T1DM. Although an overlap in reactivity to certain proteins was observed (defined by molecular weight only), some reactivity clearly differed in magnitude between LADA and T1DM. The authors suggested that these differences could reflect those of the pathogenic processes that lead to the different courses of disease in these two forms of autoimmune diabetes. Determining whether LADA can be characterized by T cells that can react to specific antigens or epitopes in a distinct manner to T1DM awaits improvements in current T cell assay technology. Seissler et al. showed that differences in ICA subspecificities (identified by blocking experiments) exist between T1DM and LADA (Seissler et al. 1998). They found that preincubation of sera with a mixture of GAD 65 and IA-2 completely blocked ICA staining in 60% of T1DM patients. However, when analyzing sera of LADA patients, only 37.5% inhibited the total ICA reactivity following preincubation with these autoantigens. This suggested that in LADA, the majority of ICA could be directed to an uncharacterized target antigen. A higher frequency of single autoantibody positivity versus >2 autoantibodies is seen in LADA compared with T1DM patients (Hosszufalusi et al. 2003; Seissler et al. 1998). In view of the fact that multiple autoantibody positivity is more common in T1DM, some have hypothesized that these patients lose tolerance to a greater number of islet antigens than do LADA patients, resulting in a more aggressive autoimmune attack.

GADA Titer

Simultaneous presence of multiple antibodies and high GADA titer compared with single and low GADA titer was associated with an early age of onset, low fasting C-peptide values, and high predictive value for future insulin requirement. Some studies highlighted that GADA titer are useful to categorize LADA patients in two different distinct groups with characteristic clinical picture, autoimmune features, and genetic signature. In NIRAD Study, analysis of GADA titer was independent of diabetes duration and showed a bimodal distribution. Consistent with this observation, LADA patients were divided into subgroups representing the two distributions,

namely, low (taken to be ≤32 U) and high (>32 U) GADA titers (Buzzetti et al. 2007) . Patients with high GADA titers with phenotypic similarities to T1DM had more prominent characteristics of insulin deficiency and a profile of more severe and extended autoimmunity. Compared with those with low GADA titers, patients with high GADA titers had higher HbA1C and significantly lower BMI, total cholesterol, and triglycerides (Table 3).

Compared with T2DM patients, differences in age of diagnosis, BMI, waist circumference, fasting glucose, HbA1c, and uric acid were more pronounced in patients with high GADA titers than with low GADA titers. On the other hand, in patients with low GADA titers, total cholesterol and triglycerides were similar to those in T2DM patients (Table 3).

High GADA titer patients showed a higher frequency of diabetes-specific antibodies (IA-2_{IC}, IA-$2_{256–760}$ and ZnT8) compared to low GADA titer and T2DM. High GADA titer was associated, also, with a higher frequency of other organ-specific antibodies. Subjects with high GADA titer compared with low GADA titer subjects showed a significantly higher prevalence of thyroid peroxidase (TPO) and antiparietal cell (APC) antibodies. Subjects with high GADA titer, compared with T2DM, showed a significantly higher prevalence of TPO, tTG, and APC antibodies. Antibodies to steroid 21-hydroxylases (21-OH) showed a prevalence of 3.4% (4 of 116) in high GADA titer and were not present either in low GADA titer or in T2DM (Zampetti et al. 2012) (Fig. 1).

Moreover, it was observed during a follow-up of 7 years that the progression to insulin requirement in LADA patients was significantly higher and, above all, occur sooner in high GADA titer subjects compared with those with low GADA titer (Zampetti et al. 2014). These findings provided novel insights into the heterogeneity of LADA: the bimodal distribution of GADA titers has allowed to identify a first

Table 3 Clinical characteristics of LADA patients with high or low GADA titer and T2DM

	High GADA titer	Low GADA titer	T2DM	*p* for trend
n (male/female)	**49/45**	**50/47**	**2100/1947**	
Age of diagnosis (years)	49.1 ± 12.29	51.5 ± 13.13	55.6 ± 10.81	<0.001
HbA1c (%)	7.8 ± 1.7	7.2 ± 1.8	6.8 ± 1.6	<0.001
BMI (kg/m^2)	26.29 ± 5.16	28.43 ± 5.01	29.9 ± 5.4	<0.001
Waist circumference (cm)	92.86 ± 12.6	96.37 ± 13.37	101 ± 13.29	<0.001
Fasting glucose (mg/dl)	170.4 ± 63.4	166 ± 53	149.38 ± 44.47	<0.001
Triglycerides (mg/dl)	116 ± 106	171 ± 102	161 ± 119	
HDL (mg/dl)	49.7 ± 14.4	50.5 ± 12.3	47.7 ± 12.5	
Total cholesterol (mg/dl)	186 ± 44.8	207 ± 47	209 ± 43.3	
Uric acid (mg/dl)	4.38 ± 1.71	4.62 ± 1.16	5.13 ± 1.44	<0.001

Adapted from Buzzetti et al. (2007)
Data are expressed as media and SD
All comparisons are adjusted for age of recruitment, duration of disease, gender, and therapy

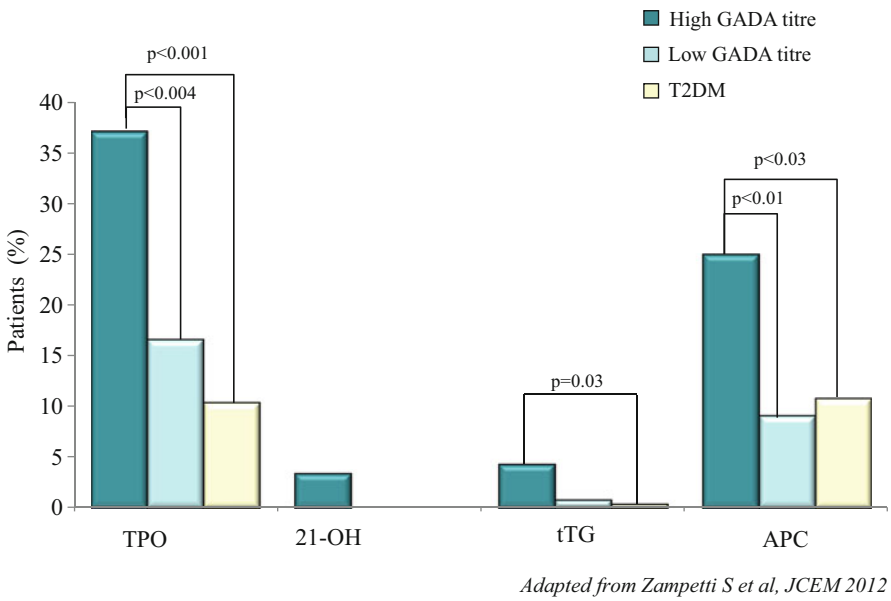


Fig 1 Frequency of organ-specific autoantibodies in LADA subdivided according to GADA titer

group of patients with a high GADA titer similar to T1DM, in which the autoimmune process is presumably strong enough to induce diabetes with no major contribution by other concomitant factors and a second group with low GADA titers more similar to T2DM, reflecting a less intense autoimmune process, with associated features of insulin resistance.

Low Grade Inflammation

The proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), and the anti-inflammatory IL-1 receptor antagonist (IL-1RA) and IL-10 were increased in T2DM patients while levels in patients with LADA and T1DM were similar (Pham et al. 2011).

A Chinese study detected some differences in C-reactive protein and adiponectin comparing T1DM, LADA, and T2DM with the majority of immune mediators similar in T1DM and LADA (Xiang et al. 2011).

Systemic concentrations of adhesion molecules (sE-selectin, sICAM-1, and sVCAM-1), related to cardiovascular risk factors, were higher in T2DM patients, while they are similar between LADA and T1DM (Pham et al. 2012).

It is also becoming increasingly evident that many factors that are involved in the T1DM specific process are also integral to the beta-cell lesion in T2DM, including IL-1, Fas, nuclear factor-B, and increased expression of c-Myc. Moreover, it also has been shown a macrophage infiltration in islets of T2DM subjects (Cnop et al. 2005).

The mechanisms leading to cytokine-induced beta-cell dysfunction in T1DM and to nutrient-induced beta-cell dysfunction in T2DM may share common final pathways, including IL-1 signaling (Donath et al. 2008). Autoimmune aspects in T2DM are not solely restricted to autoantibodies; they include the self-reactive T cells or defects in regulatory T cells (Tregs), which have been detected in autoantibody-negative T2DM patients (Itariu and Stulnig 2014). One contributor to the autoimmune activation in T2DM seems to be the chronic inflammatory state, characteristic of this disease. Upon inflammation-induced tissue destruction, cryptic "self" antigens can trigger an autoimmune response, which in turn accelerates beta-cell death. Both innate and adaptive immune system components, specifically macrophages and self-reactive T cells, contribute to an increased secretion of inflammatory cytokines involved in inflammatory and autoimmune processes. Obesity and insulin resistance have been considered as part of the mechanisms responsible in the progression of beta-cell autoimmunity; this issue is raised by the fact that LADA patients have residual beta-cell activity and it seems to be compromised as weight increase, very similar to what Wilkin proposed in his accelerator hypothesis for T1DM. Wilkin first introduced the hypothesis that obesity and weight gain are crucial for inducing beta-cell apoptosis (Wilkin 2001).

The accelerator hypothesis is a singular, unifying concept that argues that T1DM and T2DM are the same disorder of insulin resistance, set against different genetic backgrounds. The hypothesis does not deny the role of autoimmunity, only its primacy in the process. It distinguishes T1DM and T2DM only by tempo, the faster tempo reflecting the more susceptible genotype and (inevitably) earlier presentation. Insulin resistance is closely related to the rise in overweight and obesity, a trend that the hypothesis seems central to the rising incidence of all diabetes in the developed and developing world. Rather than overlap between the two types of diabetes, the accelerator hypothesis envisages overlay each a subset of the general population differing from each other only by genotype. Indeed, the "accelerator hypothesis" suggests that although on different genetic backgrounds, T1DM and T2DM are basically one and the same disorder distinguished only by the rate of beta-cell destruction and the causal factors ("accelerators") leading to beta-cell loss such as high intrinsic rate of apoptosis, insulin resistance, and autoimmunity, which act in various degrees in different individuals. From this perspective, diabetes does not differ in type, but varies in tempo. A separate classification for LADA becomes unnecessary; LADA simply occupies the middle ground of a causal spectrum defined by the relative contributions to beta-cell loss of two accelerators, metabolic (insulin demand, the driver) at one end and genetic (immune response, the modulator) at the other (Wilkin et al. 2016).

Risk Factors for LADA

Results from HUNT study suggest that traditional risk factors such as family history of diabetes and obesity are strong risk factors for LADA as for T2DM (Carlsson et al. 2007a, b). Recent findings also suggest an increased risk linked to poor

psychosocial well-being and sleeping problems (Olsson et al. 2012). On the other hand, a protective effect seems to be conferred by physical activity and, also in correspondence with T2DM, by moderate alcohol consumption. Risk factors for LADA thus include factors known to affect insulin sensitivity. These findings fit with other data indicating that LADA is characterized by insulin resistance. LADA thus shares features of T2DM despite its coupling to type 1-like autoimmunity. This highlights the interactions that are bound to take place between insulin resistance and faulty insulin secretion (for LADA due to autoimmunity) in the process of developing diabetes. There are however discrepancies; smoking is associated with a reduced risk of LADA, possibly through an inhibitory effect of nicotine on autoimmunity. The risk of LADA is also twice as high for people with high socioeconomic status, while T2DM, in this and many previous studies is more common for people with low socioeconomic status. Similar results have been reported previously for classical T1DM, which was more prevalent in children of high-income families. The increased risk which we observed was not explained by traditional risk factors such as family history, obesity, and smoking, but may result from other environmental factors that differ between socioeconomic groups. High education was also associated with higher levels of GADA. This suggests that environmental factors linked to development of autoimmunity may explain the excess risk associated with high education. Proposed explanations for this finding includes "the hygiene hypothesis" which assigns importance to a lower prevalence of infections early in life, or differences in dietary pattern habits, that may affect an autoimmune process. Unfortunately, there are limited data on early infections and diet in HUNT Study, and additional studies are therefore needed to further test the hypothesis (Carlsson et al. 2013).

Genetics

One way to shed light on the classification of LADA would be to determine to what extent LADA shares genetic similarities with T1DM and T2DM. There is some support for the view that LADA shares susceptibility genes with T1DM, but there are only a limited number of reports, which have been a sufficient sample size to address this issue.

HLA Genes

T1DM is genetically determined as shown by family, twin, and genetic studies, and the disease is more frequent in siblings of diabetic patients than in the general population (0.4% by the age of 30 years), with the concordance rate being higher in identical than nonidentical twins (Field 2002).

The HLA gene region, localized in the region on 6p21 chromosome, is the major susceptibility locus in T1DM, accounting for 42% of the total familial risk; primary susceptibility is conferred by the HLA-DRB1 and HLA-DQB1 genes, and the

highest risk is from DRB1*04-DQB1*0302 and DRB1*0301-DQB1*0201 haplotypes, present in ~90% of T1DM patients compared to 20% of the general population. HLA-DR2 haplotype is also thought to have some protective values (Atkinson and Eisenbarth 2001).

Several studies have reported an association of T1DM with high risk genes, HLA-DR3, HLA-DR4, and their alleles DQB1*0302 and DQB1*0201 HLA (Buzzetti et al. 2004). The prevalence of these genes has been linked with age at onset of diabetes. It has been observed that there is a decreased presence of these alleles in adult patients diagnosed with T1DM as compared to younger onset T1DM (Leslie and Delli Castelli 2004). These HLA genes have also been implicated in the susceptibility to LADA. Several studies have shown increased frequencies of T1DM associated high-risk HLA genotypes in patients with LADA, thereby concluding that LADA represents a subgroup of T1DM (Desai et al. 2006, 2007; Turner et al. 1997). In two large European studies on LADA, the highest risk HLA haplotypes (DRB1*04-DQB1*0302 and DRB1*0301-DQB1*0201) for T1DM were more prevalent in LADA patients than in control subjects, consistent with the known genetic predisposition to islet autoimmunity (Buzzetti et al. 2007; Tuomi et al. 1999). The frequency of high risk genotype (DR3-DQB1*0201/DR4-DQB1*0302) in LADA patients was higher compared to T2DM and controls but lower when compared to T1DM patients.

Some studies prove that HLA-DR3/DR4 alleles are more frequent in T1DM than in LADA; others suggest that occurrence of alleles DR3, DR4, and DQB1*0201 and 0302 is similar to the T1DM patients. In general, the frequency of high risk haplotypes as well as significance levels was more pronounced for T1DM than for LADA, although differences were attenuated when comparing late age-at-onset T1DM and LADA (Pettersen et al. 2010). UKPDS showed that the prevalence of DR3/DR4-DQB1*0302 was found to decrease as the age at diagnosis increased (Horton et al. 1999).

It could be hypothesized that the architecture of HLA-conferred susceptibility to LADA is similar to that observed in T1DM, although individual effect sizes may differ (Desai et al. 2007).

The DQB1*0602 protective allele, which was rarely detected in T1DM, was more frequently found in LADA patients. It also has been suggested that the protective mechanism of DR2-DQB1*0602 in adult autoimmune diabetes (LADA) is less effective (Vatay et al. 2002).

In Chinese population, as for Europeans, the frequency of diabetes-susceptibility haplotypes was significantly higher in LADA (63.9%) than in both T2DM (47.1%) and control (43.2%) subjects, while the frequency of diabetes-protective haplotypes in LADA (22.8%) was significantly lower than in both T2DM patients (33.3%) and controls (32.7%) (Zhou et al. 2013).

The HLA DRB1 and DQB1 association is dependent on the strength of positivity for GADA, since patients with LADA and high GADA titer had risk genotypes more often and protective genotypes less often than did those with low GADA titer.

In NIRAD study, there was an increasing linear trend in the frequency of high/moderate HLA risk genotypes from T2DM patients to patients with low GADA titer and to high GADA titer (Buzzetti et al. 2007). Patients with high GADA titer displayed the

highest frequency of DRB1*03-DQB1*0201 (50%) compared with those with low GADA titer (26.8%, $p < 0.001$) and T2DM (Fig. 2). The DQB1*0602 protective allele, also, showed a linear trend but with the highest frequency in T2DM patients and decreasing in those with low GADA titer and then in those with high GADA titer.

Genes Outside HLA

CTLA-4

Some non-HLA genes have also been linked to LADA. The cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a costimulatory molecule, which is located on chromosome 2. It encodes a glycoprotein receptor of the immunoglobulin family expressed on the surface of activated T cells and act as an important negative regulator of T cell activation, playing a protective role in autoimmunity (Nisticò et al. 1996). A single nucleotide polymorphism of CTLA-4 rs231775 has been identified as potential risk factors contributing to the development of T1DM.

A number of studies have assessed the association between the polymorphism of CTLA-4 rs231775 and LADA in different populations (Kisand and Uibo 2012; Pettersen et al. 2010); however, the individual study may not have enough statistical power to detect a true association. In a recent meta-analysis performed in six case-control studies, with 528 cases and 2687 controls to determine the effects of the rs231775polymorphisms of CTLA4 on the LADA, it was observed individuals

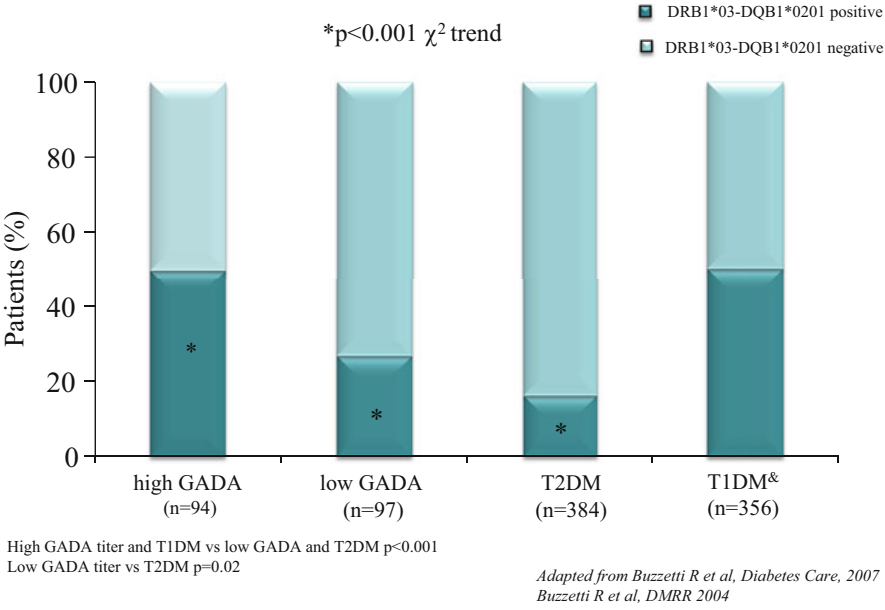


Fig. 2 Haplotype distribution of DRB1*03-DQB1*0201 in LADA patients with high and low GADA titer, T2DM, and T1DM

carrying the risk allele G in rs231775 may lead to an increasing risk of having LADA by 39% compared with allele A (Dong et al. 2014). The results suggest association in Caucasians, that is, carriage of G in the CTLA-4 rs231775 increases 45% risk relative to carriage of A allele. Asian populations showed an unrelated result; this difference may be due to the different genetic backgrounds.

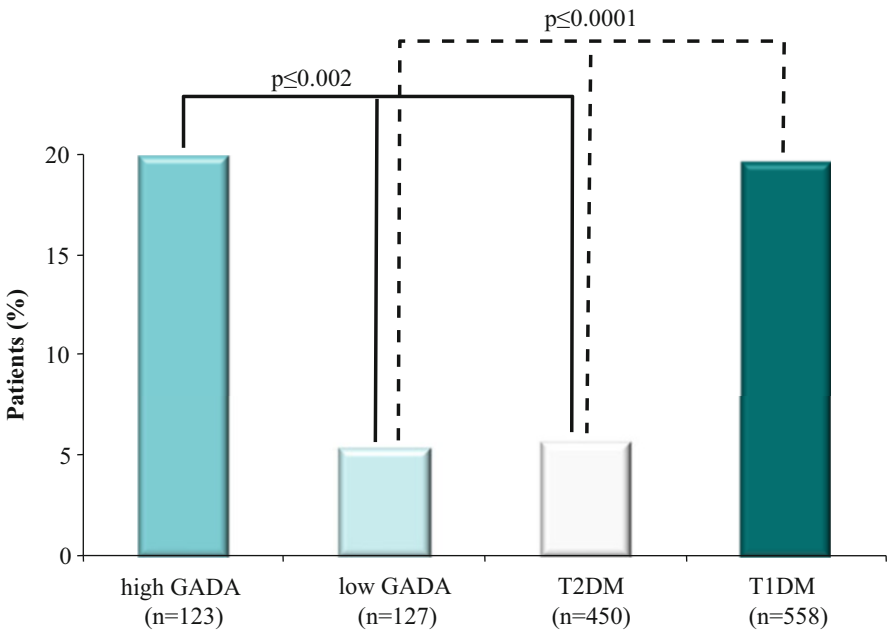
PTPN22

Protein tyrosine phosphatase non-receptor type 22 (PTPN22) encodes a lymphoid-specific phosphatase known as LYP, a powerful inhibitor of T cell activation. A missense single nucleotide polymorphism (SNP), C1858T, in the PTPN22 gene was found associated with T1DM and other autoimmune diseases (Bottini et al. 2004). Vang et al. demonstrated that the Arg620Trp variant (which corresponds to the C1858T polymorphism) is a gain of function form of the protein (Vang et al. 2005), but the mechanism by which the PTPN22 Trp20 variant exerts the disease promoting effect has yet to be established. Several studies reported that PTPN22 risk genotypes were increased in LADA patients compared to T2DM and controls, although the risk genotypes were much less common than in childhood-onset T1DM. PTPN22 has been associated with both LADA in general and with high GADA titer (Petrone et al. 2008). NIRAD study observed that the PTPN22 1858T variant in LADA patients was associated with high GADA titer only, providing evidence of genetic background to clinical heterogeneity identified by GADA titer. The frequency of 1858T carriers was significantly increased in high GADA titer group (20.3%) compared to low GADA titer (5.5%), T2DM (5.8%), and control group (9%) ($p < 0.001$ comparisons) conferring an O.R. of 2.6 (Fig. 3).

A large meta-analysis based on six case-control studies showed that individuals who carried the T minor allele in rs2476601 had 52% increased risk of developing LADA relative to those carrying C allele (Dong et al. 2014).

Insulin Gene

The insulin gene (INS) maps on chromosome 11 and regulates the expression of proinsulin in the thymus and thereby the selection of proinsulin-reactive T cells and the acquisition of immune tolerance to proinsulin. The variable number of tandem repeats (VNTR) is located 596-bp upstream of the INS translation initiation site and can be divided into two principal classes: a short class I (26–63 repeats) and a longer class III (141–209 repeats) in terms of the lengths and compositions of the repeat sequences (Ramos-Lopez et al. 2008). Short class I VNTR alleles predispose to T1DM, whereas longer class III alleles are protective. INS gene has been widely considered to influence the development of T1DM and LADA (Cervin et al. 2008; Desai et al. 2006). However, the available data are in disagreement, due to differences in research design, sample size, and population stratification. A significant association between insulin VNTR and LADA was shown by findings from the UKPDS Study (Desai et al. 2006) and a Swedish study (Cervin et al. 2008), but not in studies from Finland (Andersen et al. 2010) or Norway (Pettersen et al. 2010). The studies differed in



Adapted from Petrone A et al., Diabetes Care 2008

Fig. 3 Phenotype frequencies of the PTPN22 C1858T polymorphism in LADA patients according to GADA titre, T2DM, and T1DM

patients recruitment, as inclusion of patients with adult-onset T1DM in the LADA groups of the UK and Swedish studies could account for this difference.

A recent meta-analysis evidenced that individuals with short class I VNTR alleles are at equally increased risks of developing T1DM or LADA (Zhang et al. 2015).

Even if some studies show discrepancy possibly due to population differences, statistical power, or different criteria in patient selection, the results of all studies performed on HLA, CTLA-4, PTPN22, and INS seem to suggest a genetic susceptibility continuum in LADA extending from a marked effect in childhood-onset T1DM to a significant, but far less pronounced, effect of the same genes in LADA.

Gene Associated with T2DM

TCF7L2

In 2008, Cervin et al. first proposed that LADA is genetically an admixture of T1DM and T2DM after they had found an association between LADA and transcription factor 7-like 2 (TCF7L2) gene (10q25.3), a variant (rs7903146 C-to-T polymorphism) of which is the most common susceptibility locus for T2DM (Cervin et al. 2008). Thus, the identification of the TCF7L2 gene as the strongest candidate gene

for T2DM has opened for the possibility to test whether LADA also shares genetic features with T2DM. LADA patients showed the same increased frequency of risk genotypes in the TCF7L2 gene as T2DM patients. The mechanism by which variation in the TCF7L2 gene contributes to diabetes is unclear; however, the intestinal proglucagon gene shows binding sites for TCF7L2 and a potential mechanism could involve the incretin axis. It has been shown that carriers of the risk T allele show impaired insulin secretion, impaired incretin effect, and enhanced expression of the TCF7L2 gene in human islets. Also in previous studies, the TCF7L2 variants have been associated with impaired insulin secretion. It is thus likely that similar non-autoimmune mechanisms are operative in islets from both T2DM patients and LADA, causing impaired insulin secretion.

In Europeans, the intronic TCF7L2 rs7903146 variant is the strongest identified genetic risk factor for T2DM (Lukacs et al. 2012). Findings from several studies have shown increased frequency of the T2DM-associated rs7903146 C → T allele of TCF7L2 in patients with LADA, but the HUNT study did not replicate this finding (Pettersen et al. 2010). A meta-analysis from six European populations showed strong evidence that the TCF7L2 variant rs7903146 C-to-T polymorphism contributes to susceptibility to LADA (Lukacs et al. 2012). A north–south geographic gradient was seen in the frequency of the disease-associated T allele, both in LADA and control populations. It suggests that the non-autoimmune mechanisms have an increasing role in the pathogenesis of LADA toward the south. Despite these substantial differences in risk allele frequencies, the effect size of TCF7L2 gene on LADA risk was very similar across the populations. The absence of between study heterogeneity may indicate that the effect of TCF7L2 gene is population-independent among Europeans. In addition, a comparable magnitude of gene effect was observed between LADA and T2DM populations.

The level of GADA have a distinct effect on the genetic associations, in fact it is becoming increasingly clear that the magnitude of the association between LADA and TCF7L2 is correlated with antibody titer, where the lower it is, the stronger is the association. NIRAD Study reported that TCF7L2 common genetic variants of susceptibility are associated only with low GADA titer in LADA patients (Zampetti et al. 2010). The risk allele of the rs7903146 SNPs was increased in low GADA titer and T2DM compared with high GADA titer, T1DM, and control subjects (Fig. 4).

In agreement with the Italian NIRAD study a meta-analysis performed on Swedish LADA and Finnish LADA patients showed stronger TCF7L2 association in patients with low GADA titer (Andersen et al. 2014). This GADA-level effect was, however, driven by the Swedish data set, while the association in the Finnish data set was independent of GADA titer. This discrepancy may reflect differences in the differentiation between LADA and T1DM in Finnish and Swedish patients.

FTO

LADA, as T2DM, is associated with increased frequencies of the obesity-associated variant of FTO. Some evidence suggests that the association is stronger in low GADA titer patients (Lundgren et al. 2013; Pettersen et al. 2010).

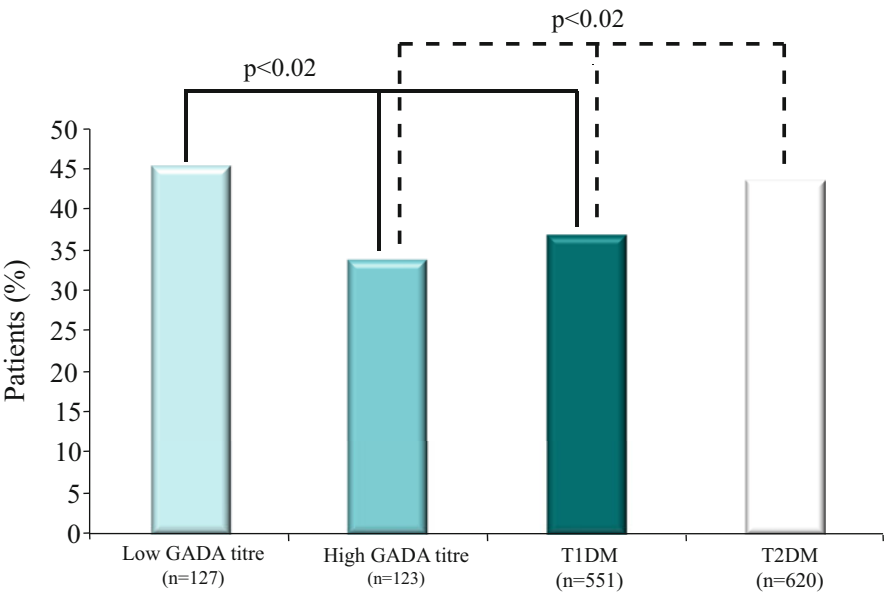


Fig. 4 Allelic frequencies of the rs7903146 of TCF7L2 gene in LADA patients according to GADA titer, T1DM, and T2DM

In conclusion, all these reported studies add evidence that LADA is associated with the same genetic features as T1DM (HLA, INS VNTR, PTPN22, and CTLA-4) and T2DM (TCF7L2 and FTO), although at lower degree. These findings, thus, suggest that LADA may represent a genetic admixture of the two types of diabetes. It remains to be clarified whether such genetic admixture represents a distinct disease syndrome or is part of an autoimmune continuum. So far all genetic studies of LADA have been candidate-gene based and have focused on T1DM and T2DM associated variants, no novel loci for this disease have been described to date, thus it is still unknown whether LADA harbors its own unique risk variants. A genome-wide association studies in a larger sample of LADA patients should be needed to answer this question.

Criteria for Diagnosis

Although LADA has been reported for about 20 years, the diagnostic criteria of this disease remain controversial. Various studies have used different inclusion criteria and markers for disease definition, and thus drawing conclusions is difficult. In the earlier stages of the disease, people affected by LADA are often wrongly diagnosed as having developed T2DM, as a result of the concomitant insulin resistance state and the absence of clinical information on GADA and other antibodies.

In the attempt to standardize the diagnosis of LADA, the Immunology of Diabetes Society (IDS) in 2004 has proposed three main following criteria: (a) adult age of onset (>30 years); (b) presence of at least one islet circulating autoantibody; and (c) insulin independence for the first 6 months after diagnosis.

However, all of these criteria have some pitfalls: criteria 1 and 3 are not categorical traits and are highly dependent on physicians' decisions, and criterion 2 is not specific for LADA.

Major Points of LADA Diagnosis Criteria

Criterion 1: Adult Age at Onset (>30 Years)

Various cutoff ages have arbitrarily been used (between 25 and 45 years), but the proposed lower limit is now 30 years of age. Nevertheless, since adulthood starts earlier in life, this limit might not be all inclusive.

Criterion 2: Presence of Circulating Islet Autoantibodies (At Least One)

The diagnosis of LADA mainly relies on sero-positivity of antibodies. There are five serum autoantibodies reflecting humoral immunity of LADA: ICA, GADA, IAA, IA-2A, and ZnT8A.

However, autoantibody criteria lack specificity because they are based on autoantibodies associated with childhood-onset T1DM, which lack 100% specificity, even in the best laboratories (Schlosser et al. 2010). Of the autoantibodies associated with LADA, GADA is the most prevalent. It is also the most sensitive marker, as it is present in early stages of the disease and has a long duration in the serum. The assay of GADA is the most standardized of all autoantibodies. The determination of GADA is useful for clinical classification of diabetes. Patients with high GADA titer are more similar to T1DM subjects, while those with low GADA titer are more similar to T2DM (Buzzetti et al. 2007). GADA titers decrease in the disease duration, but can be found positive after more than 10–20 years (Borg et al. 2002). IAA, IA-2A, and ZnT8A were also found to be alternative immune parameters for the diagnosis of LADA. Multiple autoantibodies in combination are able to improve the positivity of LADA. The radioligand assays for islet autoantibodies have the highest sensitivity and specificity among the currently established islet autoantibodies assays.

The definition of autoantibody positivity is not unequivocal and different cut-off points have been applied in different studies. The clinical significance of borderline positivity remains unsettled.

Moreover, there could be false positive results. False positives may be limited by setting a higher cut-off or by repeating positive measurements. Longitudinal studies observe changing autoantibody status over time, and even though the majority of patients are positive for only one type of autoantibody, existing autoantibodies may be lost and other autoantibodies may develop (Laugesen et al. 2015).

Some LADA patients had no serum antibodies at the time of diagnosis, but abnormal T cell function could be detected. So these have been linked to the diagnosis of LADA, which quickly becomes a hotspot. Some researchers proposed

the term "T-LADA" to define the autoantibody-negative LADA, the evidence of T cell autoreactivity can be found in them and antibody positive subjects were termed as B-LADA (Naik et al. 2009).

Criterion 3: Lack of Insulin Requirement for at Least 6 Months After Diagnosis

This criterion is used to distinguish LADA patients from those with T1DM, but reports indicate that there is a high bias in the time to insulin treatment initiation and it does not depend on disease process, but rather on physicians' clinical judgment (Brophy et al. 2008). Since that judgment is based on the presence of GADA, it follows that to define LADA on GADA positivity and the lack of initial need for insulin treatment is fraught with difficulties since the one often precludes the other. In addition, the natural history of the disease, the timing of the diagnosis in relation to it, as well as clinical features at diagnosis (e.g., presence or absence of symptoms), are factors that influence the period of insulin independence.

Asymptomatic individuals diagnosed with diabetes on the basis of raised blood glucose alone are more likely to meet the criterion of insulin independence for a minimum period than those diagnosed with diabetes after becoming symptomatic. The current diagnostic classification is therefore biased, often excluding patients who are symptomatic and/or have a delayed diagnosis of diabetes. Someone with asymptomatic undiagnosed diabetes for many months who eventually presents with symptoms is likely to be immediately commenced on insulin injections and, thus, considered to have classic T1DM. If diagnosed with diabetes earlier on the basis of blood glucose alone, this person would be insulin independent initially and, if islet antibody-positive, likely to be classified as having LADA, not classic T1DM.

Moreover, the decision to treat LADA patients with oral agents or insulin reflects also the judgment of the treating physician. Some patients with marked insulin deficiency will be treated with oral hypoglycemic agents when they should have been treated with insulin from the outset.

Conversely, some patients with adequate endogenous insulin production will be treated with insulin from the outset when they could have achieved adequate glycemic control with oral agents. Thus, an overriding factor is whether or not the treating physician is proactive with regard to insulin treatment. Instead of "insulin independence," some researchers described it as "without occurrence of ketosis or ketoacidosis in 6 months of onset of diabetes." But this classification standard is also in dispute, because under the effect of certain incentives, such as infection, LADA patients may also develop ketosis or ketoacidosis.

Although there is no consensus regarding diagnostic criteria, patients are generally designated as having adult onset autoimmune diabetes in the presence of diabetes associated autoantibodies without ketoacidosis at diagnosis, irrespective of insulin treatment (Brophy et al. 2008).

Who Has to Be Screened for LADA?

Despite the frequency of LADA, there are no universal recommendations regarding testing for islet antibodies in adult-onset diabetes. A reliable clinical strategy is

required to identify which adults with diabetes have a high likelihood of LADA and need testing for islet antibodies.

Currently, many physicians test for islet antibodies only if they suspect LADA, generally on the basis of body weight. Overweight adults with diabetes are presumed to have T2DM and are not tested, whereas normal-weight adults are considered to potentially have LADA and may be tested. However, this approach neglects the many studies in which LADA has been documented with mean BMI in the overweight or even obese category. Moreover, with increasing obesity in adults worldwide, it will become even more difficult to distinguish LADA from T2DM based on BMI.

Monge et al. also were the first to propose a clinically oriented approach for LADA screening which was based on body weight and/or BMI along with fasting blood glucose and HbA1c, and gave a prevalence of 31.8% (Monge et al. 2004). Some studies have emphasized on potential role of C-peptide in early detection of LADA patients, reserving more expensive antibody testing for high suspect cases. One such study by Aggarwal et al. showed decreased C-peptide levels in patients suspected of having LADA as compared to classic T2DM (Aggarwal et al. 2010).

More recently, a retrospective study performed in Australia identified five characteristics that are related to LADA (Fourlanos et al. 2006):

1. Manifestation of diabetes below age 50 years
2. Acute symptoms at diagnosis
3. Body mass index <25 kg/m^2
4. Positive personal history of autoimmune disease
5. Positive family history for autoimmune diseases

If two of these criteria were satisfied, specificity for diagnosis of LADA was 71%. Furthermore, the presence of less than two distinguishing clinical features (LADA clinical risk score ≥ 1) was a highly reliable method for excluding LADA (negative predictive value 99%). Nevertheless, these criteria have not yet been validated with populations outside Australia.

To date, LADA patients are easily still misdiagnosed as having T2DM, and for this reason it would be important to determine the optimum screening strategy for them.

Complications

Chronic complications associated with diabetes are also present in LADA; however, there are few data relating to chronic complications in LADA which reported inconsistent results.

Microvascular Complication

In the Botnia Study, LADA patients had a similar prevalence of retinopathy of T2DM (Isomaa et al. 1999). However, only in LADA group, HbA1c was positively

associated with retinopathy, suggesting that glycemic control was a more important risk factor in LADA than in T2DM patients in whom there was no such association. A study performed in Turkish LADA patients showed a higher prevalence of nephropathy and retinopathy compared toT2DM (Arikan et al. 2005). A recent study found that the prevalence of microvascular complications between LADA and T2DM was related to the duration of diabetes (Lu et al. 2015). When duration of diabetes was <5 years, the prevalence of diabetic nephropathy and retinopathy were significantly lower in LADA than in T2DM; the difference in the prevalence of microvascular complications between the two groups became nonsignificant when the duration of diabetes was ≥5 years (Lu et al. 2015). Of note, the prevalence of microvascular complications increased rapidly with the duration of diabetes in LADA patients.

Similar conclusions could be drawn from previous studies. Among patients with a median disease duration of 4 years, Myhill et al. reported a significantly lower prevalence of diabetic nephropathy but nonsignificantly lower prevalence of diabetic retinopathy in LADA patients compared to T2DM (Myhill et al. 2008). Two other studies reported, also, a lower prevalence of nephropathy and retinopathy, although not statistically significant, in LADA compared with T2DM (Li et al. 2003; Roh et al. 2013). Whereas in patients with a median disease duration of approximately 10 years or more, patients with LADA had a similar, or even higher, prevalence of microvascular complications compared to T2DM patients.

In addition, in a prospective study performed by the Collaborative Atorvastatin Diabetes Study (Card Study), patients with LADA had a similar frequency of microvascular diseases compared with T2DM patients (Hawa et al. 2014).

The higher prevalence of microvascular complication in T2DM at the early stage of diabetes may lie in different preclinical periods. T2DM frequently goes undiagnosed for many years as the hyperglycemia develops gradually and at earlier stages and is often not severe enough for the patient to notice any of the classic diabetes symptoms. These patients with preclinical diabetes are at increased risk of developing microvascular complications (Lu et al. 2015). Nevertheless, LADA may have a shorter preclinical phase because the rate of beta-cell destruction is more rapid in LADA than in T2DM (Lu et al. 2015).

The frequencies of retinopathy, nephropathy (microalbuminuria), and neuropathy were similar in LADA and T1DM patients diagnosed for more than 10 years (Isomaa et al. 1999). Microvascular complications in all forms of diabetes are thought to be related to the degree of hyperglycemia. Hyperglycemia exerts chronic effects on the underlying pathophysiology of microvascular complications, and intensive glycemic control was reported to reduce the incidence of microvascular complications in the UKPDS (25% decrease in microvascular complications).

Macrovascular Complications

LADA patients generally have a more favorable cardiovascular risk profile than those with T2DM. However, studies to date have not found evidence for a lower risk

of macrovascular disease in LADA patients. Macrovascular complication rates were reported to be similar in long-standing LADA and T2DM, but far lower in T1DM of similar duration (Isomaa et al. 1999). However, the T1DM patients were younger, and age is likely to be an important variable. The independent associations of hypertension, hyperlipidemia, obesity, and hyperglycemia with macrovascular disease in diabetic patients are well established. It is interesting that hypertension, hyperlipidemia, and obesity were less common in LADA than T2DM, yet the rates of macrovascular complications were similar. The prevalence of carotid plaques and cardiovascular disease were comparable between LADA patients and T2DM, regardless of the duration of diabetes (Lu et al. 2015). Possible explanations include differences in pathogenesis or treatment. Given the autoimmune pathology, LADA patients may have greater systemic inflammation, implicated in vascular pathology. LADA patients might also be suboptimally treated because they often start treatment with insulin later than is clinically indicated, due to unrecognized insulin deficiency and a reluctance to change from oral therapies to injections. They are also likely to have a shorter duration of treatment with metformin, an oral agent associated with a lower rate of ischemic heart disease in the UKPDS (1998).

Prevention

Since LADA seem to show a pathogenic process similar to T1DM but with beta-cells destruction not so rapid, it could be an ideal model for studies on total beta-cells destruction prevention (Cernea et al. 2009).

In LADA, as in T1DM, autoimmune T cells attack on insulin-producing beta-cells eventually causing hyperglycemia. The common view is that to prevent LADA, one must interfere with the autoimmune process. To avoid hazardous side effects, the intervention should not cause a generalized immune suppression but favor an immune modulation (Guglielmi et al. 2012). The autoimmune process can be mitigated inducing tolerance to autoantigens, targeting them through the administration of autoantigen and deviation of the Th1 phenotype of antigen-reactive cells toward a Th2 phenotype. Antigens that have been used so far as tolerogens in LADA are GAD65, heat shock protein (HSP), and their constituent peptides. Critical issues for a successful outcome include variables such as HLA, age at diagnosis, metabolic control, and the residual beta-cell function present at diagnosis (Spoletini et al. 2007).

Immune Modulation

DiaPep277

The basic mechanism of action of this compound is to induce tolerance to a peptide of 24 amino acids of HSP60 involved in the process of beta-cell destruction.

HSP60 is a ubiquitous protein, part of a highly conserved family of intracellular chaperones, also located in the mitochondria and mature insulin secretory granules

of pancreatic beta-cells, with relevant regulatory role in the innate immune system (Birk et al. 1996) and considered as an important autoantigen in T1DM. The dominant epitope of HSP60 was found to be the HSP277 peptide, and its modified form, Diapep277 (generated to increase its stability in vivo), has been used in patients with recent onset T1DM for prevention of further beta-cell loss (Raz et al. 2001, 2007). It activates anti-inflammatory effector cells through TLR2 leading to a shift from an inflammatory to a regulatory immune response. Patients with recent onset T1DM and basal C-peptide concentrations above 0.1 nM treated with subcutaneous injections DiaPep277 tended to preserve endogenous insulin production (at 10 months, mean C-peptide concentrations had fallen in the placebo group but were maintained in the DiaPep277 group, and need for exogenous insulin was higher in the placebo than in the DiaPep277 group) (Raz et al. 2001).

In the same trial, T cell reactivity to HSP60 and p277 in the DiaPep277 group was associated with an enhanced T-helper-2 cytokine phenotype (Raz et al. 2001). A phase II double-blind multicenter RCT was conducted in 60 patients with LADA, 30–50 years old and within 2–60 months after diagnosis for evaluation of safety, tolerability, and clinical, metabolic, and immunological efficacy of multiple subcutaneous doses of DiaPep277. Results have not been published, but a brief report suggests good safety and tolerability, and lymphocyte response to DiaPep277 in treated patients with generation of a Th2 cytokine phenotype (Pozzilli and Guglielmi 2006).

GAD65 (Diamyd)

An interesting antigen-specific immune modulatory approach to LADA is represented by the subcutaneous administration of GAD.

The 64-kD pancreatic beta-cell autoantigen, which is a target of autoantibodies associated with early as well as progressive stages of beta-cell destruction, was identified as the gamma-aminobutyric acid-synthesizing enzyme glutamic acid decarboxylase. GAD65 is mainly found in beta-cells and other tissues, and it is considered a major autoantigen in autoimmune diabetes. GAD65 autoantibodies are found in 70–75% of T1DM patients and are considered the most sensitive autoantibody marker in LADA (Falorni et al. 2005). In a phase II randomized, double-blind, placebo-controlled, clinical trial, subcutaneous vaccination with recombinant human GAD65 formulated with aluminum hydroxide (GAD-alum) was used to determine whether this intervention was safe and can improve beta-cell function in GADA positive LADA patients (Agardh et al. 2005). Fasting C-peptide levels at 24 weeks were increased compared with placebo in the 20 μg group. In addition, both fasting and stimulated C-peptide levels increased from baseline to 24 weeks in the 20 μg dose group. These changes were accompanied by an increase of the purported Tregs subsets (CD4-CD25/CD4-CD25 cell ratio) in the peripheral blood. No change in HbA1c or plasma glucose or decrease in beta-cell function was observed in any of the dose groups and no study-related adverse effects were reported. However, subsequent phase II/III clinical trials in recent onset T1DM patients treated with GAD-alum have shown discordant results. While the first trial showed that treatment with two doses of 20 μg GAD-alum induces tolerance to

GAD65 resulting in preservation of beta-cell insulin secretion in a subgroup of patients who were recruited within 6 months of diagnosis (Ludvigsson et al. 2008, 2011); these effects could not be reproduced by two subsequent larger clinical trials that used the same drug for intervention (Ludvigsson et al. 2012; Wherrett et al. 2011). The reasons for these discrepancies are still unclear.

Recently, Krause et al. examined GADA affinity in LADA patients participating in the GAD65 vaccination trial to evaluate whether antibody affinity was similarly restricted to high affinity (Krause et al. 2014).

It has been observed that subcutaneous injection of different GAD-alum had no effect on GADA affinity. Patients with low-affinity GADAs had increased fasting and stimulated C-peptide concentrations and lower HbA1c levels at baseline and retained relatively high fasting C-peptide concentrations over a time course of 30 months. Concordantly, all patients who started insulin treatment during this time course had high-affinity GADAs. High GADA affinity could be a marker for reduced beta-cell function in LADA patients and may improve the ability to identify single GADA-positive patients who are at highest risk of requiring insulin therapy (Krause et al. 2014).

Immune Therapy

Theoretically, immunotherapy in antibody-positive patients might prevent or modify the underlying disease process. Yet, in childhood-onset T1DM, immunological approaches have had limited success at reducing the loss of C-peptide secretion. Agents that have been shown to be of benefit include cyclosporine (an inhibitor of T cell activation), abatacept (a CTLA-4 inhibitor), Rituximab (anti-CD20), and anti-CD3 monoclonal antibodies.

Anti-CD3 Monoclonal Antibodies

The rationale of this approach is to delete T lymphocytes (T cells) in the host and thus halt pathogenesis. It is well established that islet antigen-specific T cells are among the cellular players that lead to islet beta-cell destruction and resulting T1DM. The CD3 molecule is expressed on all T cells, and once bound by a specific antibody (anti-CD3), the T cells become unresponsive or killed, and can thus no longer contribute to pancreatic damage. Because the initial antigenic repertoire as the primary target of the immune attack in autoimmune diabetes is still not well defined, considerable efforts have been devoted to nonantigenic immune interventions (Cernea et al. 2009). Although the exact mechanisms responsible for the actions of the anti-CD3 are still not fully elucidated, there are several possibilities: induction of antigenic modulation, anergy, and/or apoptosis in activated cells and immune tolerance through adaptive Tregs (Chatenoud and Bluestone 2007).

Noteworthy outcomes have been seen in two studies in new-onset T1DM using two different humanized anti-CD3, and both have reported preservation of beta-cell function with maintenance of higher endogenous insulin secretion assessed by CPR and concomitant reduction in HbA1c levels and insulin usage in the treated group

over at least 1 year (Herold et al. 2002; Keymeulen et al. 2005). This could be a possible beneficial intervention also for LADA patients, but further studies are required to confirm the feasibility of anti-CD3 therapy for this group.

Treatment

The potential best therapeutic option for LADA patients should aim not only to obtain good metabolic control but also to allow better preservation of residual beta-cell function. It has been shown that maintenance of some endogenous insulin production is associated with improved metabolic control and better long-term disease outcome.

The key question is which drug (or combination of drugs) is most effective in obtaining these goals. Unfortunately, there are no therapeutic guidelines for LADA so far, and they are currently treated as patients with T2DM.

Diet

Diet treatment in LADA is similar to that of classical T1DM. Obese LADA patients benefit from restriction in calorie consumption and increased levels of physical activity (Davis et al. 2005). Lifestyle intervention with diet and exercise improves glycemic control in T1DM and T2DM. Two studies, the Finnish Diabetes Prevention Study (Lindström et al. 2003) and the Diabetes Prevention Program Study reported that lifestyle intervention improved beta-cell function in people with impaired glucose tolerance (Knowler et al. 2002). Lifestyle intervention would presumably improve short-term glycemic control in LADA, but studies are required to determine if it preserves beta-cell function in the long term. Preventative strategies for T1DM including immune-based therapies may also be applicable to LADA. If beta-cell destruction is slowly progressive in LADA, this implies a wider therapeutic window for prevention.

Sulfonylurea

Sulfonylureas are commonly used for the treatment of T2DM and act by stimulating insulin release from the pancreatic beta-cells to lower blood glucose levels. The insulin secretion is triggered by binding of sulfonylureas to a specific site on the ATP sensitive K^+ channels at the level of plasma membrane, which leads to their closure and subsequent opening of the calcium channels and activation of an effector system of insulin release (Malaisse and Lebrun 1990). Despite their initial efficacy, there is a progressive reduction in insulin producing capacity of pancreatic beta-cells and deterioration of glycemic control over time. The cause might be exhaustion or desensitization of beta-cells by prolonged exposure to sulfonylureas and possibly acceleration of oxidative stress and apoptosis (Cernea et al. 2009). It has also been

suggested that stimulation of insulin release might be associated with increased autoantigen expression, which could be deleterious in LADA because it might accentuate the ongoing autoimmune process (Björk et al. 1992).

One medium-term (12 months) randomized control trial (RCT) compared insulin with sulfonylureas (glibenclamide) plus insulin treatment, by evaluating metabolic control (fasting blood glucose), insulin secretion (fasting C-peptide), and markers of autoimmunity (ICA and GADA) at baseline and at the end of study (Cabrera-Rode et al. 2002). After 1 year of treatment, the group receiving insulin alone had better metabolic control than the sulfonylureas plus insulin group and had also improved the markers of autoimmunity. Similarly, a study examining the effect of adding insulin to sulfonylureas (glibenclamide) and of withdrawal of sulfonylureas on glycemic control in T2DM patients seemed to support the exclusion of sulfonylureas in autoantibody-positive subjects, who were less likely to respond to it (Landstedt-Hallin et al. 1999). A long-term RCT compared conventional treatment (primarily with diet) to sulfonylureas and to insulin, and sulfonylureas with insulin. A total of 60% of the autoantibody-positive patients treated with sulfonylureas progressed to insulin requirement within 2 years compared with 15% of the autoantibody-negative patients (Davis et al. 2005). This again suggests that the use of sulfonylureas may accelerate insulin requirement when compared with conventional intervention.

There are two randomized controlled trials (RCTs) conducted in Japan comparing glibenclamide with insulin treatment in LADA patients. The first was a pilot RCT study that included ICA subjects and examining insulin alone versus glibenclamide alone (Kobayashi et al. 1996). It reported that two of five patients treated with sulfonylureas required insulin treatment within 24 months due to failure of treatment with secondary oral hypoglycemic agents. At the end of study (30 months), the sulfonylureas group had a worsening of metabolic control and showed a progressive deterioration of beta-cell function.

The second multicenter, randomized, nonblinded clinical study (the Tokyo study), which included GADA subjects, reported that the group receiving sulfonylurea therapy progressed in greater proportion to the insulin-dependent stage during 57 months of follow-up (Maruyama et al. 2008). Longitudinal analysis showed that the sum of serum C-peptide values during the oral glucose tolerance test was better preserved in the insulin group than in the sulfonylurea group.

Even though it is difficult to generalize these data because the studies had different selection criteria and ethnicity as well as different outcome parameters and follow-up durations, taken together, they do suggest that sulfonylureas accelerate (or at least do not protect against) progressive beta-cell failure and are similar to (or worse than) insulin in obtaining good metabolic control. Therefore, sulfonylureas should not be used as first-line therapy in patients with LADA.

Insulin Sensitizers, Metformin, and Thiazolidinediones

Metformin is the initial choice of drug in T2DM patients. It acts by decreasing the hepatic glucose output and sensitizing peripheral tissues to the action of insulin. Unlike

sulfonylureas, it does not cause β-cell exhaustion. Since (at least some) patients with LADA have features of metabolic syndrome and a certain degree of insulin resistance, they might benefit from therapy with an insulin-sensitizing drug (Cernea et al. 2009).

Despite its widespread use as primary treatment in T2DM, the specific role of metformin in LADA is not known since there are no controlled studies on the effects of metformin alone in patients with LADA (Cernea et al. 2009). In addition, a potential risk associated with its use is occurrence of lactic acidosis in patients that progress toward insulin dependency (Pozzilli and Di Mario 2001).

The thiazolidinediones (TZDs) are in turn a more appealing therapeutic approach. They decrease insulin resistance and enhance glucose uptake by upregulating GLUT4 channels via peroxisome proliferator activated receptor-γ. Apart from their effect on glucose homeostasis and lipid metabolism, they decrease insulin demand and β-cell exhaustion, have anti-inflammatory effects, protecting cells from oxidative stress and apoptosis, and even facilitate β-cell proliferation (Cernea et al. 2009). Data from animal models suggest that TZD administration has favorable effects on preservation and augmentation of β-cell mass through a combination of enhanced proliferation and decreased apoptosis (Finegood et al. 2001; Holloway et al. 2008). This might be significant for the clinical management of LADA in therapeutic efforts aimed at β-cell protection.

A RCT compared rosiglitazone plus insulin with insulin alone in LADA patients over a total follow-up period of 18 months (Zhou et al. 2005). Results of 17 patients at 12 months showed no significant change in HbA1C in the insulin group and a significant decrease from baseline in the rosiglitazone plus insulin group, but at 18 months, this improvement in glycemic control was no longer seen. Even though rosiglitazone plus insulin did not improve metabolic control significantly more than insulin alone, it appeared to have a beneficial effect in terms of maintaining C-peptide levels (especially stimulated C-peptide) in the long term.

In NIRAD study, insulin sensitizers maintained the insulin-free period longer than sulfonylurea. The proportion of LADA patients who required insulin therapy was significantly higher in the group treated also with sulfonylurea in the first year after diagnosis than in those treated with diet and/or insulin sensitizers alone (Zampetti et al. 2012).

Insulin

It seems somehow paradoxical to initiate early insulin treatment in LADA, since this disease is characterized by lack of insulin requirement at onset. The rationale for early insulin therapy though would be to improve glycemic control while protecting β-cells (Cernea et al. 2009). The exact mechanisms for the apparent beneficial effects of insulin treatment are yet to be fully understood, but it is thought that administration of exogenous insulin would allow beta-cells to rest and decrease insulitis at least by decreasing their metabolism and by relieving hyperglycemic stress (Argoud et al. 1987).

The consequence is a decrease in the severity of insulitis and in the number of infiltrative antigen-presenting cells in and around the pancreatic islets (Jansen et al.

1996). A number of experiments suggested that active beta-cells, producing high amounts of insulin, are more susceptible to immune-mediated killing and are also associated with higher antigen expression (Björk et al. 1992; Aaen et al. 1990). Thus, a reduction of beta-cell function and of inflammatory processes in the islets would lead to decreased antigen expression on beta-cells and subsequent reduction of T cell responses (Schloot and Eisenbarth 1995). Other possible explanations would be that exposure to exogenous insulin would actually promote Th2 immunity in humans, as indicated by an increase in IgG1 and IgG4-IA (antibodies to insulin) (although no secondary spreading to other autoantigens) and induce an activation of insulin specific regulatory T-cells (Tregs) (Füchtenbusch et al. 2000). Finally, as insulin is a major autoantigen in diabetes, it is thought that immunization with exogenous insulin would determine immune modulation possibly by tolerance induction or "bystander" suppression of autoreactive T cells through the local release of regulatory cytokines.

Some studies conducted in LADA patients have shown that insulin treatment is associated with better outcome in terms of metabolic control, insulin secretion, and autoimmune responses against pancreatic beta-cells. In two studies, patients receiving insulin monotherapy had improved markers of autoimmunity (Cabrera-Rode et al. 2002; Kobayashi et al. 1996). Two studies carried out in Japan demonstrated preservation of beta-cell function with insulin compared with sulfonylurea in ICA positive and GAD autoantibody-positive phenotypic T2DM subjects (Kobayashi et al. 2002, 2003).

Subgroup analysis suggested that patients with high GADA titers and preserved C-peptide response at baseline were less likely to progress to the insulin dependency, with early initiation of insulin. Overall, these results are encouraging because they imply that the insulin treated patients maintain better beta-cell function. The optimal insulin regimen is not clear. If rapid loss of insulin release occurs early in LADA, replacement with multiple doses of insulin might be beneficial. However, from a practical point of view, it is difficult to initiate multiple insulin injection therapy very early in LADA patients, especially if their blood glucose levels are moderately increased. Thus, a long-acting insulin injection might be a good alternative (Cernea et al. 2009).

A 3-year follow-up study has shown that early insulin treatment in LADA not only preserves the level of metabolic control, but is also safe and well tolerated (Thunander et al. 2011).

The GADA titer could be useful to determine the early intervention in LADA patients. Subjects with high GADA titer could benefit from early insulin intervention for their high risk of beta-cell failure. GADA titer decreases in the disease duration, but can be found positive after more than 10–20 years.

GLP-1 Receptor Agonists

Incretin mimetics are a new class of pharmacologic agents developed to improve metabolic control in T2DM patients. The most advanced drug of this class is

exendin-4, which acts as a full agonist at the glucagon-like peptide (GLP)-1 receptor and has glucoregulatory actions similar to the incretin hormones, as well as slows gastric emptying and reduces food intake (Gautier et al. 2005). In addition, exendin-4 has been shown in vitro and in animal models to have trophic effects on the pancreas, since it modifies the susceptibility to apoptotic injury and stimulates beta-cell proliferation and islet neogenesis from precursor cells (Xu et al. 1999). Exendin-4 has islet growth-promoting effects through regulation of genes controlling proliferation, growth, and differentiation, apparently by targeting different components of the epigenetic machinery (Ghanaat-Pour and Sjöholm 2009). It induces multiple signaling pathways intrinsic to beta-cells (including expression of Pdx-1), which results in expansion of beta-cell mass through promoting differentiation of precursor into mature beta-cells and stimulation of mature beta-cell proliferation (Song et al. 2008). Therefore, the reports of exenatide increasing the mass of beta-cells, in addition to its glucose-lowering effects, provide encouragement for its use in the treatment of LADA. There are a few studies evaluating GLP-1 (and exendin-4) in T1DM subjects, and they showed reduction of fasting hyperglycemia and glycemic excursions after a meal, accompanied by inhibition of abnormal rises of blood levels of glucagon (Dupre 2005). Additionally, in islet transplant recipients, exendin-4 has stimulated insulin secretion and demonstrated an ability to reduce exogenous insulin requirements.

DPP-4 Inhibitors

Dipeptidyl peptidase 4 (DPP-4) inhibitors are a class of oral antidiabetic agent that has been shown to preserve beta-cell function in mouse models of T2DM, T2DM patients, and even in patients with impaired fasting glucose (Zhao et al. 2014). Linagliptin, a DPP-4 inhibitor, has a direct protective effect on beta-cell function under diabetogenic conditions in vitro. Furthermore, DPP-4 inhibition reduces insulitis and stimulates beta-cell function in nonobese diabetic mouse model of autoimmune diabetes, a classic model of T1DM (Zhao et al. 2014). Three DPP-4 inhibitors (sitagliptin, linagliptin, and saxagliptin) have been studied in patients with LADA in three trials (Buzzetti et al. 2016; Johansen et al. 2014), and the other, a prospective study (Zhao et al. 2014). In the prospective study, Chinese patients with LADA were given insulin glargine and randomized to either sitagliptin or placebo. Sitagliptin-treated patients had a minimal and insignificant decline in C-peptide over 1 year, whereas the placebo-treated group had a significant decrease. The 2-h C-peptide level in the sitagliptin-treated patients was significantly higher than in the placebo-treated patients at 12 months (Zhao et al. 2014). Whether DPP-4 inhibitors alter beta-cell function, independent of their acute insulin-stimulating action, remains unknown.

A recent study performed to assess the efficacy and tolerability of saxagliptin and C-peptide secretion in LADA and T2DM patients showed that saxagliptin was effective in lowering blood glucose levels and generally well tolerated in GADA-positive patients. Interestingly, saxagliptin appears to improve β-cell function in

these patients, although a longer treatment duration may be needed to confirm this finding (Buzzetti et al. 2016).

Summary

- LADA describes a subgroup of patients who develop phenotypic T2DM but with markers of autoimmunity typical of T1DM.
- The WHO diabetes classification does not differentiate LADA as distinct entity including it in the T1DM classification.
- The prevalence of LADA in patients with apparent T2DM, which varies significantly in different ethnic groups, ranges between 4% and 14%.
- LADA is associated with the same genetic features as T1DM (HLA, PTPN22, CTLA-4, and insulin gene) and T2DM (TCF7L2 and FTO) although at lower degree, suggesting that it may represent a genetic admixture of the two types of diabetes.
- A major question still to be clarified is whether LADA is a distinct form of autoimmune diabetes or just a part of a phenotypic continuum with a similar disease pathogenesis as T1DM.
- The IDS has proposed the following criteria to standardize the definition of LADA patients: (1) $\geq$30 years of age, (2) positive for at least one of the T1DM-associated antibodies, and (3) not requiring insulin therapy within the first 6 months after diagnosis.
- GADA has become the main islet autoantibody for LADA screening and the most sensitive autoimmune marker for LADA diagnosis.
- There are a few data relating to chronic complications in LADA and reported inconsistent results.
- Since LADA seem show a pathogenic process similar to T1DM but with beta-cells destruction not so rapid, it could be an ideal model for immunotherapy/modulation studies.
- Insulin sensitizers are the first choice of therapy in LADA. The GADA titer could be useful to determine the choice of the first intervention in LADA patients. Subjects with high GADA titers could benefit from early insulin intervention for their high risk of beta-cell failure.

References

Aaen K, Rygaard J, Josefsen K, Petersen H, Brogren CH, Horn T, Buschard K. Dependence of antigen expression on functional state of beta-cells. Diabetes. 1990;39(6):697–701.

Adeleye OO, Ogbera AO, Fasanmade O, Ogunleye OO, Dada AO, Ale AO, Abatan FM. Latent autoimmune diabetes mellitus in adults (LADA) and it's characteristics in a subset of Nigerians initiallymanaged for type 2 diabetes. Int Arch Med. 2012;5(1):23.

Agardh CD, Cilio CM, Lethagen A, Lynch K, Leslie RD, Palmér M, Harris RA, Robertson JA, Lernmark A. Clinical evidence for the safety of GAD65 immunomodulation in adult onset autoimmune diabetes. J Diabetes Complicat. 2005;19(4):238–46.

Aggarwal S, Goel A, Jain A. Role of C-peptide in identification of patients suspected of having latent autoimmune diabetes in adults (LADA) in north Indian type 2 diabetes mellitus population. Int J Pharm Biosci. 2010;1:3.

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–53.

Andersen MK, Lundgren V, Turunen JA, et al. Latent autoimmune diabetes in adults differs genetically from classical T1DM diagnosed after the age of 35 years. Diabetes Care. 2010;33:2062–4.

Andersen MK, Sterner M, Forsén T, Käräjämäki A, Rolandsson O, Forsblom C, Groop PH, Lahti K, Nilsson PM, Groop L, Tuomi T. Type 2 diabetes susceptibility gene variants predispose to adult-onset autoimmune diabetes. Diabetologia. 2014;57(9):1859–68.

Argoud GM, Schade DS, Eaton RP. Insulin suppresses its own secretion in vivo. Diabetes. 1987;36 (8):959–62.

Arikan E, Sabuncu T, Ozer EM, Hatemi H. The clinical characteristics of latent autoimmune diabetes in adults and its relation with chronic complications in metabolically poor controlled Turkish patients with type 2 diabetes mellitus. J Diabetes Complicat. 2005;19:254–8.

Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet. 2001;358:221–9.

Barinas-Mitchell E, Pietropaolo S, Zhang YJ, Henderson T, Trucco M, Kuller LH, Pietropaolo M. Islet cell autoimmunity in a triethnic adult population of the Third National Health and Nutrition Examination Survey. Diabetes. 2004;53(5):1293–302.

Birk OS, Elias D, Weiss AS, Rosen A, Van-der Zee R, Walker MD, Cohen IR. NOD mouse diabetes: the ubiquitous mouse hsp60 is a beta-cell target antigen of autoimmune T cells. J Autoimmun. 1996;9(2):159–66.

Björk E, Kämpe O, Andersson A, Karlsson FA. Expression of the 64 kDa/glutamic acid decarboxylase rat islet cell autoantigen is influenced by the rate of insulin secretion. Diabetologia. 1992;35(5):490–3.

Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like (islet cell antigen 512) as the insulindependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. J Immunol. 1995;155(11):5419–26.

Borg H, Gottsäter A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in patients with adult-onset diabetes. Diabetes. 2002;51(6):1754–62.

Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet. 1974;2(7892):1279–82.

Bottazzo GF, Bosi E, Cull CA, Bonifacio E, Locatelli M, Zimmet P, Mackay IR, Holman RR. IA-2 antibody prevalence and risk assessment of early insulin requirement in subjects presenting with type 2 diabetes (UKPDS 71). Diabetologia. 2005;48(4):703–8.

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, Mac-Murray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36:337–8.

Brooks-Worrell BM, Juneja R, Minokadeh A, Greenbaum CJ, Palmer JP. Cellular immune responses to human islet proteins in antibody-positive type 2 diabetic patients. Diabetes. 1999;48(5):983–8.

Brophy S, Yderstraede K, Mauricio D, Hunter S, Hawa M, Pozzilli P, Schernthaner G, Schloot N, Buzzetti R, Davies H, Leslie D, Williams R, Action LADA Group. Time to insulin initiation cannot be used in defining latent autoimmune diabetes in adults. Diabetes Care. 2008;31 (3):439–41.

Buzzetti R, Galgani A, Petrone A, Del Buono ML, Erlich HA, Bugawan TL, Lorini R, Meschi F, Multari G, Pozzilli P, Locatelli M, Bottazzo G, Di Mario U. Genetic prediction of T1DM in a population with low frequency of HLA risk genotypes and low incidence of the disease (the DIABFIN study). Diabetes Metab Res Rev. 2004;20(2):137–43.

Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C, Capizzi M, Arpi ML, Bazzigaluppi E, Dotta F, Bosi E, Non Insulin Requiring Autoimmune Diabetes Study Group. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care. 2007;30(4):932–8.

Buzzetti R, Spoletini M, Zampetti S, Campagna G, Marandola L, Panimolle F, Dotta F, Tiberti C, NIRAD Study Group (NIRAD 8). Tyrosine phosphatase-related islet antigen 2(256–760) autoantibodies, the only marker of islet autoimmunity that increases by increasing the degree of BMI in obese subjects with type 2 diabetes. Diabetes Care. 2015a;38(3):513–20.

Buzzetti R, Pozzilli P, Frederich R, Iqbal N, Hirshberg B. Saxagliptin improves glycaemic control and C-peptide secretion in latent autoimmune diabetes in adults (LADA). Diabetes Metab Res Rev. 2016;32(3):298–96.

Cabrera-Rode E, Perich P, Diaz-Horta O, Tiberti C, Molina G, Arranz C, Martin JM, Licea M, De Leiva AD, Puig-Domingo M, Di Mario U. Slowly progressing type 1 diabetes: persistence of islet cell autoantibodies is related to glibenclamide treatment. Autoimmunity. 2002;35 (7):469–74.

Campbell-Thompson M, Fu A, Kaddis JS, Wasserfall C, Schatz DA, Pugliese A, Atkinson MA. Insulitis and β-cell mass in the natural history of type 1 diabetes. Diabetes. 2016;65(3):719–31.

Carlsson S, Midthjell K, Grill V. Influence of family history of diabetes on incidence and prevalence of latent autoimmune diabetes of the adult: results from the Nord-Trøndelag Health Study. Diabetes Care. 2007a;30(12):3040–5.

Carlsson S, Midthjell K, Tesfamarian MY, Grill V. Age, overweight and physical inactivity increase the risk of latent autoimmune diabetes in adults: results from the Nord-Trøndelag Health Study. Diabetologia. 2007b;50(1):55–8.

Carlsson S, Midthjell K, Grill V. LADA (latent autoimmune diabetes in adults) in Norway – occurrence, risk factors, treatment and complications. Nor Epidemiol. 2013;23(1):39–44.

Cernea S, Buzzetti R, Pozzilli P. Beta-cell protection and therapy for latent autoimmune diabetes in adults. Diabetes Care. 2009;32(Suppl 2):S246–52.

Cervin C, Lyssenko V, Bakhtadze E, et al. Genetic similarities between latent autoimmune diabetes in adults, type 1 diabetes, and type 2 diabetes. Diabetes. 2008;57(5):1433–7.

Chatenoud L, Bluestone J. CD3-specific antibodies: a portal to the treatment of autoimmunity. Nat Rev Immunol. 2007;7:622–32.

Cnop M, Welsh N, Jonas JC, Jörns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes. 2005;54(Suppl 2):S97–107.

Davis TM, Wright AD, Mehta ZM, Cull CA, Stratton IM, Bottazzo GF, Bosi E, Mackay IR, Holman RR. Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). Diabetologia. 2005;48(4):695–702.

Deng C, Xiang Y, Tan T, Ren Z, Cao C, Huang G, Wen L, Zhou Z. Altered peripheral B-lymphoctye subsets in T1DM and latent autoimmune diabetes in adults. Diabetes Care. 2016;39(3):434–40.

Desai M, Zeggini E, Horton VA, Owen KR, Hattersley AT, Levy JC, Hitman GA, Walker M, Holman RR, McCarthy MI, Clark A. The variable number of tandem repeats upstream of the insulin gene is a susceptibility locus for latent autoimmune diabetes in adults. Diabetes. 2006;55 (6):1890–4.

Desai M, Zeggini E, Horton VA, Owen KR, Hattersley AT, Levy JC, Walker M, Gillespie KM, Bingley PJ, Hitman GA, Holman RR, McCarthy MI, Clark A. An association analysis of the HLA gene region in latent autoimmune diabetes in adults. Diabetologia. 2007;50(1):68–73.

Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Ehses JA. Islet inflammation in type 2 diabetes. Diabetes Care. 2008;31(Suppl 2):S161–4.

Dong F, Yang G, Pan HW, Huang WH, Jing LP, Liang WK, Zhang N, Zhang BH, Wang M, Liu Y, Zhang LJ, Zhang SH, Li H, Chen C, Nie LH, Jing CX. The association of PTPN22 rs2476601 polymorphism and CTLA-4 rs231775 polymorphism with LADA risks: a systematic review and meta-analysis. Acta Diabetol. 2014;51(5):691–703.

Dowse GK, Zimmet PZ, Spark RA, Mavo B, Rowley MJ, Mackay IR. Lack of antibodies to glutamic acid decarboxylase in young adults of the high diabetes prevalence Wanigela people of Papua New Guinea. Diabetes Res Clin Pract. 1994;24(3):195–8.
Dupre J. Glycaemic effects of incretins in T1DM mellitus: a concise review, with emphasis on studies in humans. Regul Pept. 2005;128(2):149–57.
Falorni A, Brozzetti A. Diabetes-related antibodies in adult diabetic patients. Best Pract Res Clin Endocrinol Metab. 2005;19(1):119–33.
Falorni A, Calcinaro F. Autoantibody profile and epitope mapping in latent autoimmune diabetes in adults. Ann N Y Acad Sci. 2002;958:99–106.
Field LL. Genetic linkage and association studies of type 1 diabetes: challenges and rewards. Diabetologia. 2002;45:21–35.
Finegood DT, McArthur MD, Kojwang D, Thomas MJ, Topp BG, Leonard T, Buckingham RE. Beta-cell mass dynamics in Zucker diabetic fatty rats: rosiglitazone prevents the rise in net cell death. Diabetes. 2001;50(5):1021–9.
Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC. Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia. 2005;48(11):2206–12.
Fourlanos S, Perry C, Stein MS, Stankovich J, Harrison LC, Colman PG. A clinical screening tool identifies autoimmune diabetes in adults. Diabetes Care. 2006;29(5):970–5.
Füchtenbusch M, Kredel K, Bonifacio E, Schnell O, Ziegler AG. Exposure to exogenous insulin promotes IgG1 and the T-helper 2-associated IgG4 responses to insulin but not to other islet autoantigens. Diabetes. 2000;49(6):918–25.
Gale EA. Latent autoimmune diabetes in adults: a guide for the perplexed. Diabetologia. 2005;48(11):2195–9.
Gautier JF, Fetita S, Sobngwi E, Salaün-Martin C. Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with type 2 diabetes. Diabetes Metab. 2005;31(3):233–42.
Genovese S, Bazzigaluppi E, Gonçalves D, Ciucci A, Cavallo MG, Purrello F, Anello M, Rotella CM, Bardini G, Vaccaro O, Riccardi G, Travaglini P, Morenghi E, Bosi E, Pozzilli P. Clinical phenotype and β-cell autoimmunity in Italian patients with adult-onset diabetes. Eur J Endocrinol. 2006;154(3):441–7.
Ghanaat-Pour H, Sjöholm A. Gene expression regulated by pioglitazone and exenatide in normal and diabetic rat islets exposed to lipotoxicity. Diabetes Metab Res Rev. 2009;25(2):163–84.
Guglielmi C, Palermo A, Pozzilli P. Latent autoimmune diabetes in the adults (LADA) in Asia: from pathogenesis and epidemiology to therapy. Diabetes Metab Res Rev. 2012;28(Suppl 2):40–6.
Hampe CS, Kockum I, Landin-Olsson M, Törn C, Ortqvist E, Persson B, Rolandsson O, Palmer J, Lernmark A. GAD65 antibody epitope patterns of patients with type 1.5 differ from that of T1DM patients. Diabetes Care. 2002;25(8):1481–2.
Hampe CS, Hall TR, Agren A, Rolandsson O. Longitudinal changes in epitope recognition of autoantibodies against glutamate decarboxylase 65 (GAD65Ab) in prediabetic adults developing diabetes. Clin Exp Immunol. 2007;148(1):72–8.
Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R, Mauricio D, De Leiva A, Yderstraede K, Beck-Neilsen H, Tuomilehto J, Sarti C, Thivolet C, Hadden D, Hunter S, Schernthaner G, Scherbaum WA, Williams R, Brophy S, Pozzilli P, Leslie RD, Action LADA Consortium. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. Diabetes Care. 2013;36(4):908–13.
Hawa MI, Buchan AP, Ola T, Wun CC, DeMicco DA, Bao W, Betteridge DJ, Durrington PN, Fuller JH, Neil HA, Colhoun H, Leslie RD, Hitman G. LADA and CARDS: a prospective study of clinical outcome in established adult-onset autoimmune diabetes. Diabetes Care. 2014;37(6):1643–9.
Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA. Anti-CD3 monoclonal antibody in new-onset T1DM mellitus. N Engl J Med. 2002;346(22):1692–8.

Hillman M, Törn C, Thorgeirsson H, Landin-Olsson M. IgG4-subclass of glutamic acid decarboxylase antibody is more frequent in latent autoimmune diabetes in adults than in type 1 diabetes. Diabetologia. 2004;47(11):1984–9.

Holloway AC, Petrik JJ, Bruin JE, Gerstein HC. Rosiglitazone prevents diabetes by increasing beta-cell mass in an animal model of type 2 diabetes characterized by reduced beta-cell mass at birth. Diabetes Obes Metab. 2008;10(9):763–71.

Horton V, Stratton I, Bottazzo GF, Shattock M, Mackay I, Zimmet P, Manley S, Holman R, Turner R. Genetic heterogeneity of autoimmune diabetes: age of presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS 43). Diabetologia. 1999;42:608–16.

Hosszufalusi N, Vatay A, Rajczy K, Prohaszka Z, Pozsonyi E, Horvath L, Grosz A, Gero L, Madacsy L, Romics L, Karadi I, Fust G, Panczel P. Similar genetic features and different islet cell autoantibody pattern of latent autoimmune diabetes in adults (LADA) compared with adult-onset T1DM with rapid progression. Diabetes Care. 2003;26(2):452–7.

Irvine WJ, Gray RS, McCallum CJ, Duncan LJP. Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetics treated with oral hypoglycaemic agents. Lancet. 1977;1(8020):1025–7.

Isomaa B, Tuomi T, Almgren P, Groop L, Henricsson M, Sarelin M. Chronic complications in patients with slowly progressing autoimmune type 1 diabetes (LADA). Diabetes Care. 1999;22:1347–53.

Itariu BK, Stulnig TM. Autoimmune aspects of type 2 diabetes mellitus – a mini-review. Gerontology. 2014;60(3):189–96.

Jansen A, Rosmalen JG, Homo-Delarche F, Dardenne M, Drexhage HA. Effect of prophylactic insulin treatment on the number of ER-MP23+ macrophages in the pancreas of NOD mice: is the prevention of diabetes based on beta-cell rest? J Autoimmun. 1996;9(3):341–8.

Johansen OE, Boehm BO, Grill V, Torjesen PA, Bhattacharya S, Patel S, Wetzel K, Woerle HJ. C-peptide levels in latent autoimmune diabetes in adults treated with linagliptin versus glimepiride: exploratory results from a 2-year double-blind, randomized, controlled study. Diabetes Care. 2014;37(1):e11–2.

Juneja R, Palmer JP. Type 1 1/2 diabetes: myth or reality? Autoimmunity. 1999;29(1):65–83.

Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med. 2005;352(25):2598–608.

Kisand K, Uibo R. LADA and T1D in Estonian population – two different genetic risk profiles. Gene. 2012;497(2):285–91.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.

Kobayashi T, Nakanishi K, Murase T, Kosaka K. Small doses of subcutaneous insulin as a strategy for preventing slowly progressive beta-cell failure in islet cell antibody-positive patients with clinical features of NIDDM. Diabetes. 1996;45(5):622–6.

Kobayashi T, Maruyama T, Shimada A, Kasuga A, Kanatsuka A, Takei I, Tanaka S, Yokoyama J. Insulin intervention to preserve beta cells in slowly progressive insulindependent (type 1) diabetes mellitus. Ann N Y Acad Sci. 2002;958:117–30.

Kobayashi T, Shimada A, Kanatsuka A, Kasuga A, Takei I, Yokoyama J, Kobayashi T. Multicenter prevention trial of slowly progressive IDDM with small dose of insulin (the Tokyo study). Ann N Y Acad Sci. 2003;1005:362–9.

Kolb H. Benign versus destructive insulitis. Diabetes Metab Rev. 1997;13(3):139–46.

Krause S, Landherr U, Agardh CD, Hausmann S, Link K, Hansen JM, Lynch KF, Powell M, Furmaniak J, Rees-Smith B, Bonifacio E, Ziegler AG, Lernmark A, Achenbach P. GAD autoantibody affinity in adult patients with latent autoimmune diabetes, the study participants of a GAD65 vaccination trial. Diabetes Care. 2014;37(6):1675–80.

Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, Di Pietro S, Songini M, Bonicchio S, Giorgino F, Bonifacio E, Bosi E, Buzzetti R, Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study Group. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes: Non Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care. 2010;33(1): 104–8.

Landstedt-Hallin L, Arner P, Lins PE, Bolinder J, Olsen H, Groop L. The role of sulphonylurea in combination therapy assessed in a trial of sulphonylurea withdrawal. Scandinavian Insulin-Sulphonylurea Study Group Research Team. Diabet Med. 1999;16(10):827–34.

Laugesen E, Østergaard JA, Leslie RD, Danish Diabetes Academy Workshop and Workshop Speakers. Latent autoimmune diabetes of the adult: current knowledge and uncertainty. Diabet Med. 2015;32(7):843–52.

Lee SH, Kwon HS, You SJ, Ahn YB, Yoon KH, Cha BY, Lee KW, Son HY. Identifying latent autoimmune diabetes in adults in Korea: the role of C-peptide and metabolic syndrome. Diabetes Res Clin Pract. 2009;83(2):e62–5.

Leslie RD, Delli Castelli M. Age-dependent influences on the origins of autoimmune diabetes: evidence and implications. Diabetes. 2004;53(12):3033–40.

Li X, Yang L, Zhou Z, et al. Glutamic acid decarboxylase 65 autoantibody levels discriminate two subtypes of latent autoimmune diabetes in adults. Chin Med J. 2003;116(11):1728–32.

Li X, Zhou Z, Huang G, Peng J, Yan X, Yang L, Wang JP, Deng ZM. Study on the positive frequency and distribution of glutamic acid decarboxylase antibody in phenotypic type 2 diabetic patients. Chin J Epidemiol. 2005a;26(10):800–3.

Li X, Zhou Z, Huang G, Su H, Yan X, Yang L. Metabolic syndrome in adult-onset latent autoimmune diabetes. Metab Syndr Relat Disord. 2005b;3(2):174–80.

Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention Study Group. The Finnish Diabetes Prevention Study (DPS): lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care. 2003;26(12):3230–6.

Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA, Seissler J. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia. 2001;44(8):1005–10.

Lohmann T, Nietzschmann U, Kiess W. 'Lady-like': is there a latent autoimmune diabetes in the young? Diabetes Care. 2008;23(11):1707–8.

Lu J, Hou X, Zhang L, Hu C, Zhou J, Pang C, Pan X, Bao Y, Jia W. Associations between clinical characteristics and chronic complications in latent autoimmune diabetes in adults and type 2 diabetes. Diabetes Metab Res Rev. 2015;31(4):411–20.

Ludvigsson J, Faresjo M, Hjorth M, Axelsson S, Chéramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Ortqvist E, Zerhouni P, Casas R. GAD treatment and insulin secretion in recent-onset type 1 diabetes. N Engl J Med. 2008;359 (18):1909–20.

Ludvigsson J, Hjorth M, Cheramy M, Axelsson S, Pihl M, Forsander G, Nilsson NÖ, Samuelsson BO, Wood T, Aman J, Ortqvist E, Casas R. Extended evaluation of the safety and efficacy of GAD treatment of children and adolescents with recent-onset type 1 diabetes: a randomised controlled trial. Diabetologia. 2011;54(3):634–40.

Ludvigsson J, Krisky D, Casas R, et al. GAD65 antigen therapy in recently diagnosed T1DM mellitus. N Engl J Med. 2012;366(5):433–42.

Lukacs K, Hosszufalusi N, Dinya E, Bakacs M, Madacsy L, Panczel P. The type 2 diabetes-associated variant in TCF7L2 is associated with latent autoimmune diabetes in adult Europeans and the gene effect is modified by obesity: a meta-analysis and an individual study. Diabetologia. 2012;55:689–93.

Lundgren VM, Andersen MK, Isomaa B, Tuomi T. Family history of type 1 diabetes affects insulin secretion in patients with 'type 2' diabetes. Diabet Med. 2013;30:e163–9.

MacCuish AC, Irvine WJ, Barnes EW, Duncan LJP. Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet. 1974;2(7896):1529–31.

Maddaloni E, Lessan N, Al Tikriti A, Buzzetti R, Pozzilli P, Barakat MT. Latent autoimmune diabetes in adults in the United Arab Emirates: clinical features and factors related to insulin-requirement. PLoS One. 2015;10(8):e0131837.

Maioli M, Pes GM, Delitala G, Puddu L, Falorni A, Tolu F, Lampis R, Orrù V, Secchi G, Cicalò AM, Floris R, Madau GF, Pilosu RM, Whalen M, Cucca F. Number of autoantibodies and HLA genotype, more than high titers of glutamic acid decarboxylase autoantibodies, predict insulin dependence in latent autoimmune diabetes of adults. Eur J Endocrinol. 2010;163(4):541–19.

Malaisse WJ, Lebrun P. Mechanisms of sulfonylurea-induced insulin release. Diabetes Care. 1990;13(Suppl 3):9–17.

Maruyama T, Tanaka S, Shimada A, Funae O, Kasuga A, Kanatsuka A, Takei I, Yamada S, Harii N, Shimura H, Kobayashi T. Insulin intervention in slowly progressive insulin-dependent (type 1) diabetes mellitus. J Clin Endocrinol Metab. 2008;93(6):2115–21.

Mohatt J, Gilliam LK, Bekris L, Ebbesson S, Lernmark A. Type 1 diabetes-related autoantibodies are rare in Alaska native populations. Int J Circumpolar Health. 2002;61(1):21–31.

Monge L, Bruno G, Pinach S, Grassi G, Maghenzani G, Dani F. A clinically orientated approach increases the efficiency of screening for latent autoimmune diabetes in adults (LADA) in a large clinic-based cohort of patients with diabetes onset over 50 years. Diabet Med. 2004;21(5): 456–9.

Myhill P, Davis WA, Bruce DG, Mackay IR, Zimmet P, Davis TME. Chronic complications and mortality in community-based patients with latent autoimmune diabetes in adults: the Fremantle Diabetes Study. Diabet Med. 2008;25:1245–50.

Naik RG, Brooks-Worrell BM, Palmer JP. Latent autoimmune diabetes in adults. J Clin Endocrinol Metab. 2009;94(12):4635–44.

Nisticò L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Tosi R, Pozzilli P, Todd JA. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. Hum Mol Genet. 1996;5(7): 1075–80.

Olsson L, Ahlbom A, Grill V, Midthjell K, Carlsson S. Sleep disturbances and low psychological well-being are associated with an increased risk of autoimmune diabetes in adults. Results from the Nord-Trøndelag Health Study. Diabetes Res Clin Pract. 2012;98(2):302–11.

Park Y, Hong S, Park L, Woo J, Baik S, Nam M, Lee K, Kim Y, KNDP Collaboratory Group. LADA prevalence estimation and insulin dependency during follow-up. Diabetes Metab Res Rev. 2011;27(8):975–9.

Petrone A, Suraci C, Capizzi M, Giaccari A, Bosi E, Tiberti C, Cossu E, Pozzilli P, Falorni A, Buzzetti R. The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with high GAD antibody titer in latent autoimmune diabetes in adults: Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study 3. Diabetes Care. 2008;31(3):534–8.

Pettersen E, Skorpen F, Kvaloy K, Midthjell K, Grill V. Genetic heterogeneity in latent autoimmune diabetes is linked to various degrees of autoimmune activity: results from the Nord-Trondelag Health Study. Diabetes. 2010;59(1):302–10.

Pham MN, Hawa MI, Pfleger C, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, Scherbaum WA, Seissler J, Kolb H, Hunter S, Leslie RD, Schloot NC, Action LADA Study Group. Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: Action LADA 4. Diabetologia. 2011;54(7):1630–8.

Pham MN, Hawa MI, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, Scherbaum WA, Seissler J, Hunter S, Leslie RD, Kolb H, Schloot NC, Action LADA Study Group. Increased serum concentrations of adhesion molecules but not of chemokines in patients with type 2 diabetes compared with patients with type 1 diabetes and latent autoimmune diabetes in adult age: Action LADA 5. Diabet Med. 2012;29(4):470–8.

Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. Diabetes Care. 2001;24(8):1460–7.

Pozzilli P, Guglielmi C. Immunomodulation for the prevention of SPIDDM and LADA. Ann N Y Acad Sci. 2006;1079:90–8.

Qi X, Sun J, Wang J, Wang PP, Xu Z, Murphy M, Wang J, Xie Y, Xu W. Prevalence and correlates of latent autoimmune diabetes in adults in Tianjin China: a population based cross-sectional study. Diabetes Care. 2011;34(1):66–70.

Radtke MA, Midthjell K, Nilsen TI, Grill V. Heterogeneity of patients with latent autoimmune diabetes in adults: linkage to autoimmunity is apparent only in those with perceived need for insulin treatment: results from the Nord-Trondelag Health (HUNT) study. Diabetes Care. 2009;32(2):245–50.

Ramos-Lopez E, Lange B, Kahles H, Willenberg HS, Meyer G, Penna-Martinez M, Reisch N, Hahner S, Seissler J, Badenhoop K. Insulin gene polymorphisms in type 1 diabetes, Addison's disease and the polyglandular autoimmune syndrome type II. BMC Med Genet. 2008;9:65.

Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta cell function in new-onset T1DM and immune-modulation with a heatshock protein peptide (Dia-Pep277): a randomised, doubleblind, phase II trial. Lancet. 2001;358(9295):1749–53.

Raz I, Avron A, Tamir M, Metzger M, Symer L, Eldor R, Cohen IR, Elias D. Treatment of new-onset T1DM with peptide DiaPep277 is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. Diabetes Metab Res Rev. 2007;23(4):292–8.

Roh MO, Jung CH, Kim BY, Mok JO, Kim CH. The prevalence and characteristics of latent autoimmune diabetes in adults (LADA) and its relation with chronic complications in a clinical department of a university hospital in Korea. Acta Diabetol. 2013;50(2):129–34.

Sachan A, Zaidi G, Sahu RP, Agrawal S, Colman PG, Bhatia E. Low prevalence of latent autoimmune diabetes in adults in northern India. Diabet Med. 2015;32(6):810–3.

Schloot N, Eisenbarth GS. Isohormonal therapy of endocrine autoimmunity. Immunol Today. 1995;16(6):289–94.

Schlosser M, Banga JP, Madec AM, Binder KA, Strebelow M, Rjasanowski I, Wassmuth R, Gilliam LK, Luo D, Hampe CS. Dynamic changes of GAD65 autoantibody epitope specificities in individuals at risk of developing type 1 diabetes. Diabetologia. 2005;48(5):922–30.

Schlosser M, Mueller PW, Torn C, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for insulin autoantibodies. Diabetologia. 2010;53(12):2611–20.

Seissler J, de Sonnaville JJ, Morgenthaler NG, Steinbrenner H, Glawe D, Khoo-Morgenthaler UY, Lan MS, Notkins AL, Heine RJ, Scherbaum WA. Immunological heterogeneity in type 1 diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. Diabetologia. 1998;41(8):891–7.

Shimada A, Imazu Y, Moringa S, Funae O, Kasuga A, Matsuoka K. T-cell insulitis found in anti-GAD65+ diabetes with residual β-cell function: a case report (Letter). Diabetes Care. 1999;22 (4):615–7.

Signore A, Chianelli M, Ronga G, Pozzilli P, Beverley PC. In vivo labelling of activated T lymphocytes by i.v. injection of 123I-IL2 for detection of insulitis in type 1 diabetes. Prog Clin Biol Res. 1990;355:229–38.

Signore A, Capriotti G, Chianelli M, Bonanno E, Galli F, Catalano C, Quintero AM, De Toma G, Manfrini S, Pozzilli P, Action LADA Group. Detection of insulitis by pancreatic scintigraphy with 99mTc-labeled IL-2 and MRI in patients with LADA (Action LADA 10). Diabetes Care. 2015;38(4):652–8.

Song WJ, Schreiber WE, Zhong E, Liu FF, Kornfeld BD, Wondisford FE, Hussain MA. Exendin-4 stimulation of cyclin A2 in beta-cell proliferation. Diabetes. 2008;57(9):2371–81.

Sørgjerd EP, Skorpen F, Kvaløy K, Midthjell K, Grill V. Time dynamics of autoantibodies are coupled to phenotypes and add to the heterogeneity of autoimmune diabetes in adults: the HUNT study, Norway. Diabetologia. 2012;55:1310–8.

Soriguer-Escofet F, Esteva I, Rojo-Martinez G, Ruiz de Adana S, Catalá M, Merelo MJ, Aguilar M, Tinahones F, García-Almeida JM, Gómez-Zumaquero JM, Cuesta-Muñoz AL, Ortego J, Freire JM. Prevalence of latent autoimmune diabetes of adults (LADA) in Southern Spain. Diabetes Res Clin Pract. 2002;56(3):213–20.

Spoletini M, Petrone A, Zampetti S, Capizzi M, Zavarella S, Osborn J, Foffi C, Tuccinardi D. Low-risk HLA genotype in T1DM is associated with less destruction of pancreatic B-cells 12 months after diagnosis. Diabet Med. 2007;24(12):1487–90.

Sutanegara D, Budhiarta AA. The epidemiology and management of diabetes mellitus in Indonesia. Diabetes Res Clin Pract. 2000;50(Suppl 2):S9–S16.

Szepietowska B, Głębocka A, Puch U, Górska M, Szelachowska M. Latent autoimmune diabetes in adults in a population-based cohort of Polish patients with newly diagnosed diabetes mellitus. Arch Med Sci. 2012;8(3):491–5.

Takeda H, Kawasaki E, Shimizu I, Konoue E, Fujiyama M, Murao S, et al. Clinical, autoimmune, and genetic characteristics of adultonset diabetic patients with GAD autoantibodies in Japan (Ehime Study). Diabetes Care. 2002;25(6):995–1001.

Thai AC, Ng WY, Loke KY, Lee WR, Lui KF, Cheah JS. Anti-GAD antibodies in Chinese patients with youth and adultonset IDDM and NID. Diabetologia. 1997;40(12):1425–30.

Thunander M, Thorgeirsson H, Torn C, Petersson C, Landin-Olsson M. β-cell function and metabolic control in latent autoimmune diabetes in adults with early insulin versus conventional treatment: a 3-year follow-up. Eur J Endocrinol. 2011;164(2):239–44.

Tiberti C, Giordano C, Locatelli M, Bosi E, Bottazzo GF, Buzzetti R, Cucinotta D, Galluzzo A, Falorni A, Dotta F. Identification of tyrosine phosphatase 2(256–760) construct as a new, sensitive marker for the detection of islet autoimmunity in type 2 diabetic patients: the non-insulin requiring autoimmune diabetes (NIRAD) study 2. Diabetes. 2008;57(5):1276–83.

Tree TI, Roep BO, Peakman M. A mini meta-analysis of studies on CD4+CD25+ T cells in human type 1 diabetes: report of the Immunology of Diabetes Society T Cell Workshop. Ann N Y Acad Sci. 2006;1079:9–18.

Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes. 1993;42(2):359–62.

Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissén M, Ehrnström BO, Forsén B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes. 1999;48(1):150–7.

Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet. 1997;350(9087):1288–93.

UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352:854–65.

Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, Nika K, Tautz L, Tasken K, Cucca F, Mustelin T, Bottini N. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet. 2005;37:1317–9.

Vatay A, Rajczy K, Pozsonyi E, Hosszúfalusi N, Prohászka Z, Füst G, Karádi I, Szalai C, Grósz A, Bártfai Z, et al. Differences in the genetic background of latent autoimmune diabetes in adults (LADA) and T1DM mellitus. Immunol Lett. 2002;84:109–15.

Wenzlau JM, Moua O, Sarkar SA, Yu L, Rewers M, Eisenbarth GS, Davidson HW, Hutton JC. SIC30A8 is a major target of humoral autoimmunity in T1DM and a predictive marker in prediabetes. Ann N Y Acad Sci. 2008;1150:256–9.

Wherrett DK, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Herold KC, Marks JB, Monzavi R, Moran A, Orban T, Palmer JP, Raskin P, Rodriguez H, Schatz D, Wilson DM, Krischer JP, Skyler JS, T1DM TrialNet GAD Study Group.

Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised doubleblind trial. Lancet. 2011;378(9788):319–27.
Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. Diabetologia. 2001;44(7):914–22.
Wilkin T, Greene S, McCrimmon R. Testing the accelerator hypothesis: a new approach to T1DM prevention (adAPT 1). Diabetes Obes Metab. 2016;18(1):3–5.
Xiang Y, Zhou P, Li X, Huang G, Liu Z, Xu A, Leslie RD, Zhou Z. Heterogeneity of altered cytokine levels across the clinical spectrum of diabetes in China. Diabetes Care. 2011;34 (7):1639–41.
Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes. 1999;48:2270–6.
Yang Z, Zhou Z, Huang G, Ling H, Yan X, Peng J, Li X. The CD4 regulatory T-cells is decreased in adults with latent autoimmune diabetes. Diabetes Res Clin Pract. 2007;76(1):126–31.
Zampetti S, Spoletini M, Petrone A, Capizzi M, Arpi ML, Tiberti C, Di Pietro S, Bosi E, Pozzilli P, Giorgino F, Buzzetti R, NIRAD Study Group. Association of TCF7L2 gene variants with low GAD autoantibody titre in LADA subjects (NIRAD study 5). Diabet Med. 2010;27(6):701–4.
Zampetti S, Capizzi M, Spoletini M, Campagna G, Leto G, Cipolloni L, Tiberti C, Bosi E, Falorni A, Buzzetti R, NIRAD Study Group. GADA titer-related risk for organ-specific autoimmunity in LADA subjects subdivided according to gender (NIRAD study 6). J Clin Endocrinol Metab. 2012;97(10):3759–65.
Zampetti S, Campagna G, Tiberti C, Songini M, Arpi ML, De Simone G, Cossu E, Cocco L, Osborn J, Bosi E, Giorgino F, Spoletini M, Buzzetti R, NIRAD Study Group. High GADA titer increases the risk of insulin requirement in LADA patients: a 7-year follow-up (NIRAD study 7). Eur J Endocrinol. 2014;171(6):697–704.
Zhang N, Huang W, Dong F, Liu Y, Zhang B, Jing L, Wang M, Yang G, Jing C. Insulin gene VNTR polymorphisms -2221MspI and -23HphI are associated with T1DM and latent autoimmune diabetes in adults: a meta-analysis. Acta Diabetol. 2015;52(6):1143–55.
Zhao Y, Yang L, Xiang Y, Liu L, Huang G, Long Z, Li X, Leslie RD, Wang X, Zhou Z. Dipeptidyl peptidase 4 inhibitor sitagliptin maintains beta-cell function in patients with recent-onset latent autoimmune diabetes in adults: one year prospective study. J Clin Endocrinol Metab. 2014;99 (5):E876–80.
Zhou Z, Li X, Huang G, Peng J, Yang L, Yan X, Wang J. Rosiglitazone combined with insulin preserves islet beta cell function in adult-onset latent autoimmune diabetes (LADA). Diabetes Metab Res Rev. 2005;21(2):203–8.
Zhou J, Ma XJ, Bao YQ, Pan XP, Lu W, Hu C, Xiang KS, Jia WP. Study on prevalence of latent autoimmune diabetes in adults and its relationship with metabolic syndrome. Zhonghua Yi Xue Za Zhi. 2009;89(18):1250–4.
Zhou Z, Xiang Y, Ji L, Jia W, Ning G, Huang G, Yang L, Lin J, Liu Z, Hagopian WA, Leslie RD, on behalf of LADA China Study Group. Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. Diabetes. 2013;62(2):543–50.
Zinman B, Kahn SE, Haffner SM, O'Neill MC, Heise MA, Freed MI, ADOPT Study Group. Phenotypic characteristics of GAD antibody-positive recently diagnosed patients with type 2 diabetes in North America and Europe. Diabetes. 2004;53(12):3193–200.

Monogenic Diabetes

9

Katharine R. Owen

Contents

Abstract

Monogenic forms of diabetes can be associated with diabetes diagnosed in neonatal life or in young adulthood.

The commonest presentation of monogenic diabetes is maturity-onset diabetes of the young (MODY), which typically presents in young adult life with familial,

K. R. Owen (✉)
Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK
e-mail: Katharine.owen@drl.ox.ac.uk

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_10

non-autoimmune diabetes without insulin resistance. *HNF1A* and *GCK* mutations account for most cases.

Mitochondrial diabetes presents at a similar age with diabetes associated with deafness, myopathy, and neurological features.

In the first 6 months of life, diabetes is nearly always caused by single gene mutations. Neonatal diabetes is highly heterogeneous, but the commonest causes are mutations in *KCNJ11* and *ABCC8* encoding the beta-cell K_{ATP} channel components and methylation defects in the chromosome 6q24 region.

The most important reason for diagnosing monogenic diabetes is that the genetic aetiology alters treatment. Mutations in *HNF1A, HNF4A, ABCC8*, and *KCNJ11* all lead to diabetes responsive to sulfonylurea therapy, while GCK-MODY does not require treatment.

When diabetes arises as part of a multisystem disorder such as in Wolcott-Rallison syndrome or HNF1B-MODY, the genetic diagnosis may give important insight into prognosis and development of other features.

New sequencing technologies such as exome sequencing can be used to search for new genes which cause diabetes, but interpreting novel genetic variants in both familiar and less well-known genes remains extremely challenging.

Keywords
Monogenic diabetes · MODY · Neonatal diabetes · Mitochondrial diabetes · Precision medicine

Introduction

The majority of cases of diabetes can be categorized as either type 1 (T1D) or type 2 (T2D) diabetes; however there are a large number of less common aetiologies (American Diabetes, Association 2017), including monogenic diabetes. Monogenic diabetes is an inherited form of diabetes caused by single gene variants. Increased access to molecular diagnostics has allowed a shift in the classification of diabetes from purely clinical to include genetic causes, and variants in a long and increasing list of genes have found to be causative for monogenic diabetes.

Monogenic diabetes is associated with a number of different phenotypes including young adult onset beta-cell dysfunction (including maturity-onset diabetes of the young (MODY) and mitochondrial diabetes), neonatal diabetes, and severe insulin resistance. Identifying those who have monogenic diabetes rather than classic type 1 or type 2 is important because it guides treatment decisions, gives patients information about prognosis, and defines risk to family members. Diagnostic genetic testing is available in many countries, but despite this there is still a lack of awareness among clinicians about monogenic diabetes, meaning that uptake of genetic testing is variable (Shields et al. 2010).

Gene Discovery in Monogenic Diabetes

The approaches to gene discovery have changed with the technological advances in molecular genetics and sequencing. In the early 1990s, the first MODY genes were discovered after large families with MODY underwent linkage studies to identify loci of interest (Yamagata et al. 1996a, b; Froguel et al. 1992). As direct sequencing became more available, this allowed candidate gene approaches and the investigation of many beta-cell genes (Flanagan et al. 2014). One of the most important findings from this era of investigation was the discovery that mutations in *KCNJ11*, the gene encoding for the Kir6.2 component of the beta-cell K_{ATP} channel, were a common cause of neonatal diabetes (Gloyn et al. 2004). More recently the advent of exome and whole genome sequencing has applied an unbiased approach to gene discovery. This has been most successful in neonatal diabetes, which has the advantage of a clearly defined diabetes phenotype and includes a number of distinct syndromes which feature neonatal diabetes (De Franco et al. 2015).

Following gene discovery, a common paradigm is observed of a spectrum of phenotypes of differing severity seen with a number of genes. Glucokinase exemplifies this – common variation in the gene has a small effect on fasting plasma glucose, heterozygous inactivating mutations lead to autosomal dominantly inherited fasting hyperglycemia (glucokinase-MODY), and homozygous mutations cause permanent neonatal diabetes – but there are a number of other examples.

Maturity-Onset Diabetes of the Young (MODY)

MODY is the term for diabetes (or hyperglycemia) caused by monogenic beta-cell dysfunction. MODY was recognized clinically many years before the genes responsible were found. Some young-onset, lean individuals with a strong family history did not present acutely like type 1 diabetes and could be managed without insulin treatment (Tattersall 1974). The collection of large multigenerational families allowed linkage studies, and the causal genes for MODY subtypes started emerging in the 1990s. This lead to the rapid development of diagnostic molecular testing and therapeutic recommendations (Ellard et al. 2008).

The clinical term MODY refers to diabetes with the following characteristics:

- Onset generally in the 2nd–4th decade of life
- Frequently exhibit autosomal-dominant pattern of inheritance
- Preservation of endogenous insulin secretion (C-peptide present)
- Absence of beta-cell autoimmunity
- Lean with absence of insulin resistance

Mutations in a number of genes are associated with a MODY phenotype, although most of them are very rare (<1% of MODY) and some reported in less than five families. In most clinical settings, the majority of MODY is accounted for

by mutations in the transcription factor hepatocyte nuclear factor 1-alpha (HNF1A) and the glycolytic enzyme glucokinase (GCK). Together they represent about 80–90% of MODY cases in adults. The relative prevalence of MODY subtypes varies between countries, reflecting that routine screening for diabetes is performed in countries such as Germany, Italy, France, and Spain, where the generally asymptomatic GCK-MODY predominates (Shields et al. 2010; Estalella et al. 2007; Lorini et al. 2009).

Initially, MODY was estimated at around 2% of those with diabetes (Ledermann 1995; Panzram and Adolph 1981); however this was based on clinical features rather than molecular genetic diagnosis. An assessment of MODY prevalence in the UK, based on data from about 10 years of diagnostic genetic testing, estimated the MODY prevalence as a minimum of 68–108 cases per million of the general population (Shields et al. 2010) which corresponds to 0.1–0.15% of people with diabetes in the UK. Studies based on defined populations in Norway (Eide et al. 2008) and the UK (Kropff et al. 2011) reported prevalence of HNF1A-MODY as 0.07–0.4% of the diabetic population. Other studies have concentrated on the arguably slightly easier discrimination of MODY in pediatric populations (Moritani et al. 2016; Johansson et al. 2017; Pihoker et al. 2013). Recently the UNITED study, using defined populations in the UK and screening for a wide range of MODY genes using a next-generation sequencing panel, estimated the proportion of MODY as 3.6% of adults or children with diabetes diagnosed before age 30 (Shields et al. 2017).

Glucokinase MODY (MODY2)

This is the commonest form of MODY in children and is also detected more often when routine glucose testing is performed. Glucokinase (GCK), sometimes known as the pancreatic β-cell glucose sensor, is a key regulatory enzyme in glucose-stimulated insulin secretion. It catalyzes the first step of glycolysis, the conversion of glucose to glucose-6-phosphate, and maintains fasting glucose at the correct homeostatic level. Heterozygous inactivating mutations raise the threshold at which insulin secretion is initiated leading to a phenotype of mild, often subclinical, nonprogressive, fasting hyperglycemia (fasting glucose 5.5–8.0 mmol/L, HbA1c 40–60 mmol/mol), which is present from birth (Steele et al. 2013). Diagnosis of GCK-MODY is often incidental and depends on the level of glucose screening in the population. It is commonly identified in pregnancy.

The key finding in GCK-MODY is that insulin secretion remains intact and regulated, albeit that glucose levels are shifted 2–3 mmol/L higher than usual. This results in low postprandial glucose excursions compared to other forms of diabetes. It was observed annecdotally that families with GCK-MODY had a low level of vascular complications and this was confirmed in a large observational study (Steele et al. 2014) of individuals with GCK-MODY who had reached middle age and so been exposed to several decades of hyperglycemia. There were no significant microvascular complications, and there did not seem to be a higher

risk of macrovascular complications than age-matched nondiabetic individuals (although a much larger study would be required to prove this). Additionally treatment with insulin or oral hypoglycemic agents does not appear to alter HbA1c (Stride et al. 2014), probably due to the continued regulation of glucose levels.

Treatment is therefore not generally considered necessary in GCK-MODY. There are two possible exceptions to this: firstly in pregnancy when the birthweight depends on the interaction of the maternal and fetal genotype. Where the mother is affected and the fetus is not, the mild hyperglycemia in the mother leads to an increased insulin secretion in the fetus and thus increased birthweight (Spyer et al. 2009). Where both mother and fetus have the *GCK* mutation, the glucose level is appropriate for both and normal growth occurs. As usually the *GCK* status of the fetus is unknown, monitoring the fetal abdominal circumference (AC) in the final trimester is recommended and treatment with insulin considered if the AC is increased. In a case where the fetus has inherited the *GCK* mutation from the father, the birthweight tends to be lower (Hattersley et al. 1998).

Treatment in those with glucokinase mutations should also be considered if HbA1c rises above the individual's usual baseline due to obesity (essentially a mixed picture of type 2 diabetes and GCK-MODY), in which case metformin would be an appropriate first-line treatment.

Possession of two inactivating mutations in *GCK* (either homozygous in consanguineous families or compound heterozygote) is a rare cause of permanent neonatal diabetes (Njolstad et al. 2003). Affected children are insulin requiring, while family members may have GCK-MODY (Kavvoura et al. 2014).

HNF1A-MODY (MODY3)

This is the commonest type of MODY in adults. Heterozygous mutations in the transcription factor hepatocyte nuclear factor 1α (*HNF1A)* cause progressive β-cell dysfunction, resulting in hyperglycemia and diabetes diagnosed in the 2nd–4th decade of life. In contrast to GCK-MODY, β-cell decline is progressive leading to increasing treatment requirement over a period of years.

Similarly to T1D and T2D, microvascular and macrovascular complications are common unless glycemic targets are met; therefore monitoring and follow-up are required.

One of the most important features of HNF1A-MODY is the sensitivity seen to sulfonylurea (SU) drugs. This was first noted anecdotally, and then confirmed in a small RCT (Pearson et al. 2003), where it was shown that there was a fourfold greater response to low-dose gliclazide than to metformin in those with *HNF1A* mutations, whereas the effect of the two drugs was comparable in T2D. The reason for this is not completely understood but is probably because the defects in HNF1A affect ATP production in the β-cell. This is overcome by stimulation of insulin secretion via the K-ATP channel where SU drugs bind. Therefore low-dose SU

represents the first-line treatment for HNF1A-MODY, and in patients commenced on other treatments, including insulin, SUs can frequently be safely substituted with as good, or better, control as insulin achieved (Shepherd et al. 2009; Bacon et al. 2015; Bellanne-Chantelot et al. 2011). Starting doses equivalent to 20–40 mg gliclazide daily are usually effective and can be reduced if hypoglycemia occurs. Typically HbA1c rises considerably on other oral agents. The glinide secretagogues can also be used (Tuomi et al. 2006) and are useful in those who experience hypoglycemia on very low doses of SU agents.

Secondary failure of SUs eventually occurs in most, and metformin or DPP4 inhibitors can be added once SUs are maximized, with no particular evidence base for second-line treatment. Theoretically SGLT2 inhibitors are likely to be ineffective, or cause dehydration, as people with HNF1A-MODY already have reduced *SGLT2* expression which causes glycosuria. Most patients eventually do require insulin as β-cell dysfunction progresses.

Extra-pancreatic features of HNF1A-MODY include low renal threshold for glucose due to the effect on *SGLT2* expression, and early glycosuria as a response to a carbohydrate load is a noninvasive way of monitoring glucose in children at risk of HNF1A-MODY (Stride et al. 2002). HNF1A also regulates many genes in the liver, and in some cases this leads to a measurable difference in serum levels. Both low levels of C-reactive protein (Thanabalasingham et al. 2011) and altered plasma glycan profile (Thanabalasingham et al. 2013) have been suggested as biomarkers for HNF1A-MODY (see below).

HNF4A-MODY (MODY1)

Mutations in *HNF4A* cause a similar phenotype to HNF1A- MODY, but are less common, accounting for about 10% of MODY in adults. One difference is that *HNF4A* mutations cause hyperinsulinemia in utero and neonatal life, leading to macrosomia and neonatal hypoglycemia (Pearson et al. 2007). This is generally transient, with normoglycaemia in childhood and then development of diabetes in young adult life. The reason for this change in phenotype is unknown but is likely to be related to the different isoforms of HNF4A that are expressed in fetal and adult life.

HNF4A-MODY can also be successfully treated with low doses of SU. Renal glucose threshold and CRP are normal.

The R114W *HNF4A* variant has been reported to show a decreased penetrance, leading to older age of onset, reduced sensitivity to SU drugs, and lack of association with neonatal hyperinsulinism (Laver et al. 2016). It is also observed in a higher than expected frequency in population exome-sequencing databases such as EXAC. The OR for diabetes for the variant is >30, significantly higher than seen in common variants, suggesting it is a genuinely rare, lower penetrance variant. It is likely that similar variants exist for *HNF1A* and this will become clearer as the correlation between variants reported as MODY, but also seen in publically available population sequencing data, is better understood.

HNF1B MODY (MODY5)

Mutations or deletions in *HNF1B* cause about 10% of MODY cases and lead to a syndrome of developmental anomalies in the kidneys, pancreas, genitourinary, and biliary systems (Bellanne-Chantelot et al. 2004; Kanthimathi et al. 2015; Raile et al. 2009). The syndrome of features is also known as RCAD (renal cysts and diabetes). Renal abnormalities can be evident from screening in early gestational life and are frequently the first presentation. Nondiabetic renal failure can occur. GU abnormalities such as hypospadia and bicornate uterus are commonly reported. Pancreatic atrophy leads to both diabetes and pancreatic exocrine insufficiency in adult life. In contrast to HNF1A-MODY, patients are not SU sensitive and progress to insulin treatment relatively quickly.

About half of cases are due to spontaneous deletions in chromosome 17q12 which contains HNF1B (Bellanne-Chantelot et al. 2005), and the deletions frequently involve other genes. This leads to an extended neurological phenotype in the cases involving deletions, such as learning disability, autism, and schizophrenia (Rasmussen et al. 2016; Clissold et al. 2016).

Rare Forms of MODY

Mutations in *KCNJ11*, *ABCC8*, and *INS* cause up to 1% of MODY cases, but they are far better known for their role in neonatal diabetes (see below).

Homozygous or compound heterozygous mutations in the insulin promoter factor 1 (*IPF1)* gene, an important transcription factor in pancreatic development, are rare causes of pancreatic agenesis. In the family of a proband diagnosed with pancreatic agenesis (Stoffers et al. 1997), diabetes co-segregated with heterozygous carriers of the mutation, leading to IPF1 being designated as "MODY4." However subsequently most variants investigated in *IPF1* have not been conclusively demonstrated to be the cause of diabetes.

Mutations in another pancreatic transcription factor, *NEUROD1*, have in a small number of cases appeared to co-segregate with a MODY phenotype (Szopa et al. 2016), and also are a rare cause of autosomal recessive neonatal diabetes (Rubio-Cabezas et al. 2010) but this too has not been shown to be a major cause of MODY.

Two Norwegian MODY pedigrees, who also had exocrine pancreatic dysfunction, were shown to have deletions in a VNTR region of the carboxyl ester lipase (CEL) gene (Raeder et al. 2006), a similar phenotype has been described in one other European family.

Mutations in the Wolfram syndrome gene *WFS1* appear to also cause MODY occasionally. Exome sequencing has identified *WFS1* variants in two families (Johansson et al. 2012; Bonnycastle et al. 2013) with young-onset non-autoimmune diabetes. Common variation in *WFS1* is also associated with T2D.

Recently heterozygous *RFX6* protein-truncating variants have been reported to cause MODY with a reduced penetrance (Patel et al. 2017). RFX6 is a further transcription factor involved in the development of the endocrine pancreas (Smith

et al. 2010) so is a good candidate for causing monogenic diabetes. Earlier, homozygous mutations in *RFX6* were found to cause neonatal diabetes with gut and gallbladder anomalies (Smith et al. 2010), but no phenotype had previously been identified with heterozygous variants. The RFX6 variants were identified following investigation of UK cases with a clinical diagnosis of MODY using a next-generation sequencing panel of 29 genes that included all the known causes of monogenic diabetes (Ellard et al. 2013). The findings were then confirmed in a Finnish cohort (Patel et al. 2017). Variants were found to co-segregate with diabetes, but with an increased age of onset compared to HNF1A- MODY as only 27% had developed diabetes by age 25, compared to 55% of HNF1A-MODY cases.

Proteins with a known role in glucose homeostasis are also considered candidate genes for monogenic diabetes when filtering the many variants found in next-generation sequencing. Exome sequencing in 60 families from the USA and Italy recently identified 2 families with loss of function variants in *APPL1*, a molecule in the insulin and adiponectin signaling pathway (Prudente et al. 2015). Co-segregation studies showed that most with hyperglycemia shared the mutations, while unaffected mutation carriers came from the youngest generation. Functional work was supportive that the variants were loss of function and they were absent from population databases. It will remain to be seen whether *APPL1* variants will be reported in other MODY pedigrees.

Other genes such as *PAX4*, *KLF11*, and *BLK* have been implicated in MODY families; however they have been reported in a handful of families each, and the genetic evidence is uncertain or lacking or has not been replicated outside the original study.

Mitochondrial Diabetes

Diabetes is a common feature of mitochondrial gene mutations or deletions. The syndrome maternally inherited diabetes and deafness (MIDD) was used to describe early pedigrees (van den Ouweland et al. 1994); however the phenotype often includes other neurological features and myopathies (Murphy et al. 2008). The A3243G missense mutation is the commonest mitochondrial mutation described and causes a range of presentations from diabetes alone to the severe syndrome of MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes). The different presentations are thought to be due to heteroplasmy: each mitochondrion has several copies of the DNA and not all contain the mutation. The level of heteroplasmy varies in different organs due to the distribution of mitochondrial DNA during early fetal development.

Classically mitochondrial diabetes presents in young adult life (3rd–5th decade) with a predominantly β-cell defect, so patients are lean without insulin resistance. Other features are commonly present at diagnosis, and patients should ideally receive care within a specialist clinical environment so that associated conditions such as cardiomyopathy can be monitored (http://mitochondrialdisease.nhs.uk).

There is little evidence base for treatment recommendations. Patients with mitochondrial diabetes can usually be managed with oral agents initially but progress to insulin treatment relatively rapidly. As mitochondrial disease can be associated with raised lactate levels, theoretically metformin treatment should be avoided, or monitored closely, particularly in those with neurological disease.

Neonatal Diabetes

Neonatal diabetes is a rare condition defined as diabetes diagnosed in the first 6 months of life and has an incidence of around 1 in 100,000 live births (Iafusco et al. 2012). Typically there is a low birthweight as a consequence of decreased insulin secretion in utero. Neonatal diabetes is a heterogeneous condition which can be transient or permanent and comprise diabetes alone, or diabetes as a feature of a syndrome. Inheritance patterns are also variable. Transient neonatal diabetes (TNDM) is a subtype which remits in infancy or early childhood but may relapse in adolescence or early adulthood.

Until 2004, diabetes in neonatal life was mainly considered an early onset of type 1 diabetes, but since the identification of mutations in *KCNJ11* as the most common cause of neonatal diabetes (Gloyn et al. 2004), there has been a hugely successful discovery effort to identify responsible genes. This has been facilitated by the collection of DNA samples from large numbers of cases with phenotypic information, allowing putative genes to be investigated and replicated quickly (De Franco et al. 2015). It is now apparent that a genetic aetiology can be found in more than 80% of cases of neonatal diabetes (De Franco et al. 2015). Supporting this, those presenting with neonatal diabetes do not have any evidence of increased genetic susceptibility for T1D, such as high-risk HLA (Iafusco et al. 2002) or positive autoantibodies. As in MODY, establishing the genetic aetiology influences the first-line medical therapy. Additionally as neonatal diabetes may be the first feature in a syndromic case, genetic testing at presentation allows prediction of, and screening for, other features of the syndrome (De Franco et al. 2015).

Neonatal Diabetes Due to Potassium Channel Gene Mutations

The commonest type of permanent neonatal diabetes (PNDM), accounting for 40% of cases, is caused by activating mutations in the *KCNJ11* and *ABCC8* genes, encoding the Kir6.2 and SU receptor 1 (SUR1) subunits, respectively, of the pancreatic β-cell K_{ATP} channel. Defects cause the channel to remain permanently open, leading to reduction or lack of insulin secretion (Gloyn et al. 2004). The molecular defect can be overcome by the use of high doses of sulfonylurea drugs, which restore insulin secretion (Babiker et al. 2016). Most, although not all, individuals with K_{ATP} channel mutations are able to transfer successfully from insulin to glibenclamide, usually with an improved HbA1c (Pearson et al. 2006; Ashcroft et al.

2017). It is also observed that sulfonylurea treatment appears to have an enduring therapeutic effect in many patients.

As K_{ATP} channels are also expressed in the central nervous system, some mutations are associated with a more severe syndrome of psychomotor developmental delay, epilepsy, and diabetes (DEND syndrome). There are some anecdotal reports of improvement in neurological symptoms after use of SU drugs (Battaglia et al. 2012).

Mutations in the K_{ATP} channel genes cause about 20% of cases of transient neonatal diabetes (Flanagan et al. 2007). There are some differences between this form of TNDM and that caused by 6p24 defects (see below) which can help guide first-line genetic testing.

It's also apparent that the phenotypes associated with *KCNJ11* and *ABCC8* mutations can be of variable penetrance within and between families (Patch et al. 2007). Some individuals present in adulthood with a MODY-like phenotype, and as they are likely to be effectively treated with SU drugs, it is important to consider K_{ATP} channel mutations as a cause of MODY when mutations in more common genes have been excluded. The dose of SU required in diabetes due to K_{ATP} mutations varies according to the presentation and level of C-peptide. High doses are used in permanent neonatal diabetes where C-peptide is usually undetectable (e.g., Glibenclamide dose range 0.1-1 mg/kg), although these tend to reduce with time (Transferring Patients with Diabetes due to a KIR6.2 Mutation from Insulin to Sulphonylureas 2017). C-peptide is usually measurable in TNDM, and for these babies, or relapsed adults, small doses of SU can be commenced and the dose titrated up as necessary.

Insulin Gene Mutations

Insulin gene (*INS*) mutations can cause several phenotypes via different mechanisms. The most common presentation is permanent neonatal diabetes, but a MODY-like diabetes is also seen and much more rarely hyperproinsulinemia and hyperinsulinemia (Stoy et al. 2007; Edghill et al. 2008). Permanent neonatal diabetes is usually caused by heterozygous mutations which are predicted to lead to abnormal processing and folding of the proinsulin molecule through interfering with disulfide bonding as it passes through the endoplasmic reticulum (ER) of the β-cell. This mechanism is seen in animal studies (Izumi et al. 2003) and causes ER stress due to accumulation of the misfolded protein and subsequent apoptosis of the β-cell. More rarely homozygous *INS* mutations have been identified in neonatal diabetes, generally causing a more severe insulin deficiency and early onset of diabetes (Garin et al. 2010). In both cases treatment with insulin is generally required for affected children.

About 1% of individuals with MODY have *INS* mutations (Molven et al. 2008; Boesgaard et al. 2010). They have variable age of onset from childhood to middle age and may be treated with diet and oral agents initially. It is advised to avoid stimulating the β-cell to produce insulin because theoretically this should reduce

production of the misfolded insulins and help preserve β-cell function. Thus insulin-sparing agents such as metformin or SGLT2i would be preferred to a sulfonylurea, an important distinction from HNF1A/HNF4A-MODY and the K_{ATP} channel mutations.

Less commonly *INS* mutations lead to insulin molecules that have reduced activity through impaired binding to the insulin receptor – they lead to hyper-insulinemia or hyperproinsulinemia and may not necessarily cause diabetes.

Transient Neonatal Diabetes Due to 6q24 Defects

About 70% of cases of transient neonatal diabetes are caused by a methylation defect at chromosome 6q24 which leads to overexpression of paternally inherited genes *ZAC* and/or *HYMAI* (Temple and Shield 2002). The mechanism of this can include paternal uniparental disomy, a paternal 6q24 duplication or hypomethylation on the maternal chromosome. Apart from low birthweight and diabetes, there are other features such as macroglossia, umbilical hernia, and developmental delay. Some of these additional features may be caused by other imprinting defects. Diabetes tends to present earlier than in the potassium channel mutations and invariably remits in the first months of life. Insulin treatment is required. About half of cases relapse in young adulthood with a non-insulin requiring diabetes.

Rare Causes of Neonatal Diabetes

There are a number of other genetic causes of neonatal diabetes (De Franco et al. 2015). Many of these are associated with rare multisystem disease. Wolcott-Rallison Syndrome is the commonest cause of neonatal diabetes in consanguineous families and caused by recessive mutations in *EIF2AK3* (Senee et al. 2004). The X-linked IPEX syndrome, caused by mutations in FOXP3, is considerably rarer (Bennett et al. 2001), but in both cases neonatal diabetes is the first manifestation of the syndrome, and identifying the genetic defect through systematic screening of the known genes associated with neonatal diabetes allows anticipation and treatment of other features of the syndrome (De Franco et al. 2015).

Single gene disorders may also be responsible for very early-onset autoimmune diabetes (Johnson et al. 2017). In these cases the autoimmunity is not associated with classic HLA-linked genetic risk for type 1 diabetes.

Pancreatic Agenesis

Complete absence of the pancreas is extremely rare and leads to insulin-dependent neonatal diabetes and pancreatic exocrine insufficiency. Most cases of pancreatic agenesis are caused by mutations in transcription factors involved in pancreatic development, e.g., GATA6, PTF1A, and PDX1 (Flanagan and De Franco 2015).

Diagnosing Monogenic Diabetes

Molecular diagnosis of monogenic diabetes is beneficial because it informs treatment decisions and allows risk to family members or future pregnancies to be defined. Translation of research findings into clinical diagnostics has occurred rapidly, and this has been particularly marked for neonatal diabetes when it became quickly clear that children with potassium channel mutations could be treated with sulfonylureas (Pearson et al. 2006). International collaboration has assisted this process. As there is an almost complete lack of classic autoimmune diabetes arising under 6 months of age, and monogenic diabetes becomes much less common after 6–12 months, there is a clear evidence base for genetic testing in all those diagnosed under 1 year of age (Rubio-Cabezas et al. 2014).

Most diabetes in children is type 1, but studies show that 1–5% of patients actually have MODY. Differentiation from type 1 can be made on the basis of negative β-cell antibodies at diagnosis and persistence of endogenous insulin secretion (C-peptide). Ten to twenty percent of children will be β-cell antibody negative at diagnosis, and while most of these will still not have MODY, antibody negativity highlights those in which further consideration of the aetiology is indicated (Pihoker et al. 2013; Shields et al. 2017). C-peptide is not useful at diagnosis because most with newly diagnosed type 1 continue to secrete some insulin for months or years.

Discrimination of MODY in young adults becomes increasingly difficult when the differential diagnosis includes type 1 and a rising prevalence of young type 2 diabetes as well as monogenic diabetes. In our experience there are many "gray cases" where the aetiology remains uncertain even after investigation.

As MODY is a β-cell dysfunction, screening those with absence of insulin resistance is one approach to select cases (Thanabalasingham et al. 2012). Another approach is to define non-genetic biomarkers that are associated with extra-pancreatic features of the defective protein or transcription factor. For example, *HNF1A* regulates many hepatic genes, and it was found that plasma levels of C-reactive protein (CRP) were lower in those with HNF1A-MODY than other forms of diabetes (Owen et al. 2010). CRP levels appear to discriminate HNF1A-MODY from young type 2 diabetes (Thanabalasingham et al. 2011; McDonald et al. 2011), but in another study did not offer an advantage over clinical features alone (Bellanne-Chantelot et al. 2016). Similarly *HNF1A* regulates the process of glycosylation, and mutations in *HNF1A* lead to a distinctive pattern of plasma glycan profile (Thanabalasingham et al. 2013).

An attempt to identify the best clinical features for distinguishing those with MODY from other young adults lead to the development of a MODY probability calculator (Shields et al. 2012). The most discriminative features were defined using logistic regression modeling of characteristics from groups of individuals with strictly defined MODY, type 1 and type 2 diabetes. The calculator, now available as an app, uses age of onset, parent with diabetes, HbA1c, gender, treatment and time to insulin, and BMI to calculate a probability of MODY compared to the test group and taking into account background prevalence of MODY in those initially diagnosed as type 1 or type 2 diabetes. The calculator helps guide non-experts in choosing who to request genetic testing on, although it is limited to those diagnosed

before age 35 and white European patients, and assumes the features of the MODY group used in development of the model as a gold standard.

Challenges in Interpretation of Genetic Variants

More data exists about the human genome than ever before, but this increased knowledge has made interpretation of genetic testing results more challenging rather than less. This is especially relevant to missense variants, which could be neutral to the protein function and therefore not disease-causing, despite being rare and nonsynonymous.

Data from over 60,000 individuals sequenced as part of the Exome Aggregation Consortium (ExAC) (Lek et al. 2016) showed that each exome in ExAC contained an average of 7.6 rare nonsynonymous variants (MAF $< 0.1\%$) in well-characterized Mendelian disease genes, of which clearly not all cause penetrant autosomal-dominant disease. ExAC data has been used to inform the interpretation of rare variant alleles that otherwise may have been assigned as disease-causing (Walsh et al. 2017).

Examination of population cohorts with and without diabetes by Flannick et al. aimed to characterize the spectrum of low-frequency variation (MAF $< 1\%$) in the main seven MODY genes in 4003 individuals (Flannick et al. 2013). The authors found that genetic variants previously reported to cause MODY in Human Gene Mutation Database (HGMD) were present in 1.5–1.8% of individuals from population-based cohorts. Of those, 0.5% carried variants, which were described in HGMD as causal for MODY, were evolutionally conserved, predicted by bioinformatics as damaging, and absent in the 1000 Genomes Project. Two thirds of the HGMD MODY variant carriers in this study did not have diabetes.

To reduce the risk of misinterpretation of genetic variants in clinical and research settings, guidelines for investigating causality of sequence variance in humans were published in 2014. The authors recommended providing all positive and negative evidence for rare genetic variants: use of genetic, bioinformatics, and experimental data if available; take advantage of public data sets of genomic variants such as ExAC; and not to regard previous reports as definitive (MacArthur et al. 2014). Even if good evidence exists for the variant to cause the disease, full penetrance of the variant allele may not be observed.

This makes the interpretation of sequence variation extremely complex even for genes such as *HNF1A*, where the clinical phenotype associated with rare penetrant variants is well described and many individuals have been sequenced. Assessing potential "new" MODY genes such as *APPL1* is much more challenging.

Summary

Monogenic diabetes may present in neonatal life or young adulthood. In diabetes diagnosed before 6 months of age, single gene causes are found in the majority of cases. In later life, type 1 or type 2 diabetes predominates, but monogenic diabetes

remains an important differential diagnosis. The most compelling reason for considering and testing for monogenic diabetes is the potential to alter management, e.g., the use of sulfonylureas in the potassium channel gene mutations, *KCNJ11* and *ABCC8*, and in HNF1A and HNF4A-MODY. Identifying single gene causes of diabetes also allows accurate delineation of risk for family members and may provide information about prognosis or extra-pancreatic features of multisystem syndromes. Diagnostic testing is increasingly available, but caution is required in assessing genetic variants even in genes such as *HNF1A* where variants have been well characterized previously.

References

American Diabetes, Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2017;40(Suppl 1):S11–24.

Ashcroft FM, Puljung MC, Vedovato N. Neonatal diabetes and the KATP Channel: from mutation to therapy. Trends Endocrinol Metab. 2017;28(5):377–87.

Babiker T, et al. Successful transfer to sulfonylureas in KCNJ11 neonatal diabetes is determined by the mutation and duration of diabetes. Diabetologia. 2016;59(6):1162–6.

Bacon S, et al. Successful maintenance on sulfonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. Diabet Med. 2015;33:976.

Battaglia D, et al. Glyburide ameliorates motor coordination and glucose homeostasis in a child with diabetes associated with the KCNJ11/S225T, del226-232 mutation. Pediatr Diabetes. 2012;13(8):656–60.

Bellanne-Chantelot C, et al. Clinical spectrum associated with hepatocyte nuclear factor-1beta mutations. Ann Intern Med. 2004;140(7):510–7.

Bellanne-Chantelot C, et al. Large genomic rearrangements in the hepatocyte nuclear factor-1beta (TCF2) gene are the most frequent cause of maturity-onset diabetes of the young type 5. Diabetes. 2005;54(11):3126–32.

Bellanne-Chantelot C, et al. Clinical characteristics and diagnostic criteria of maturity-onset diabetes of the young (MODY) due to molecular anomalies of the HNF1A gene. J Clin Endocrinol Metab. 2011;96(8):E1346–51.

Bellanne-Chantelot C, et al. High-sensitivity C-reactive protein does not improve the differential diagnosis of HNF1A-MODY and familial young-onset type 2 diabetes: a grey zone analysis. Diabetes Metab. 2016;42(1):33–7.

Bennett CL, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27(1):20–1.

Boesgaard TW, et al. Further evidence that mutations in INS can be a rare cause of maturity-onset diabetes of the young (MODY). BMC Med Genet. 2010;11:42.

Bonnycastle LL, et al. Autosomal dominant diabetes arising from a Wolfram syndrome 1 mutation. Diabetes. 2013;62(11):3943–50.

Clissold RL, et al. Chromosome 17q12 microdeletions but not intragenic HNF1B mutations link developmental kidney disease and psychiatric disorder. Kidney Int. 2016;90(1):203–11.

De Franco E, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. Lancet. 2015;386(9997):957–63.

Edghill EL, et al. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes. 2008;57(4):1034–42.

Eide SA, et al. Prevalence of HNF1A (MODY3) mutations in a Norwegian population (the HUNT2 study). Diabet Med. 2008;25(7):775–81.

Ellard S, et al. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. Diabetologia. 2008;51(4):546–53.
Ellard S, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013;56(9):1958–63.
Estalella I, et al. Mutations in GCK and HNF-1alpha explain the majority of cases with clinical diagnosis of MODY in Spain. Clin Endocrinol. 2007;67(4):538–46.
Flanagan S, De Franco E. Monogenic causes of pancreatic agenesis. 2015; Diapedia 4105491820 rev. no. 2. Available from https://doi.org/10.14496/dia.4105491820.2.
Flanagan SE, et al. Mutations in ATP-sensitive K+ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. Diabetes. 2007;56(7):1930–7.
Flanagan SE, et al. Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. Cell Metab. 2014;19(1):146–54.
Flannick J, et al. Assessing the phenotypic effects in the general population of rare variants in genes for a dominant Mendelian form of diabetes. Nat Genet. 2013;45(11):1380–5.
Froguel P, et al. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature. 1992;356:162–4.
Garin I, et al. Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. Proc Natl Acad Sci U S A. 2010;107(7):3105–10.
Gloyn AL, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med. 2004;350(18):1838–49.
Hattersley AT, et al. Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet. 1998;19:268–70.
Iafusco D, et al. Permanent diabetes mellitus in the first year of life. Diabetologia. 2002;45(6):798–804.
Iafusco D, et al. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. Acta Diabetol. 2012;49(5):405–8.
Izumi T, et al. Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. Diabetes. 2003;52(2):409–16.
Johansson S, et al. Exome sequencing and genetic testing for MODY. PLoS One. 2012;7(5):e38050.
Johansson BB, et al. Targeted next-generation sequencing reveals MODY in up to 6.5% of antibody-negative diabetes cases listed in the Norwegian Childhood Diabetes Registry. Diabetologia. 2017;60(4):625–35.
Johnson MB, et al. Recessively inherited LRBA mutations cause autoimmunity presenting as neonatal diabetes. Diabetes. 2017;66(8):2316–22.
Kanthimathi S, et al. Identification and molecular characterization of HNF1B gene mutations in Indian diabetic patients with renal abnormalities. Ann Hum Genet. 2015;79(1):10–9.
Kavvoura F, et al. Reclassification of diabetes etiology in a family with multiple diabetes phenotypes. J Clin Endocrinol Metab. 2014;99:E1067. https://doi.org/10.1210/jc2013-3641.
Kropff J, et al. Prevalence of monogenic diabetes in young adults: a community-based, cross-sectional study in Oxfordshire, UK. Diabetologia. 2011;54(5):1261–3.
Laver TW, et al. The common p.R114W HNF4A mutation causes a distinct clinical subtype of monogenic diabetes. Diabetes. 2016;65(10):3212–7.
Ledermann HM. Maturity-onset diabetes of the young (MODY) at least ten times more common in Europe than previously assumed? Diabetologia. 1995;38(12):1482.
Lek M, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285–91.
Lorini R, et al. Maturity-onset diabetes of the young in children with incidental hyperglycemia: a multicenter Italian study of 172 families. Diabetes Care. 2009;32(10):1864–6.
MacArthur DG, et al. Guidelines for investigating causality of sequence variants in human disease. Nature. 2014;508(7497):469–76.

McDonald TJ, et al. High-sensitivity CRP discriminates HNF1A-MODY from other subtypes of diabetes. Diabetes Care. 2011;34(8):1860–2.
Molven A, et al. Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. Diabetes. 2008;57(4):1131–5.
Moritani M, et al. Identification of monogenic gene mutations in Japanese subjects diagnosed with type 1B diabetes between >5 and 15.1 years of age. J Pediatr Endocrinol Metab. 2016;29 (9):1047–54.
Murphy R, et al. Clinical features, diagnosis and management of maternally inherited diabetes and deafness (MIDD) associated with the 3243A>G mitochondrial point mutation. Diabet Med. 2008;25(4):383–99.
Njolstad PR, et al. Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. Diabetes. 2003;52(11):2854–60.
Owen KR, et al. Assessment of high-sensitivity C-reactive protein levels as diagnostic discriminator of maturity-onset diabetes of the young due to HNF1A mutations. Diabetes Care. 2010;33 (9):1919–24.
Panzram G, Adolph W. Heterogeneity of maturity onset diabetes at young age (MODY). Lancet. 1981;2(8253):986.
Patch AM, et al. Mutations in the ABCC8 gene encoding the SUR1 subunit of the KATP channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. Diabetes Obes Metab. 2007;9(Suppl 2):28–39.
Patel K, Laakso M, Stancakova A, Laver TW, Colclough K, Johnson MB, Kettunen J, Tuomi T, Cnop M, Shepherd MH, Flanagan SE, Ellard S, Hattersley AT, Weedon MN. Heterozygous RFX6 protein truncating variants cause Maturity-Onset Diabetes of the Young (MODY) with reduced penetrance. Nat Commun. 2017;8:888. BioRxiv beta.
Pearson ER, et al. Genetic cause of hyperglycaemia and response to treatment in diabetes. Lancet. 2003;362(9392):1275–81.
Pearson ER, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006;355(5):467–77.
Pearson ER, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. PLoS Med. 2007;4(4):e118.
Pihoker C, et al. Prevalence, characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for diabetes in youth. J Clin Endocrinol Metab. 2013;98(10):4055–62.
Prudente S, et al. Loss-of-function mutations in APPL1 in familial diabetes mellitus. Am J Hum Genet. 2015;97(1):177–85.
Raeder H, et al. Mutations in the CEL VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. Nat Genet. 2006;38(1):54–62.
Raile K, et al. Expanded clinical spectrum in hepatocyte nuclear factor 1b-maturity-onset diabetes of the young. J Clin Endocrinol Metab. 2009;94(7):2658–64.
Rasmussen M, et al. 17q12 deletion and duplication syndrome in Denmark-a clinical cohort of 38 patients and review of the literature. Am J Med Genet A. 2016;170(11):2934–42.
Rubio-Cabezas O, et al. Homozygous mutations in NEUROD1 are responsible for a novel syndrome of permanent neonatal diabetes and neurological abnormalities. Diabetes. 2010;59 (9):2326–31.
Rubio-Cabezas O, et al. ISPAD clinical practice consensus guidelines 2014. The diagnosis and management of monogenic diabetes in children and adolescents. Pediatr Diabetes. 2014;15 (Suppl 20):47–64.
Senee V, et al. Wolcott-Rallison syndrome: clinical, genetic, and functional study of EIF2AK3 mutations and suggestion of genetic heterogeneity. Diabetes. 2004;53(7):1876–83.
Shepherd M, et al. A genetic diagnosis of HNF1A diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. Diabet Med. 2009;26(4):437–41.
Shields BM, et al. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia. 2010;53(12):2504–8.

Shields BM, et al. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. Diabetologia. 2012;55 (5):1265–72.
Shields BM, et al. Population-based assessment of a biomarker-based screening pathway to aid diagnosis of monogenic diabetes in young-onset patients. Diabetes Care. 2017;40(8):1017–25.
Smith SB, et al. Rfx6 directs islet formation and insulin production in mice and humans. Nature. 2010;463(7282):775–80.
Spyer G, et al. Pregnancy outcome in patients with raised blood glucose due to a heterozygous glucokinase gene mutation. Diabet Med. 2009;26(1):14–8.
Steele AM, et al. Use of HbA1c in the identification of patients with hyperglycaemia caused by a glucokinase mutation: observational case control studies. PLoS One. 2013;8(6):e65326.
Steele AM, et al. Prevalence of vascular complications among patients with glucokinase mutations and prolonged, mild hyperglycemia. JAMA. 2014;311(3):279–86.
Stoffers DA, et al. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet. 1997;15:106–10.
Stoy J, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci U S A. 2007;104(38):15040–4.
Stride A, et al. Glycosuria at 2 h post OGTT: a screening tool for unaffected subjects in families with HNF-1a mutations. Diabet Med. 2002;19(S2):59–60.
Stride A, et al. Cross-sectional and longitudinal studies suggest pharmacological treatment used in patients with glucokinase mutations does not alter glycaemia. Diabetologia. 2014;57(1):54–6.
Szopa M, et al. A family with the Arg103Pro mutation in the NEUROD1 gene detected by next-generation sequencing – clinical characteristics of mutation carriers. Eur J Med Genet. 2016; 59(2):75–9.
Tattersall RB. Mild familial diabetes with dominant inheritance. Q J Med. 1974;43:339–57.
Temple IK, Shield JP. Transient neonatal diabetes, a disorder of imprinting. J Med Genet. 2002; 39(12):872–5.
Thanabalasingham G, et al. A large multi-centre European study validates high-sensitivity C-reactive protein (hsCRP) as a clinical biomarker for the diagnosis of diabetes subtypes. Diabetologia. 2011;54(11):2801–10.
Thanabalasingham G, et al. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. Diabetes Care. 2012;35(6):1206–12.
Thanabalasingham G, et al. Mutations in HNF1A result in marked alterations of plasma glycan profile. Diabetes. 2013;62(4):1329–37.
Transferring Patients with Diabetes due to a KIR6.2 Mutation from Insulin to Sulphonylureas. 2017. Available from: http://www.diabetesgenes.org/content/transferring-patients-diabetes-due-kir62-mutation-insulin-sulphonylureas.
Tuomi T, et al. Improved prandial glucose control with lower risk of hypoglycemia with nateglinide than with glibenclamide in patients with maturity-onset diabetes of the young type 3. Diabetes Care. 2006;29(2):189–94.
van den Ouweland JM, et al. Maternally inherited diabetes and deafness is a distinct subtype of diabetes and associates with a single point mutation in the mitochondrial tRNA(Leu(UUR)) gene. Diabetes. 1994;43(6):746–51.
Walsh R, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet Med. 2017;19(2):192–203.
Yamagata K, et al. Mutations in the hepatic nuclear factor 1 alpha gene in maturity-onset diabetes of the young (MODY3). Nature. 1996a;384:455–8.
Yamagata K, et al. Mutations in the hepatocyte nuclear factor 4 alpha gene in maturity-onset diabetes of the young (MODY1). Nature. 1996b;384:458–60.
(2017) 2. Classification and Diagnosis of Diabetes:. Diabetes Care 41 (Supplement 1):S13–S27

Methods to Assess In Vivo Insulin Sensitivity and Insulin Secretion

10

Riccardo C. Bonadonna, Linda Boselli, Alessandra Dei Cas, and Maddalena Trombetta

Contents

"Ad perceptionem cui certum & indubitatum iudicium possit inniti, non modo requiritur ut sit clara, sed etiam ut sit distincta." (DesCartes 1644a)

[For a perception to support a certain and indubitable judgment, it needs to be not merely vivid, but also clear.] (Descartes 1644b)

(René Descartes, "Principia Philosophiae": I.45; 1644)

R. C. Bonadonna (✉) · A. Dei Cas
Department of Medicine and Surgery, University of Parma, Parma, Italy

Division of Endocrinology and Metabolic Diseases, Azienda Ospedaliera Universitaria of Parma, Parma, Italy
e-mail: riccardo.bonadonna@unipr.it; bonadonna.riccardo@fastwebnet.it; alessandra.deicas@unipr.it

L. Boselli
Department of Medicine, University of Verona, Verona, Italy
e-mail: marialinda.boselli@univr.it

M. Trombetta
Department of Medicine, University of Verona, Verona, Italy

Division of Endocrinology, Diabetes and Metabolic Diseases, Azienda Ospedaliero-Universitaria Integrata of Verona, Verona, Italy
e-mail: maddalena.trombetta@univr.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_11

Abstract

The assessments of insulin sensitivity/resistance and of insulin secretion/beta cell function in humans are still significant challenges. The interest for these traits stems from their proved, or presumed, implications in a great number of pathological conditions, such as obesity, type 2 diabetes, atherosclerosis, nonalcoholic fatty liver disease (NAFLD), Alzheimer's disease. A wide number of tests and surrogate indexes have been developed and applied in many experimental and observational settings to gauge exact values for each of these two traits. In this chapter, the general principles underlying the assessments of insulin sensitivity and of beta cell function are reviewed, and the most popular tests are described with their pros and cons.

Keywords

Beta cell function · Insulin secretion · Insulin sensitivity/resistance · Diabetes Mellitus · Clinical physiology

Introduction

Insulin secretion and insulin action are believed to lie at the heart of the pathogenesis of a great number of pathological conditions, with the common denominator of being particularly widespread nowadays and of showing a worrying trend to an unabated growth in the last decades (Engin 2017). However, the two paradigmatic conditions, in which insulin secretion and action are top players, are, of course, type 1 and type 2 diabetes mellitus (American Diabetes Association 2017).

Since the time of Himsworth (1940), a deep interest to assess both traits has generated a great number of tests and surrogate indexes (Dube et al. 2013; Hannon et al. 2017; Radziuk 2000; Shankar et al. 2016). In spite of these considerable efforts, a significant amount of confusion still exists, so that the primary goal of this chapter is to clarify as much as possible what insulin action and beta cell function are, what measuring them should entail and which tests satisfy these requirements. In doing so, the interest will be limited to insulin sensitivity/resistance and to beta cell function, as measurable without the use of tracers and with relatively noninvasive techniques. Furthermore, the techniques available to measure insulin sensitivity at the organ and/or tissue level will not be reviewed. Doing otherwise would entail to outline the principles of the tracer dilution technique and of the organ balance technique, as well as of positron emission tomography (PET) and of nuclear magnetic resonance (NMR) spectroscopy, plus dwelling in a much greater number of experimental settings than the ones the reader will find here.

Thus, this chapter is far from being exhaustive of its matter, but it is hoped that the reader, and especially the unwary one to whom it is addressed, will find it useful as a prime for a better planning and/or understanding of his/her own research and, possibly, clinical activity. A special emphasis has been posed also on separating the bona fide measures of insulin action and of beta cell function from their surrogate indexes. The latter ones, although very popular and quite frequently employed also in epidemiological and genetic studies, can provide only somewhat limited descriptions of these two traits.

Since a complete and thorough assessment of beta cell function mandates to take into account the prevailing insulin sensitivity, the latter will be presented first.

Measuring Insulin Action: General Considerations

Insulin is a pleiotropic hormone. It acts in the central nervous system to modulate food intake (Dodd and Tiganis 2017), in skeletal and cardiac muscle to increase glucose uptake (Bonadonna et al. 1993a; Ferrannini et al. 1993), in liver and fat tissue to curb the release of glucose and lipolytic products (Bonadonna et al. 1990a; Bergman and Iyer 2017), respectively, in many tissues to guarantee protein homeostasis (Castellino et al. 1987; James et al. 2017), in resistance vessels to induce vasorelaxation (Bonadonna et al. 1998), just to name only a few of its biological actions. For each of these biological effects, one can envision to assess a concentration-response relationship and to compute sensitivity and maximal response to insulin (Groop et al. 1991). Hence, one can find as many insulin sensitivities (resistances) as the number of biological insulin's effects.

Although each of these actions is of clear relevance in human pathophysiology, conventional insulin sensitivity (and resistance) refers to insulin action on glucose metabolism, of which, at the whole body level, two main and distinct effects can be recognized: inhibition of endogenous glucose output and stimulation of glucose utilization (Bonadonna et al. 1990b).

In the footsteps of Jerry Radziuk (2000), one can transform these verbal definitions of insulin sensitivity of glucose metabolism in mathematical formulae:

$$\text{Insulin Sensitivity } (InsSens) = \frac{\partial R}{\partial I} \tag{1}$$

in which R is a measure of the biological response to insulin, I is insulin concentration. The use of partial derivatives is imposed by the very well-known role that glucose concentration per se plays in regulating its own metabolism, the so-called "glucose effectiveness" (Alzaid et al. 1994), often shortened as S_G. This stimulatory effect is strongly nonlinear and is less than proportional to the increase in glucose concentration (Del Prato et al. 1993).

In this formalism, insulin sensitivity is the slope of the curve relating the biological response (y axis) to insulin concentration (x axis). Studies have shown that insulin sensitivity of glucose metabolism follows curvilinear concentration-

response curves, reaching a maximal response for sufficiently high insulin concentration (Bonadonna et al. 1990a, b). This means that *InsSens*, as defined in Eq. 1, has not a fixed value but changes (declines) with increasing insulin concentrations in each individual (Mandarino et al. 2001).

This very general definition can be simplified by assuming to focus only on steady-state conditions (i.e., time invariance of fluxes and concentrations) at a fixed, predetermined glucose concentration. Under these restricted circumstances:

$$InsSens = \frac{\Delta R}{\Delta I} \tag{2}$$

To compare different individuals/groups, steady-state techniques should ensure that Eq. 2 is applied at sufficiently similar insulin (and glucose) concentrations.

Another comment is warranted about *R*. Physicians are accustomed to think that "insulin lowers blood sugar." The fall in "blood sugar," however, is a consequence of the two events which are actually caused by insulin at the cell and organ level: inhibition of glucose production and stimulation of glucose uptake (Bonadonna et al. 1990a, b, 1993b). Thus, the direct biologic *R* to insulin is not the fall in blood glucose, but the changes in glucose fluxes elicited by cell exposure to insulin. Lowering blood glucose is an indirect, not a direct, effect of insulin, in that it is secondary to a fall in endogenous glucose output and/or an increase in glucose utilization.

To sum up, the "true" biologic response to insulin is not a fall in concentration, but a change in glucose flux(es) (endogenous glucose production ± whole body glucose utilization), and its units are $mass \cdot time^{-1}$, e.g., $\mu mol \cdot min^{-1}$.

However, since glucose exerts a strong effect on its own metabolism (Alzaid et al. 1994; Del Prato et al. 1993), at each glucose concentration the flux achieved by any given level of insulin is determined also by glucose itself. Hence, the "pure" biologic response to insulin (*R*) is a flux parameter which does not include glucose mass or concentration (Dube et al. 2013; Radziuk 2000). This parameter is the metabolic glucose clearance rate (GCR; units: $ml \cdot min^{-1}$):

$$R = \frac{Glucose\ Flux}{G} = GCR \tag{3}$$

Equation 3 establishes the need to measure *GCR*. This is of help, because we can exploit the general relationship:

$$Clearance\ Rate = \frac{Dose}{Area} \tag{4}$$

in which *Dose* is a known input of the exogenously administered substance of interest (e.g., an i.v. infusion rate, an i.v. bolus injection) and *Area* is the area under the concentration curve of the substance of interest, with the possible need of some correction factor depending on the format of administration of the substance of interest and on the timing at which the area under the concentration curve is

measured (Lassen and Perl 1979). Please note that when the input is constant over time, and the concentration is constant over time, i.e., under steady-state conditions, Eq. 4 reduces to the familiar relationship endogenous output and/or infusion rate (of glucose) divided by concentration (of glucose) (Lassen and Perl 1979).

Thus, to compute insulin sensitivity, one has to measure insulin-dependent glucose clearance rate (Eqs. 3 and 4) and insulin concentration (Eq. 2).

Insulin resistance is defined as the inverse of insulin sensitivity (Radziuk 2000), i.e.,

$$\text{Insulin resistance } (InsRes) = \frac{\partial I}{\partial R} \tag{5}$$

Under steady-state conditions:

$$InsRes = \frac{\Delta I}{\Delta R} \tag{6}$$

From the above definitions, it follows that the relationship between *InsSens* and *InsRes* is inverse and curvilinear, expected quite often to be a hyperbola.

Owing to its relationship to glucose concentration, *InsSens*(*Res*) can be measured either in steady-state conditions (Eqs. 2 and 6) or with tools which allow to compute the dynamic relationship between insulin concentration, the biologic response to insulin and glucose concentration (Eqs. 1 and 5).

Measuring Net Insulin Sensitivity

Insulin typically exerts a simultaneous effect of inhibition on endogenous glucose output and of stimulation of glucose uptake (Bonadonna et al. 1990a). The algebraic sum of these two actions accounts for the net hypoglycemic action of insulin. Thus, one can focus only on the net hypoglycemic action of insulin, irrespective of the two components which it involves. This means measuring whole body net insulin sensitivity (Gutch et al. 2015). From the experimental viewpoint, a challenge experiment is needed, that is, glucose needs be administered, and by the intravenous route, in order to have a reliable assessment of the amount of glucose entering the systemic circulation and to be able to use Eq. 4.

Three primary measures are required to quantify net insulin sensitivity:

1. Glucose concentration curve
2. Insulin concentration curve
3. Glucose dose (i.e., the glucose input into the systemic circulation)

In Table 1, taken from Gutch et al. (2015), a list of tests of insulin sensitivity/resistance is presented with their pros and cons, as generally perceived. Only one of the tests of Table 1 is a true direct measure of net insulin sensitivity, the euglycemic insulin clamp. However, other three tests, not listed in Table 1, satisfy the above

Table 1 Methods to estimate/assess insulin sensitivity/resistance

Method	Formula	Normal level	Advantage	Disadvantage	Correlation coefficients with HEC
HOMA-IR	$(I_0 \times G_0)/22.5$	<2.5	Simple, minimally invasive, predicts fasting steady-state G and I levels	Insulin sensitivity in subjects treated with insulin needs further validation	Normal glucose tolerance (0.65; $P < 0.0001$), impaired glucose tolerance (0.56; $P < 0.0001$) and with type 2diabetes (0.51; $P < 0.0001$)
QUICKI	$1/\left[\begin{matrix}\log(l\mu\mathrm{U/mL}) \\ +\log(\mathrm{G\,mg/dl})\end{matrix}\right]$	0.382 ± 0.007 for nonobese, 0.331 ± 0.010 for obese and 0.304 ± 0.007 for diabetic individuals	Consistent, precise index of insulin sensitivity, minimally invasive	Normal range to be established for each laboratory due to significant inter laboratory variations in insulin assay	Correlation coefficient 0.78; $P < 2 \times 10^{-2}$
Matsuda index	$10{,}000/\sqrt{(\mathrm{fasting\,G} \times \mathrm{fasting\,I})(\mathrm{mean\,G} \times \mathrm{mean\,I})}$	<4.3 predict IR	Represents both hepatic and peripheral tissue sensitivity to insulin	Its correlation is very weak in diabetic patients	0.73 ($P < 0.0001$) in subjects with normal glucose tolerance, 0.66 ($P < 0.0001$) in subjects with impaired glucose tolerance, and 0.60 ($P < 0.0005$) in nondiabetic subjects, and in subjects with type 2 diabetes mellitus the correlation proved to be weaker 0.54 ($P < 0.0001$)

Hyperinsulinemic euglycemic glucose clamp	$ISI_{HEC} = MCR/I_{mean}$ $MCR = M_{mean}/(G_{mean} \times 0.18)$	Clamp performed at 80 mU/m^2 min, a cutoff of 5.3 mg/kg FFM + 17.7 z min (98% prediction probability) for IR	Direct measure of insulin under steady-state conditions	Laborious, involves intravenous infusion of insulin, frequent blood sampling	Gold standard method for quantifying insulin sensitivity
McAuley index	$_e$(2,63–0,28 In (I_0)-0,31 In (TAG_0)	<5.8	The combination of fasting insulin (mIU/l) and triglycerides (TAG, mmol/l) showed the best prediction of IR	Robust method, suitable for epidemiological studies	≤ 0.63 in diabetic patients
Belfiore index	$2/ISI_{Belfiore} = \frac{G_S}{G_N} \times \frac{I_S}{I_N} + 1$	Values above 1.27 indicate pathological IR	Showed normal value for basal glucose and insulin concentrations and for mean normal value for glucose and insulin areas during OGTT	Multiple blood sampling	0.65; $P < 0.01$ in subjects with normal glucose tolerance, 0.54; $P < 0.01$ in subjects with impaired glucose tolerance, and 0.48; $P < 0.01$ in subjects with diabetes type 2
Avignon index	$Sib = 10^8/\left(\frac{I_0(mU/l) \times G_0}{(mmol/l) \times VD}\right)$ $Si2h = 10^8/\left(\frac{I_{20}(mU/l) \times G_{120}}{(mmol/l) \times VD}\right)$	–	Determines glucose tolerance and insulin sensitivity in single test	Its correlation is very weak in diabetic patients	Normal glucose tolerance (0.89; $P \leq 0.0001$), with impaired glucose tolerance (0.96; $P \leq 0.0001$), and in patients with diabetes mellitus type 2 (0.69–0.83; $P \leq 0.05$)

(*continued*)

Table 1 (continued)

Method	Formula	Normal level	Advantage	Disadvantage	Correlation coefficients with HEC
Stumvoll index	$0.156 - 0.0000459 \times I_{120}$(pmol/L) $- 0.000321 \times I_0$(pmol/L) $- 0.00541 \times G_{120}$(mmol/L)	–	Utilizes demographic data like age, sex, and BMI along with plasma glucose and insulin to predict insulin sensitivity	Very robust and weekly correlate in diabetic patients	Correlation coefficients with HEC were in the range between 0.62 and 0.79 ($P < 0.001$)
Gutt index	75,000 + ($G_0 - G_{120}$) (mg/dl) $\times$ 0.19 $\times$ IBW/120 $\times$ IG_{mean} (0, 120) (mmol/L) $\times$ ILog [I_{mean}(0, 120)] (mU/L)	<45 predict IR	Good to predict onset of type 2 diabetes	Suitable for epidemiological studies	Correlation coefficients with HEC 0.63; $P < 0.001$

From Gutch et al. (2015) with publisher's permission

BW Body weight, *HEC* Hyperinsulinemic euglycemic clamp, *BMI* Basal metabolic rate, *OGTT* Oral glucose tolerance test, *TAG* Triglycerides, *IR* Insulin resistance, *FEM* Fat-free mass, *ISI* Insulin sensitivity index, *MCR* Metabolic clearance rate, $HEC - I_{mean}$ Average steady-state plasma insulin response (μIU/ml), M_{mean} Metabolized glucose expressed as average steady-state glucose infusion rate per kg of BW (mg/kg/min), G_{mean} Average steady-state blood glucose concentration (mmol/l), 0.18: Conversion factor to transform blood glucose concentration from mmol/l into mg/ml. $HOMA\text{-}IR - I_0$ Fasting insulin (mIU/l), G_0 Fasting glucose (mmol/l) concentration, $QUICKI - I_0$ Fasting insulin (mIU/l), G_0 Fasting glucose (mmol/l) concentration, $McAuley\ index - I_0$ Fasting insulin (mIU/l), *TAG* Fasting triglyceride concentration, $Matsuda\ index - I_0$ Fasting plasma insulin concentration (mIU/l), G_0 Fasting plasma glucose concentration (mg/dl), G_{mean} Mean plasma glucose concentration during OGTT (mg/dl), I_{mean} Mean plasma insulin concentration during OGTT (mU/l), 10,000: Simplifying constant to get numbers from 0 to 12, $Belfiore\ index - G_S, G_N$ Plasma glucose concentrations expressed as fasting values or as areas obtained during a standard OGTT at 0 and 2 h (0–2 h areas are equal to $G_{S,N} = G_0 + G_{120}$) or at 0, 1 and 2 h (0–2 h areas are equal to $G_{S,N}$), *Avignon index* I and G represent the plasma concentrations of insulin (mIU/l) and glucose (mmol/l) respectively, VD is the glucose distribution volume calculated using a mono compartmental model: VD = 150 ml/kg of BW, *Stumvoll index* Fasting insulin (mIU/l), G_0 Fasting glucose (mmol/l) concentration, $Gutt\ index - I_0$ Fasting plasma insulin concentration (mIU/l), G_0 Fasting plasma glucose concentration (mg/dl), G_{mean} Mean plasma glucose concentration during OGTT (mg/dl), I_{mean} Mean plasma insulin concentration during OGTT (mU/l)

listed requirements. Three out of four direct measurements of insulin sensitivity/resistance are steady-state assessments:

1. The euglycemic insulin clamp (the gold standard) (DeFronzo et al. 1979)
2. The hyperglycemic clamp (DeFronzo et al. 1979)
3. The insulin suppression test (Shen et al. 1970).

In all three of them, insulin sensitivity/resistance is computed by Eq. 2 or Eq. 6, by directly measuring ΔR and ΔI.

The fourth one is a nonsteady-state method: the IVGTT analyzed by the minimal model introduced by Bergman and Cobelli several decades ago (Bergman et al. 1981).

The Euglycemic Insulin Clamp

This is a steady-state technique, which also controls and matches both insulin concentration and glucose concentration in all study subjects. As shown in Fig. 1, an i.v. primed constant infusion raises insulin concentration up to a predetermined level; glucose concentration is kept constant by an i.v. glucose infusion, the rate of which is regulated every 5 min as per glucose reading, according to a negative feedback principle. As shown in Fig. 1, insulin concentration reaches and maintains a plateau; glucose is kept constant by the i.v. glucose infusion, which with time reaches a plateau as well. At steady state, after correcting for some slight changes in the glucose pool, the rate of glucose infusion (i.e., the Dose of Eq. 4) matches, and is a measure of, the sum of the inhibition of fasting glucose production plus the stimulation of glucose utilization by prevailing insulin concentration (Bonadonna et al. 1993b; DeFronzo et al. 1979). The net biologic response, therefore, is the steady-state rate of glucose infusion corrected for changes of the glucose pool (the *M value* of the original paper by DeFronzo et al. (1979)), normalized by glucose concentration, i.e., the net glucose clearance rate of the exogenous glucose infusion. The steady-state insulin concentration, after subtracting the baseline values, is the ΔI of Eq. 2. Hence:

$$\Delta R = \frac{M\ value}{G} = nGCR \tag{7}$$

where $nGCR$ is the net glucose clearance rate (units: $ml \cdot min^{-1}$)

$$InsSens = \frac{\Delta R}{\Delta I} = \frac{nGCR}{\Delta I} \tag{8}$$

Thus, insulin sensitivity is measured by combining primary measurements and its units are $(ml \cdot min^{-1}/pmol \cdot l^{-1})$. Quite often, since the interindividual variance of steady-state insulin concentration between individuals is rather small owing to the constant i.v. infusion and, by design, glucose is kept constant at very similar levels, the glucose infusion rate is by far the parameter with the greatest variance and the direct determinant of the variability of *InsSens*. Many investigators,

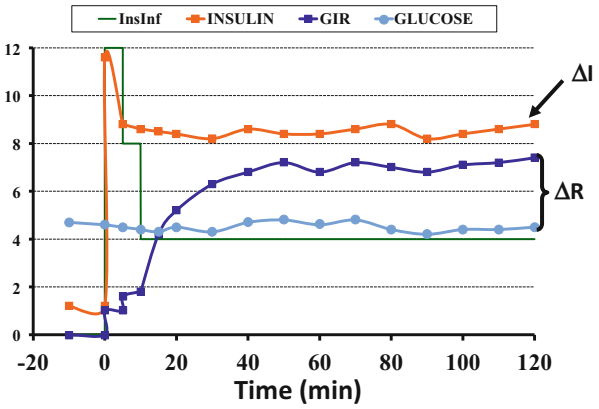


Fig. 1 Schematic description of the euglycemic insulin clamp. One antecubital vein for infusion of tests substances and one wrist/hand vein for sampling of arterialized blood are cannulated. At time 0′ a primed-continuous intravenous infusion of insulin (InsInf; green line) is started (typical rate: 40 $mU \cdot min^{-1} \cdot m^{-2}BSA$) in order to achieve a constant plasma insulin concentration (INSULIN; orange line). Plasma glucose concentration (GLUCOSE; light blue line) is kept constant at fasting euglycemia by measuring it at bedside every 5 min with a glucose analyzer and by adjusting the rate of an intravenous infusion of glucose (GIR; blue line) to maintain plasma glucose constant at fasting euglycemia according to a negative feedback principle. *InsSens* is derived at steady state according to eq. 8 by using GIR and GLUCOSE to compute *R* and INSULIN to compute *I*

therefore, are accustomed to present their own data simply as the *M value* (DeFronzo et al. 1979).

Since its introduction, thousands of papers have reported studies in which this technique was the core of the experimental design. Much of what we know about the pathophysiology of insulin sensitivity/resistance in vivo in man has been achieved through the euglycemic insulin clamp, which is almost unanimously considered as the "gold standard" method to assess insulin sensitivity/resistance.

The Hyperglycemic Clamp

As shown in Fig. 2, the hyperglycemic insulin clamp is based on an i.v. primed glucose infusion which raises and maintains plasma glucose at a desired target above the normal fasting levels by an i.v. glucose infusion in order to elicit a vivacious insulin response. The i.v. glucose infusion rate is adjusted every 5 min as per glucose readings, according to a negative feedback principle (DeFronzo et al. 1979). Please note that at variance with the euglycemic clamp, the only variable which is controlled is glucose, whereas insulin concentration depends on beta cells sensitivity to glucose, and glucose infusion rate depends on insulin sensitivity and beta cell response to glucose.

As in the case of the euglycemic clamp, at steady state, after correcting for some slight changes in the glucose pool and subtracting glycosuria, if present, the rate of i.v. glucose infusion (i.e., the Dose of Eq. 4) is the sum of the inhibition of fasting glucose production plus the stimulation of glucose utilization by prevailing insulin

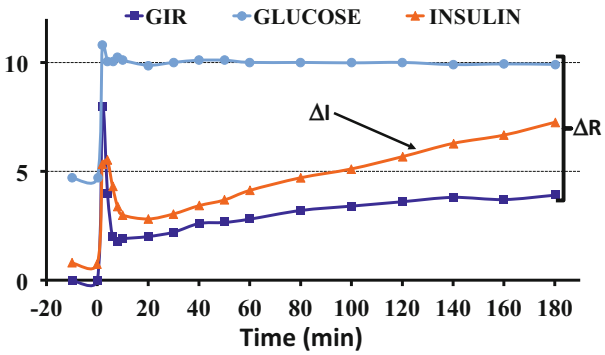


Fig. 2 Schematic description of the hyperglycemic clamp. One antecubital vein for infusion of tests substances and one wrist/hand vein for sampling of arterialized blood are cannulated. At time 0′ a primed intravenous infusion of glucose (GIR; blue line) is started to raise and maintain plasma glucose constant at a predetermined level (e.g., 10 mmol/l) by measuring it at bedside every 5 min, or less, with a glucose analyzer and by adjusting GIR according to a negative feedback principle. The body response to the square wave hyperglycemic stimulus is a time course of insulin concentration (INSULIN; orange line) characterized by an early peak (1st phase insulin response) followed by a fall and a subsequent slow and constant increase (2nd phase insulin response). Towards the end of the clamp, at near steady state, *InsSens* is derived according to eq. 8 by using GIR and GLUCOSE to compute *R* and INSULIN to compute *I*

and glucose concentration. The net biologic response, therefore, is the steady-state rate of glucose infusion, after correction for changes in glucose pool and for glycosuria, if present, divided by glucose concentration, i.e., the net glucose clearance rate (ml·min^{-1}). The steady-state insulin concentration, after subtracting the baseline values, is the ΔI of Eq. 2.

The same line of reasoning of the euglycemic insulin clamp is valid also here. Therefore, also at hyperglycemia:

$$\Delta R = \frac{M\ value}{G} = nGCR \tag{9}$$

$$InsSens = \frac{\Delta R}{\Delta I} = \frac{nGCR}{\Delta I} \tag{10}$$

Differently from the euglycemic clamp, a substantial amount of variability is attributable also to insulin concentrations. Indeed, while in the euglycemic clamp both glucose and insulin levels, two main determinants of the *M value* in the assessment of *InsSens*, are under the investigator's control, in the hyperglycemic clamp only glucose levels are tightly controlled; insulin levels are determined by the sensitivity of the beta cells to glucose and by insulin clearance. There are two consequences:

1. The *M value* cannot be used as a shortcut to assess *InsSens*; in the experimental context of the hyperglycemic clamp, its physiological significance is entirely

different form the *M value* of the euglycemic clamp, and it is akin to glucose tolerance (DeFronzo et al. 1979).

2. For the purpose of comparison, it would be desirable that the insulin concentrations achieved by different individuals/groups during the hyperglycemic clamp do not differ too much; when this requirement is not fulfilled, owing to the inverse relationship between *InsSens* of Eqs. 8 and 10 and insulin concentration, and to the insulin levels at which ΔR is assessed, people who display higher insulin response at the same hyperglycemic stimulus will undergo some underestimation of their insulin sensitivity.

However, also in this case, the investigator gains access to readily measurable variables which allow for a straightforward assessment of *InsSens*.

The Insulin Suppression Test

This test (Shen et al. 1970) has been less widely used than the clamp techniques. Briefly, an i.v. triple infusion of somatostatin, insulin, and glucose at predetermined constant rates is performed, and glucose/insulin are measured during the third hour of infusion in near steady-state conditions (Fig. 3). Somatostatin inhibits endogenous islet secretion; hence, insulin levels are quite similar between subjects. Since glucose infusion rate is the same, the *M value*, after correction for glycosuria, is similar between subjects. The variable which is left free to change is the steady-state plasma glucose concentration (*SSPG*), which has been proposed as the primary indicator of the net biologic response of the body to insulin (Shen et al. 1970).

Please notice that the previous formulae retain their validity also in this case. Therefore, also during the insulin suppression test:

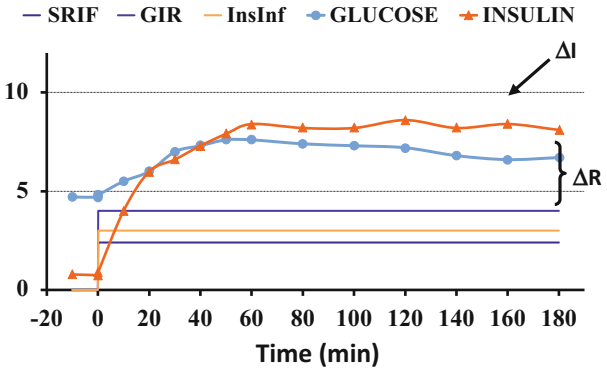


Fig. 3 Schematic description of the insulin suppression test. One antecubital vein for infusion of tests substances and one wrist/hand vein for sampling of arterialized blood are cannulated. At time 0′ three continuous intravenous infusions of glucose (GIR; blue line), insulin (InsInf; yellow line) and somatostatin (SRIF; blue line) are started and maintained until end of study. Plasma insulin (INSULIN; orange line) and glucose (GLUCOSE; light blue line) concentrations are measured at near steady state in the last 30 min of the test. *InsRes* is derived according to eq. 6 and 13 by using GIR and GLUCOSE to compute *R* and INSULIN to compute *I*

$$InsSens = \frac{\Delta R}{\Delta I} = \frac{nGCR}{\Delta I} \tag{11}$$

However, results of the insulin suppression test are not expressed as insulin sensitivity, but as SSPG, and the higher SSPG the higher is insulin resistance (Shen et al. 1970). Since:

$$InsRes = \frac{1}{InsSens} \tag{12}$$

Then, in the insulin suppression test:

$$InsRes = \frac{G \cdot \Delta I}{M\ value} \tag{13}$$

Equation 13 explains the rationale of using SSPG as an index of insulin resistance. In the insulin suppression test, ΔI and *M value* (except for the correction due to glycosuria) are similar in all subjects by experimental design; hence, steady-state plasma glucose (G in Eq. 13) is the parameter which primarily determines the between subject variance: although it is not a formal measure of insulin resistance, *SSPG* is the primary experimental metrics which reflects insulin resistance, in analogy to the relationship between the *M value* and *InsSens* in the euglycemic clamp.

Selecting a metrics of insulin resistance to present the results of the insulin suppression test is the scientifically correct choice. In the insulin suppression test (Fig. 3), the investigator constrains under experimental control both the net balance between inhibition of glucose uptake and stimulation of glucose utilization and the insulin concentration and interrogates the body regarding the glucose level which it will achieve under these experimental conditions. The question the insulin suppression test asks to the body is: what is your insulin resistance level? (For the sake of simplicity, glucose effectiveness (S_G) here is neglected, but since different SSPG are attained by different individuals, the reader should be aware that S_G is embedded in the numbers which quantify insulin resistance as assessed by the insulin suppression test.)

In contrast, in the euglycemic insulin clamp (Fig. 1), the investigator constrains under experimental control the concentrations of both glucose and insulin and interrogates the body regarding the net balance between inhibition of glucose uptake and stimulation of glucose utilization (which, at steady state, equals glucose infusion rate) it will achieve under these experimental conditions. The question the euglycemic insulin clamp asks to the body is: what is your insulin sensitivity level?

The theory predicts (Eq. 12) that the relationship between independent measures of insulin sensitivity and insulin resistance is inverse, curvilinear, and one resembling a hyperbola (Radziuk 2000). Empirical confirmation of this prediction was provided by plotting the results of the euglycemic insulin clamp (primary experimental measure: glucose infusion rate, i.e., insulin sensitivity) versus the results of the insulin suppression test (primary experimental measure: glucose concentration, i.e., insulin resistance), both performed in the same subjects (Fig. 4) (Greenfield et al. 1981).

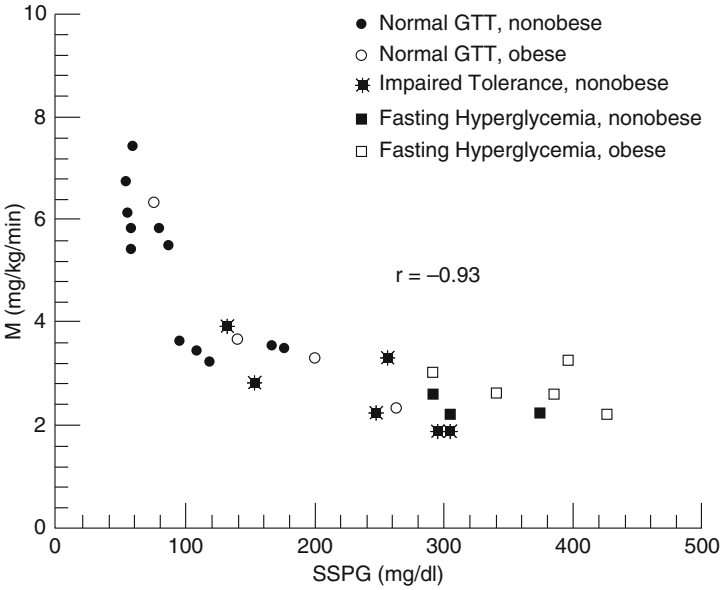


Fig. 4 Hyperbolic relationship between insulin resistance as measured by the insulin suppression test (SSPG; x axis) and insulin sensitivity (*M value*; y axis) as measured by the euglycemic insulin clamp (From Greenfield et al. (1981): publisher's permission requested)

Even this case is not perfect. Independently of any other factor, including insulin sensitivity/resistance, the higher the glucose concentration, the lower the glucose clearance is (Del Prato et al. 1997). Thus, individuals with higher glucose concentrations during the insulin suppression test are prone to an underestimation of their insulin sensitivity. Furthermore, a reduction in "glucose effectiveness" (S_G), i.e., glucose mediated glucose metabolism, is a well-established pathophysiologic feature of diabetes mellitus (Alzaid et al. 1994; Del Prato et al. 1997); hence, the higher the glucose in these patients, the greater is the role played by defective S_G and the greater is the underestimation of their insulin sensitivity.

The IVGTT

This is a nonsteady-state test. A glucose challenge (typically, 12 $g \cdot m^{-2}$ of body surface area) is rapidly injected into a peripheral vein, and blood samples are collected to measure glucose and insulin at progressively wider time intervals (Bergman et al. 1981) (Fig. 5).

As a nonsteady-state technique, Eq. 1 is the one to apply, and mathematical models are to be used, most, if not all, of which are related to the first minimal model introduced decades ago by Bergman and Cobelli (Bergman et al. 1981). For the purpose of a complete, quantitative description of the glucose-insulin system amount and timing of injected glucose (i.e., Dose of Eq. 4) are to be carefully measured. If this step is omitted, models to analyze the IVGTT are available, as in

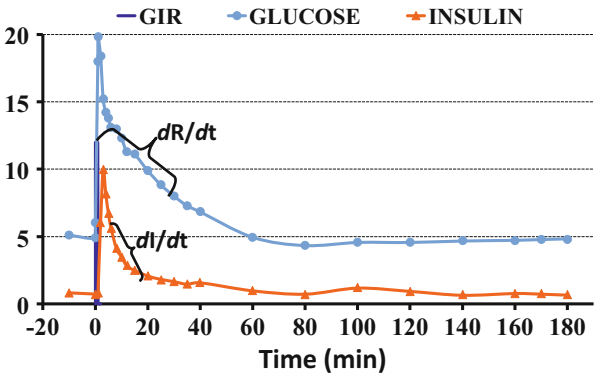


Fig. 5 Schematic description of the IVGTT for measuring insulin sensitivity. One antecubital vein for infusion of tests substances and one wrist/hand vein for sampling of arterialized blood are cannulated. At time 0′ a fast intravenous injection of glucose (GIR; blue line) (typical dose: $12\ g \cdot m^{-2}BSA$) is performed and blood is frequently sampled to measure plasma glucose (GLUCOSE; light blue line) and insulin (INSULIN;orange line) concentrations for 180′–240′. Several investigators have added at 20′ an i.v. injection of tolbutamide or insulin to further boost insulin concentration and to reportedly improve the assessment of *InsSens*. Mathematical modeling is employed to assess *InsSens* starting from the glucose bolus and the plasma glucose curve to compute *dR*/*dt* and from the plasma insulin curve to compute *dI*/*dt*

the seminal paper (Bergman et al. 1981), but the biologic response they analyze is not glucose fluxes, rather it is based on the glucose concentration dynamics.

This form of IVGTT, with no use of glucose tracer, parallels a number of features of the previous tests, in particular the one that the biologic response to insulin is the net balance of inhibition of endogenous glucose output and stimulation of glucose utilization (Bergman et al. 1981). However, the model explicitly takes into account the role of glucose per se, which, in more recent modeling evolutions, is described as strongly nonlinear (Vicini et al. 1997; Trombetta et al. 2013). Eventually, insulin sensitivity is found as:

$$InsSens = \frac{\partial nGCR}{\partial I} \tag{14}$$

in which, apart of the partial derivative symbol, the reader can easily find the familiar ratio *nGCR*/*I* which quantifies net insulin sensitivity (Fig. 6).

When the amount and the timing of glucose injected i.v. is neglected or not measured (Bergman et al. 1981), which, for the sake of keeping the experiment as simple as possible, happens in most cases, the canonical *InsSens* of Eq. 14 cannot be computed because glucose fluxes and *nGCR* cannot be estimated. In this case, strictly speaking, *R*, the net biologic response to insulin, i.e., the net balance between inhibition of glucose production and stimulation of glucose utilization, cannot be determined and insulin sensitivity cannot be quantified. This obstacle is circumvented with this line of reasoning (Bergman et al. 1981). The immediate consequence of insulin action on glucose production and/or on glucose utilization is

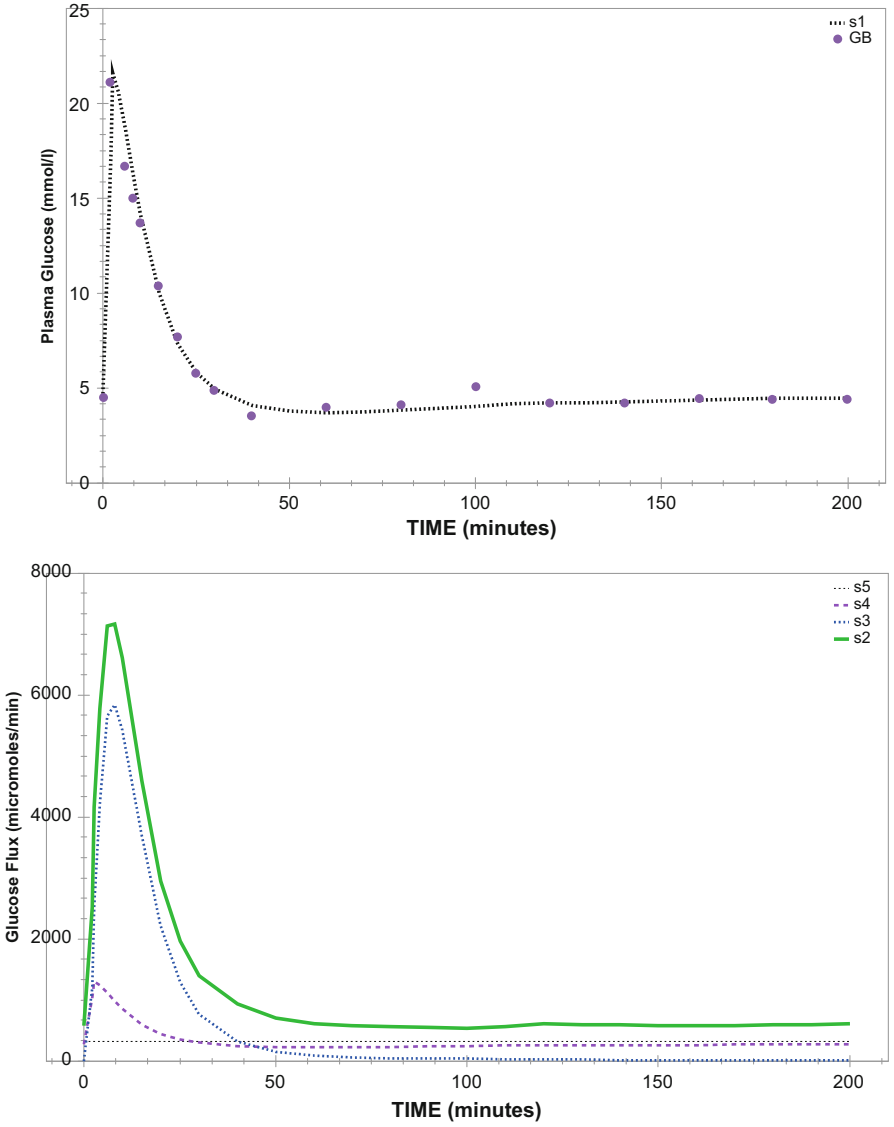


Fig. 6 Mathematical modeling of an IVGTT to assess insulin sensitivity. Left panel. Minimal model fit (black dotted line) of plasma glucose concentration (y axis; violet circles) versus time (x axis) after an IVGTT of 12 $g \cdot m^2 BSA$ in an obese individual with normal glucose tolerance. Right panel. Minimal model computation of glucose fluxes (y axis; $\mu moles \cdot min^{-1}$) during the IVGTT depicted in the left panel: total glucose disposal (s2; green continuous line), insulin-dependent glucose disposal (s3; blue dotted line), glucose effectiveness (S_G) dependent glucose disposal (s4; violet dotted line), and insulin-independent glucose disposal (s5; black dotted line) are plotted versus time (x axis). In this individual *InsSens* [units: $(ml \cdot min^{-1} \cdot m^{-2} BSA)/(pmol \cdot l^{-1})$] was 0.295, S_G [units: $(ml \cdot min^{-1} \cdot m^{-2} BSA)/(mmol \cdot l^{-1})$] was 38.4 and the volume of distribution of glucose [units: $(l \cdot m^{-2} BSA)$] was 3.77 (unpublished data)

a change in the glucose pool (units: $moles \cdot min^{-1}$). The immediate proxy of the change in the glucose pool is the change in glucose concentration (the latter is equal to *glucose pool/glucose volume of distribution*), which in turn is due to the net fractional glucose clearance, which equals *nGCR/glucose volume of distribution* (units: min^{-1}). Thus, all equations are written using glucose concentration instead of glucose mass. In this setting:

$$InsSens = \frac{\partial nfGCR}{\partial I} \tag{15}$$

in which *nfGDR* is the net fractional glucose clearance rate and the units of *InsSens* are $min^{-1}/(pmol \cdot l^{-1})$ (Bergman et al. 1981).

Several studies have demonstrated that, if correctly carried out, the IVGTT can provide the same quantitative information as the euglycemic clamp (Beard et al. 1986; Finegood et al. 1984), with the advantage that there is no need to clamp glucose concentration.

Furthermore, the IVGTT is a "closed loop test," in that the glucose-insulin system, with its negative feedback loop, is completely free to respond to the challenge (Trombetta et al. 2013). The three previous tests are "open loop" tests, because the glucose-insulin system has no more the full control of glucose and insulin concentrations. More specifically:

(i) In the euglycemic clamp, neither glucose nor insulin concentrations are free to change (DeFronzo et al. 1979).
(ii) In the hyperglycemic clamp, only insulin is free to change (DeFronzo et al. 1979).
(iii) In the insulin suppression test, only glucose is free to change (Shen et al. 1970; Greenfield et al. 1981).

In several settings a closed loop test may be more appropriate than an open loop test (clamps, insulin suppression test) (Dube et al. 2013).

A Special Case: The Intravenous Insulin Tolerance Test

The IVITT is a nonsteady state, relatively simple, and quick method to assess insulin sensitivity/resistance. In the fasting state, insulin (0.1 U/kg, up to 16 U) is injected intravenously and blood glucose is measured in the following 15 min, to compute the rate at which it falls, according to a monoexponential (i.e., single compartment) model (Bonora et al. 1989). In mathematical notation:

$$G(t) = G_{0'} \cdot e^{-k \cdot t} \tag{16}$$

in which $G_{0'}$ is glucose concentration at time $0'$ and the parameter k measures the fraction of the glucose pool which disappears in 1 min owing to insulin action. Thus,

k is a net fractional glucose clearance rate, entirely attributed to insulin action, i.e., in the IVITT:

$$InsSens_{IVITT} = k_{IVITT} = Net\ Fractional\ Glucose\ Clearance\ Rate = nfGCR \quad (17)$$

This equation, compared to Eqs. 14 and 15, neglects the role of glucose effectiveness and, in insulin sensitive individuals in whom glucose always reaches the hypoglycemic range, any significant influence of counterregulatory hormones. Furthermore, in contrast with Eqs. 14 and 15, it assumes that the variance in insulin concentration after the intravenous insulin bolus is negligible, a move which is very similar to what investigators do when they use the *M value* of the euglycemic insulin clamp as the measure of insulin sensitivity.

The units of k_{IVITT} are neither $(ml \cdot min^{-1})/(pmol \cdot l^{-1})$, the canonical units of insulin sensitivity, nor $min^{-1}/(pmol \cdot l^{-1})$, the units of *InsSens* in Eq. 15, but min^{-1}. Indeed, the IVITT is an attempt to quantify the numerator of Eq. 15, which is the *InsSens* quantified by the IVGTT analyzed by the minimal model of insulin sensitivity, when no quantitative information about the glucose i.v. injection is available or used (Bergman et al. 1981). All these (untold) assumptions detract from the accuracy of the IVITT.

In spite of these theoretic limitations, the IVITT has been repeatedly shown to correlate very well with gold standard measures of insulin sensitivity (Bonora et al. 1989; Graci et al. 1999). Its use, thus far, has been rather rare, possibly because, although it is of brief duration, people undergoing an IVITT require to be monitored for much longer time than 15′ in order to prevent hypoglycemia, which in any case, in individuals with high insulin sensitivity, may be quite profound even within the frametime of 15′ of the IVITT.

Indexes of Insulin Sensitivity/Resistance

The above described tests, although being the first step of in vivo assessment of insulin sensitivity, in that no use of glucose tracers is needed, are expensive, time-consuming, and burdensome on the patient and on the investigator. All these reasons have fueled the interest in trying and validating simpler ways to assess insulin sensitivity/resistance (Table 1). Only some of them will be reviewed in detail. From a conceptual viewpoint, they can be classified as steady-state (fasting) indexes and dynamic (usually after an oral glucose challenge) indexes.

Biomarkers of Insulin Sensitivity/Resistance Derived in the Fasting State

The HOMA insulin resistance index (*HOMAIR*) is the prototype of the fasting, steady-state surrogate indexes of insulin resistance. It was first derived as a result of a complex theoretic analysis of glucose regulation in the fasting state (Matthews et al. 1985). From a conceptual viewpoint, it belongs to the same framework of the previous tests. Indeed, according to Eq. 13, at steady state, during an i.v. glucose infusion:

$$InsRes = \frac{G \cdot \Delta I}{M\ value} \tag{18}$$

During a physiologic steady state, Eq. 13/18 is valid, but the *M value* is substituted for by endogenous glucose fluxes. In the postabsorptive steady state, the flux of glucose metabolized is equal to the endogenous glucose output and most of glucose utilization is insulin independent. Conversely, endogenous glucose output is exquisitely sensitive to liver sinusoidal insulin. Thus, in the fasting state, Eq. 13/18 becomes:

$$InsRes_{fasting} = \frac{G \cdot lsI}{EGO} \tag{19}$$

in which *lsI* is insulin concentration ($pmol \cdot l^{-1}$) in liver sinusoids. Liver sinusoidal insulin is not a simple measure to perform. It can be inferred exploiting the following equation:

$$lsI = \frac{ISR + I \cdot HPF}{HPF} \tag{20}$$

in which *ISR* is insulin secretion rate ($pmol \cdot min^{-1}$), *I* is peripheral insulin concentration ($pmol \cdot l^{-1}$), and HPF is hepatic plasma flow ($l \cdot min^{-1}$); furthermore:

$$HPF = PPF + HAPF \tag{21}$$

in which *PPF* is portal plasma flow ($l \cdot min^{-1}$) and HAPF is hepatic artery plasma flow ($l \cdot min^{-1}$). Measuring peripheral C-peptide allows to estimate ISR (see the following section on "Beta Cell Function"). *PPF* can be measured noninvasively, e.g., by Doppler US scan and hematocrit, whereas *HAPF* is a minor component of *HPF*, of which it can be assumed to be a fixed fraction.

Endogenous glucose output (EGO) can be measured by the tracer dilution technique, with a variety of labeled glucoses, of which 6,6-^{2}H-D-glucose currently is the best combination between safety and convenience.

Not surprisingly, there are rare examples of measurements of $InsRes_{fasting}$.

Investigators have assumed that *I* (but see the following section on "Beta Cell Function") can be considered a good proxy of *lsI*. Furthermore, since EGO is assumed to vary within a relatively narrow range, its contribution to the variance of $InsRes_{fasting}$ may be considered minor. Thus:

$$InsRes_{fasting} \propto G \cdot I \tag{22}$$

Indeed, the time honored first equation of *HOMAIR*, which the investigators arrived at as a result of a complex homeostatic model linking glucose and insulin (Matthews et al. 1985), is:

$$HOMAIR = \frac{G \cdot I}{k} \tag{23}$$

in which k is a constant which normalizes the value of HOMA-IR to the median value of a normal healthy population. When glucose units are mmol·l^{-1} and insulin units are mU·l^{-1}, $k = 22.4$, and the median value of *HOMAIR* in a normal healthy population should be around 1.0. This quite often is not the case, because the number 22.4 was the product of median G and median I in the control population of the original paper. It was recommended that every investigator should build his/her own control population and compute the value of k relevant to his/her study setting (Matthews et al. 1985). This suggestion has been regularly overlooked.

The equations of *InsRes* and *HOMA-IR* are similar, both mathematically and conceptually, but the difference between $InsRes_{fasting}$ and *HOMAIR* also is quite evident. Recently, the HOMA model was expanded and improved equations (HOMA2) were provided to compute *HOMA2IR* as well as *HOMA2beta* for beta cell function (Hill et al. 2013). A software (iHOMA2) also is available for download to easily compute both *HOMA2IR* and *HOMA2beta* (Oxford DTU-Uo).

HOMAIR is simple, cheap, and well correlated to net insulin sensitivity as measured by he euglycemic insulin clamp (Bonora et al. 2000), which, in its standard format, measures both peripheral and liver insulin sensitivity. Furthermore, as predicted by the theory, its relationship with clamp derived insulin sensitivity is inverse and curvilinear and becomes linear after double logarithmic transformation (Bonora et al. 2000). However, even the best reported correlation coefficients between *HOMAIR* and clamp measured insulin sensitivity rarely account for more than 50% of the *InsSens* variability (Bonora et al. 2000; Mather et al. 2001). Even making allowances for the day-to-day coefficient of variation of *InsSens* which may be as high as about 20% (Mather et al. 2001), it is clear that a large part of *InsSens* variability is not captured by *HOMAIR*. This is by no means surprising. The relationship between *HOMAIR* and clamp measured *InsSens* is driven by the very frequent biologic coexistence of hepatic and peripheral insulin resistance. When this does not hold, e.g., in people with impaired fasting glucose (IFG), *HOMA-IR* is unable to track peripheral insulin sensitivity/resistance.

Other fasting steady-state indexes include *QUICKI* (Quon 2002), the fasting insulin resistance index, plus the addition of lipid biomarkers selected with the purpose of enhancing the relationship with the gold standard *InsSens* as measured by the euglycemic clamp. None of these indexes seems to be clearly and/or consistently superior to *HOMA-IR*. In particular:

$$QUICKI = \frac{1}{\ln G + \ln I} = \frac{1}{\ln(G \cdot I)} \tag{24}$$

A simple glance to Eqs. 22 and 23 unveils the close mathematical relationship between *HOMAIR* and *QUICKI*; they convey exactly the same amount of information, derived from identical primary measures, but, in the case of *QUICKI*, under logarithmic disguise. Not surprisingly their nonparametric correlation coefficient is bound to be -1. It should be emphasized that the inverse relationship between *HOMA-IR* and *QUICKI* is only a mathematical tautology, whereas the inverse relationship between *InsRes*, as measured by the insulin suppression test, and

InsSens, as measured by the euglycemic insulin clamp, is the experimental proof that Eqs. 11, 12, and 13 adequately describe biology (Greenfield et al. 1981).

A recent report proposed a novel fasting, metabolomic derived, biomarker, compounded by fasting insulin, alpha-hydroxybutyrate (α-HB), 1-linoleoylglycerophosphocholine (L-GPC), and oleate, which was named Quantose™ (M^Q). This novel biomarker was found to be superior to *HOMA-IR* in detecting people with insulin resistance, but to be similar to the dynamic index *OGIS* (see below) in predicting the progression from normal to impaired glucose tolerance. M^Q as a fasting steady-state index appears to be superior to other time honored steady-state indexes (Cobb et al. 2013). However, it still is surrogate index and it comes at the cost of measuring three compounds (α-HB, L-GPC, and oleate), the assay of which is not readily available to most laboratories.

OGTT-Derived, or MTT-Derived, Indexes of Insulin Sensitivity

At least three indicators derived from insulin and glucose values during the OGTT have gained rather wide popularity: the Matsuda Index (Matsuda and DeFronzo 1999), the Stumvoll index (Stumvoll et al. 2000), and the oral glucose insulin sensitivity index (*OGIS*) (Mari et al. 2001a). There are conceptual difference among these three indexes. The Matsuda index was thought of as a sort of sophisticated *HOMAIR* measured with glucose and insulin levels during the OGTT (Matsuda and DeFronzo 1999). The Stumvoll index was derived as the best set of OGTT-derived predictors of clamp measured insulin sensitivity (Stumvoll et al. 2000). *OGIS* was found as a set of equations, based on a simplified model of the glucose-insulin system, which would have the best predictive power towards *InsSens* as measured by the euglycemic insulin clamp (Mari et al. 2001a). They require to carry out an OGTT; hence, they are clearly more cumbersome and costly than fasting steady state derived surrogate indexes. With the exception of *OGIS* (*Mari* et al. 2005), they were not shown to be superior to *HOMAIR* in capturing insulin resistance and/or predicting the onset of impaired glucose tolerance.

However, when it is necessary to portray both insulin sensitivity and insulin secretion and "gold standard" tests cannot be used, it is important to be able to estimate insulin sensitivity and beta cell function with sets of primary data which are independent of each other, a hardly feasible task with the use of fasting steady-state tests only.

While the above mentioned indexes have never claimed to be actual direct measurements of insulin sensitivity, in the last two decades minimal models similar to those employed for the analysis of the IVGTT have been proposed and rather thoroughly used with the goal of measuring insulin sensitivity during OGTTs (Salinari et al. 2011) or MTTs in which no use of glucose tracers is made.

The extension of the minimal model of net insulin sensitivity to OGTT and MTT (Dalla Man et al. 2002, 2005a), when no glucose tracer(s) is (are) used, requires an additional number of quantitative assumptions, especially regarding the dynamics of oral glucose appearance into the peripheral circulation (Dalla Man et al. 2002), and the estimated/assumed values of glucose effectiveness (S_G), glucose volume of distribution (Dalla Man et al. 2005b; Visentin et al. 2015), and splanchnic glucose extraction (Dalla Man et al. 2002, 2004).

All these factors, which are unmeasurable without the aid of glucose tracer(s) and/or invasive/complex experimental designs, mark a clear divide between intravenous and oral challenge tests. In the former ones, the information content of the data is self-sufficient to assess net insulin sensitivity/resistance; in the latter ones, the information content of the data is too limited to directly assess net insulin sensitivity/resistance without the aid of a number of relevant assumptions. Indeed, insulin sensitivity yielded by the oral models is fairly well correlated to those obtained by the IVGTTs, but it may overestimate insulin sensitivity (Dalla Man et al. 2002, 2005b; Caumo et al. 2000; Steil et al. 2004), as assessed by the same IVGTT. Furthermore, insulin sensitivity provided by the OGTT model has been reported to be lower than insulin sensitivity measured by the insulin clamp (Dalla Man et al. 2005c) and higher than insulin sensitivity estimated by the MTT model (Bock et al. 2007).

Thus, the minimal models of the oral challenge tests, when no glucose tracers are employed, can provide relevant information, but health and research professionals should not trust them with the same level of confidence that the intravenous tests (euglycemic and hyperglycemic clamp, insulin suppression test, and the IVGTT analyzed by the minimal model) deserve. Whether they actually are a significant improvement in comparison to the Matsuda Index, the Stumvoll Index or *OGIS*, is currently unclear.

Measuring Insulin Secretion: General Considerations

Insulin plays a unique role in glucose metabolism, being the only hormone which physiologically has a net glucose-lowering effect. Other hormones, such as IGF-1 and IGF-2, become relevant for their glucose-lowering potential only in pathological states.

This unique role of insulin and the high prevalence of diabetes mellitus explain the enormous interest that measuring insulin secretion has always raised.

In the former section devoted to insulin sensitivity, first the general definitions were stated and thereafter the most popular tests to assess insulin sensitivity/resistance were presented. Subsequently, surrogate indexes have been introduced.

In this specific case, since the most popular tests (see below) have already been presented, most of the attention will be devoted to definitions and equations. Surrogate indexes will follow. A more comprehensive list of the available tests to assess insulin secretion/beta cell function is provided by Table 2, which is taken from Shankar et al. (2016) in which the interested reader can find additional details.

Another important point needs be clarified. People quite often use insulin secretion and beta cell function (almost) as synonyms. This is a misconception.

Measuring insulin secretion is a pure description: it requires to quantify insulin output ($pmol \cdot min^{-1}$) by the pancreatic beta cells.

Measuring beta cell function implies to link insulin secretion to a physiologic goal, i.e., to assess a relationship. Not surprisingly, the same level of appropriateness of beta cell function may be met in different individuals/states with hugely different insulin secretion rates, and vice versa.

Table 2 Methods to assess beta cell function

Test	Description	Advantages	Limitations
Hyperglycemic clamp	A variable IV glucose infusion is administered to maintain the glucose level at a steady state	Provides measures of insulin secretion (first and second phases) and with modeling insulin action	No GI incretin effects
			Requires continuous adjustment of IV glucose
	Frequent blood sampling and minute-to-minute adjustments of glucose infusion rate at bedside are required	Does not require modeling of data for insulin secretion	Technically challenging to conduct testing
		Widely reported and accepted	Expertise limited to select centers
Graded glucose infusion	IV glucose is administered at progressively increasing rates (each rate maintained for ~40 min)	Provides measure of insulin secretion over a range of glucose levels	No GI incretin effects
			Not as widely studied and reported as hyperglycemic clamp, especially in the context of therapeutic interventions
		Provides measure of β-cell glucose sensitivity	
	Requires frequent blood sampling		Data analyses often require expertise in model-based methods
FSIGT	Rapid IV injection of glucose is followed 20 min later by an IV injection of insulin	Provides insulin secretion and action during rapidly changing glucose levels	No GI incretin effect
			Technically challenging to conduct
		Provides first-phase insulin release measures	Expertise to conduct limited to select centers
	Requires very frequent blood sampling		
		Insulin action results correlate well with those from euglycemic clamp	Requires computer modeling for the outcome measures,

(*continued*)

Table 2 (continued)

Test	Description	Advantages	Limitations
			requiring specialized expertise
		Widely used and reported	
		With C-peptide modeling, provides second-phase insulin release	Requires IV administration of insulin
AST	IV arginine is administered followed by combined glucose/arginine infusions	Measures of insulin secretion known to correlated with β-cell mass in islet transplant recipients	Mixed effect on incretin response
			Requires IV administration of arginine and glucose
	Frequent blood samplings over a short period of time are necessary	Provides a measure of near-maximal insulin secretion (insulin secretory reserve)	Does not inform on insulin action
Glucagon stimulation test	IV glucagon is given twice sequentially (at baseline and after glucose has been infused to achieve elevated glucose)	Robust insulin secretory response similar to that of arginine but through different mechanism of action	No oral incretin effect
			Requires IV administration of glucagon
			Does not inform on insulin action
			Side effects of nausea and vomiting are common and potentially confounding
MMTT/OGTT	Oral meal or glucose solution is ingested	Easy to administer	Assumptions must be made for rate of nutrient absorption into systemic circulation
		Effect of incretins included	
	MMTT physiologically highly relevant, mimicking oral challenges routinely encountered daily	Provides insulin secretion and action during changing glucose levels	

(*continued*)

Table 2 (continued)

Test	Description	Advantages	Limitations
			Technically challenging to model outcome measure of insulin secretion of sensitivity, requiring software and expert analysis
		OGTT standardized and simple as single substrate	
	Blood samples taken at specified intervals up to 5 h postchallenge	Insulin action and secretion results correlate with those from hyperglycemic and euglycemic clamps	
			Lack of standardized test meal
			MMTT with minimal modeling not as widely reported as the hyperglycemic and euglycemic clamps

From Shankar et al. (2016) with publisher's permission
IV intravenous, *GI* gastrointestinal, *FSIGT* frequently sampled intravenous glucose test *AST* i.v. arginine stimulation test, *MMTT* mixed meal tolerance test, *OGTT* oral glucose tolerance test

There are four tests which have gained more and more vast popularity to assess insulin secretion/beta cell function.

Two of them use the intravenous route of administration of the stimulus (IVGTT and hyperglycemic clamp); in the other two (the oral glucose tolerance test and the meal tolerance test), the stimulus is administered by the oral route. Hence, according to its traditional definition (Seufert 2017), the incretin system (and its contribution to glucose tolerance) is present only in the latter pair of tests. Furthermore, with the oral route of administration, two other factors – carbohydrate digestion/glucose absorption and first pass splanchnic glucose extraction – play major roles in determining amount and shape of the glucose challenge which reaches the systemic circulation.

Measuring Insulin Secretion/Beta Cell Function

The relationship between insulin secretion rate and insulin concentration is rather intricate due to the peculiar anatomy and the prominent role played by the liver in insulin catabolism.

The first point to clarify is that, at any given moment, in the systemic circulation:

$$Insulin\ Concentration(I) \propto \frac{Post_{Hepatic}\ Insulin\ Delivery\ Rate}{Insulin\ Clearance} \tag{25}$$

in which:

$$\begin{aligned} &Post_{Hepatic}\ Insulin\ Delivery\ Rate \\ &= (1 - Hepatic\ Fractional\ Extraction) \\ &\cdot [Insulin\ Secretion\ Rate + (I \cdot Hepatic\ Plasma\ Flow)] \end{aligned} \tag{26}$$

Rearranging and posing HFE = Hepatic Fractional Extraction of insulin, ISR = Insulin Secretion Rate and HPF = Hepatic Plasma Flow = $PPF + HAPF$:

$$I \propto (1 - HFE) \cdot \frac{[ISR + (I \cdot HPF)]}{Insulin\ Clearance} \tag{27}$$

At steady state, proportion becomes equality:

$$I = (1 - HFE) \cdot \frac{[ISR + (I \cdot HPF)]}{Insulin\ Clearance} \tag{28}$$

Equations 27 and 28 unveil that insulin concentration is directly proportional to insulin secretion rate, but also that it displays a negative linear relationship with hepatic fractional extraction of insulin and an inverse curvilinear relationship with insulin clearance. (Insulin clearance is defined as the clearance acting on the insulin molecules entering the systemic circulation; see following equation for a formal mathematical definition.)

Finally:

$$Insulin\ Clearance = (HFE \cdot HPF) + \{NHFE \cdot [CO \cdot (1 - Hct) - HPF]\} \tag{29}$$

in which $NHFE$ = Non-hepatic Fractional Extraction, i.e., insulin fractional extraction by the body tissues excluding liver, CO = cardiac output, Hct = hematocrit.

Insulin concentration of Eqs. 27 and 28 is the one which bathes all body tissues, but one, which plays a prominent role in glucose homeostasis: the liver. The latter (lsI) has been already derived in Eq. 20.

At the end of these preliminary considerations, it is clear from Eqs. 27 and 28 that insulin concentration is only a proxy of insulin secretion rate.

Insulin Secretion Rate

Early methods relied on the use of radiolabeled insulin in humans (Navalesi et al. 1978). Since the site of administration necessarily is through a peripheral vein, these methods could quantify only the posthepatic insulin delivery rate of Eq. 25. Unless ethically unacceptable methods (i.e., sampling portal vein blood to measure portal insulin levels) are employed, quantitation of insulin secretion rate was a sort of Holy Grail of clinical physiology. The situation changed with the introduction of the use of C-peptide to assess insulin secretion rate (Polonsky et al. 1986a).

C-peptide is an ideal tool to derive insulin secretion rate at the beta cell level because:

1. It is secreted in equimolar amounts to insulin (Rubenstein et al. 1969).
2. Its first pass extraction by the liver is negligible (Polonsky et al. 1983).
3. Its kinetics
   (a) is relatively "slow" (fewer samples) (Polonsky et al. 1986a, b)
   (b) is linear (Polonsky et al. 1986a, b)
   (c) can be derived from population-based equations (Van Cauter et al. 1992; Varghese et al. 2017)

The point 3c is critical because it relieves the investigators from assessing C-peptide kinetics in each individual by separate experiments which would entail the i.v. infusion of human C-peptide (Polonsky et al. 1986a).

Thus, the primary data needed to assess insulin secretion rate by the beta cells are a time series of C-peptide concentrations measured during the time window and/or the test of interest. The kinetics of C-peptide is best described by a two-compartment model (Polonsky et al. 1986a). The values of the kinetic parameters can be computed in each individual according to (Van Cauter et al. 1992). Knowing the kinetic parameters and measuring the C-peptide curve allow one to carry out the deconvolution of C-peptide (insulin) secretion rate, i.e., to step from concentration ($pmol \cdot l^{-1}$) up to secretion rate ($pmol \cdot min^{-1}$) (Polonsky et al. 1986a; Van Cauter et al. 1992).

Several algorithms are available to compute insulin secretion rate by deconvolving time series of C-peptide concentrations. In the appropriate experimental setting, this operation allows to gain significant insights.

For instance, since the very first experiments with the hyperglycemic clamp, it is common knowledge that in vivo in humans beta cells respond to a square wave hyperglycemic stimulus with a first and a second insulin secretion phase (DeFronzo et al. 1979). The evidence for this was the shape of the insulin concentration curve (Fig. 7), but Eq. 27 shows us that insulin secretion rate is just one of the determinants, albeit the prominent one, of insulin concentration. Indeed, neither hepatic fractional extraction nor insulin clearance has a fixed value, but they display nonlinear relationships to insulin concentration and they become less and less efficient the higher insulin concentration is (Pye et al. 1993). The same Eq. 27 shows us that comparing insulin concentrations amongst individuals or groups and assuming that differences or similarities closely match insulin secretion rates is hardly tenable.

Here the use of C-peptide is of great help, and it allows to compute insulin secretion rate minute by minute. In several studies of hyperglycemic clamps, deconvolution of C-peptide time series has demonstrated the presence of two temporally distinct waves of insulin secretion. During a typical OGTT in an individual with normal glucose regulation, computation of the insulin secretion rate does not discriminate between first and second phase insulin secretion, but, for instance, with the appropriate experimental design, it lends itself to quantify the incretin effect, as shown in Fig. 8 (Muscelli et al. 2008).

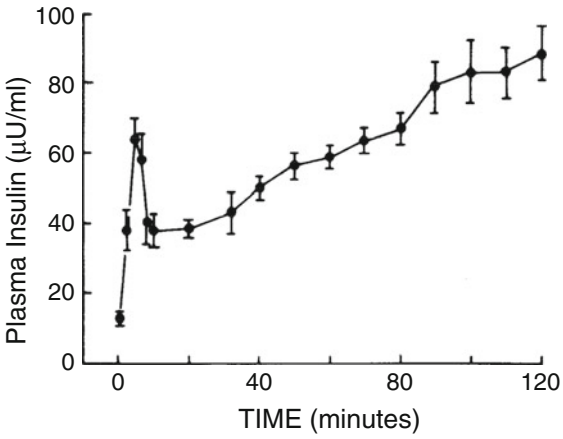


Fig. 7 Plasma insulin concentration during the hyperglycemic clamp. From DeFronzo et al. (1979): publisher's permission requested. It can be appreciated the presence of the typical early peak (1st phase insulin response), followed by a fall and a subsequent slow progressive rise, until a near steady state is achieved (2nd phase insulin response)

However, limiting the investigation of beta cell function to the pure insulin secretion rate often provides apparently paradoxical results. In Fig. 9 the median insulin secretion rates during the OGTT in individuals with progressively worsening glucose tolerance from normal glucose regulation to type 2 diabetes mellitus are plotted. The results draw a curve with a downward concavity, which in the past was interpreted as a progressive attempt of the beta cells to counteract insulin resistance. This compensation eventually fails and ushers in type 2 diabetes (DeFronzo 1988). This interpretation neglects that beta cells need be evaluated in their physiologic context (Stumvoll et al. 2003).

As shown in Fig. 10, the beta cell provides immediate response (insulin secretion) to a variety of signals, foremost among them plasma glucose concentration (Rutter et al. 2015). Insulin action is then determined by its catabolism (liver extraction and insulin clearance) and by cell sensitivity to the hormone. Insulin action, herein defined as the net result of insulin secretion, insulin catabolism and net insulin sensitivity, is not the only determinant of glucose concentration: insulin-independent glucose utilization (i.e., brain and red blood cells) and glucose-dependent glucose metabolism, i.e., glucose effectiveness (S_G) sensu stricto, also play a role. (The roles played by a number of signals, hormones and substrates in influencing glucose levels are neglected for the sake of simplicity.) Furthermore, as already mentioned above, if carbohydrates/glucose are administered through the oral route, digestion/absorption and first pass splanchnic glucose extraction also play a significant role.

In an ideal world, the beta cell function could be quantified at different hierarchical levels.

The first level is represented by its capability to respond to plasma glucose (and also other stimuli), i.e., the beta cell as a glucose sensor/transducer. This level should be *conditio* sine qua non to proceed to the following levels, in that the beta cell metrics assessed at this level should be the ones to be employed at the higher levels.

At the second level, there is the performance of the beta cell±insulin catabolism in precisely matching body's insulin sensitivity according to a negative feedback

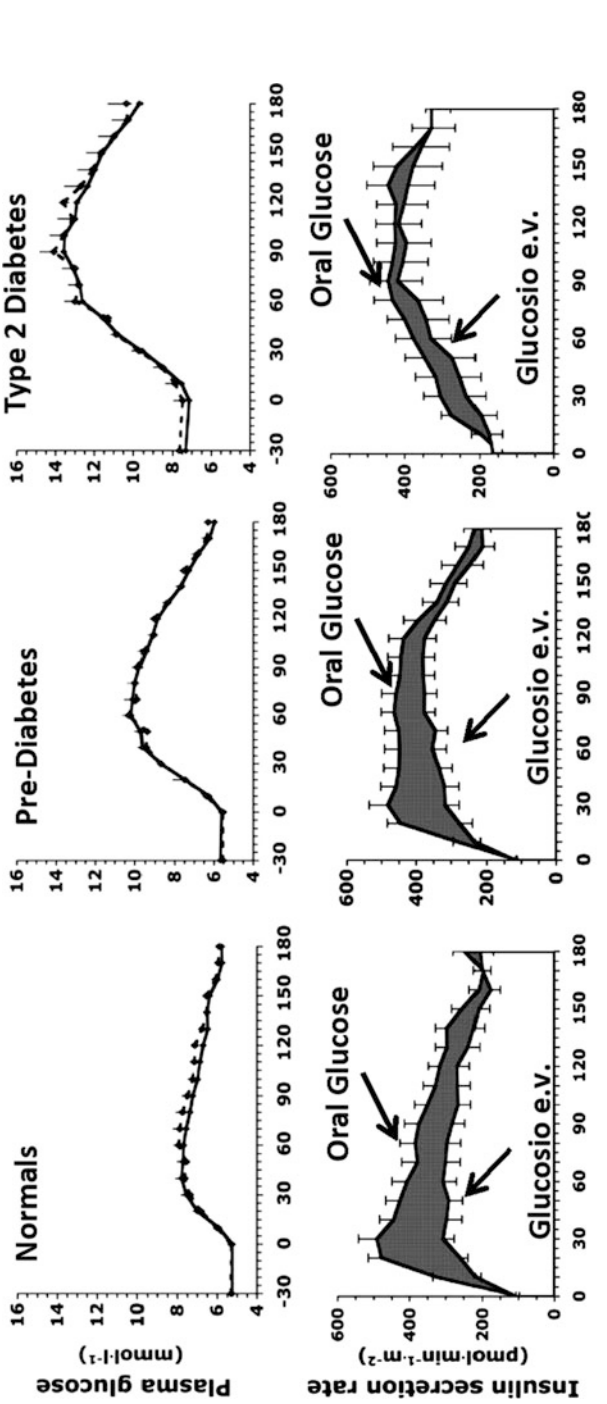


Fig. 8 Measuring insulin secretion rate to assess the incretin effect. Subjects (normals, people with prediabetes, and people with type 2 diabetes) are studied on two different occasions. In the first study they undergo a standard OGTT. In the second study, glucose is infused intravenously at a variable rate to precisely match the glucose concentration curve observed during the OGTT. Upper panels. Glucose concentration (y axis) versus time (x axis) in the two different studies: the two curves are superimposable in the three groups. Lower panels. Insulin secretion rates (y axis), obtained form deconvolution of C-peptide curves, versus time (x axis) in the two studies: secretion rates of the OGTT day are significantly higher than the i.v. glucose day; the gray area highlights the difference between oral and intravenous glucose and quantifies the incretin effect, which is found to be significantly lower going from normalcy to pre-diabetes, and form pre-diabetes to type 2 diabetes (From Muscelli et al. (2008): publisher's permission requested)

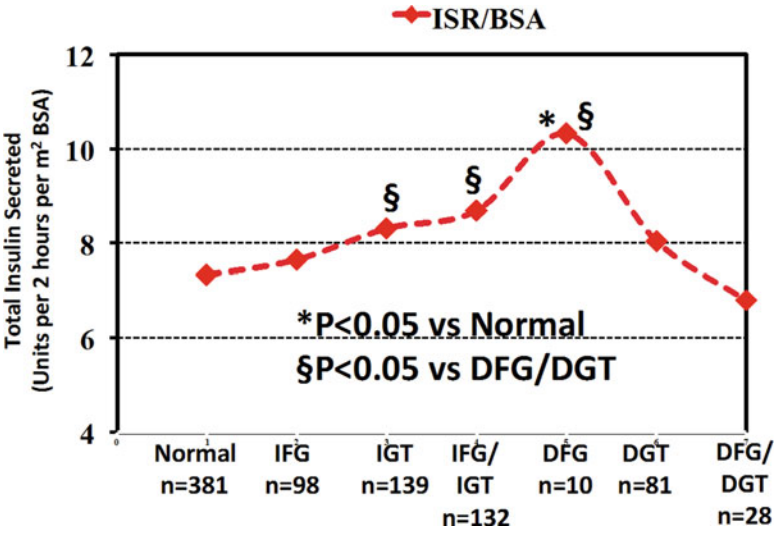


Fig. 9 Total insulin secretion rate during the first 120 min of a standard OGTT across the spectrum of glucose tolerance. Medians of the total amount of insulin secreted over the first 120 min of a standard OGTT (y axis; International Units of insulin per m^2 of BSA) versus the glucose regulation status (x axis). IFG: people with impaired fasting glucose and normal glucose tolerance; IGT: people with normal fasting glucose and impaired glucose tolerance; IFG/IGT: people with impaired fasting glucose and impaired glucose tolerance; DFG: people with diabetic fasting glucose and nondiabetic glucose tolerance; DGT: people with nondiabetic fasting glucose and diabetic glucose tolerance; people with diabetic fasting glucose and diabetic glucose tolerance. § $P < 0.05$ or less versus Normals; $P < 0.05$ versus IFG/IGT and versus DGT. Unpublished data of the GENFIEV Study (https://clinicaltrials.gov/ct2/show/NCT00879801?term=Genetics+pathophysiology&cond=type+2+diabetes&cntry1=EU%3AIT&rank=1)

principle (the lower insulin sensitivity, the higher the response of beta cell to glucose, and vice versa). Here, the metrics are required to assess the beta cell as the preserver, or the guardian, of insulin action on glucose metabolism.

At the third, and highest, level, there is the performance of the beta cell in maintaining glucose homeostasis, i.e., in precisely matching all determinants of glucose regulation. Here, the metrics need to assess the beta cell as the preserver, or the guardian, of glucose homeostasis.

These multiple layers of increasing complexity unveil that assessing the role of the pancreatic beta cell in glucose regulation is a challenge belonging to the realm of systems biology (Bergman et al. 2014), which should be met with the tools of systems analysis (Trombetta et al. 2013).

The Pancreatic Beta Cells as a Glucose Sensor/Transducer

The basic function of the beta cell is the one of a glucose sensor, which responds to glucose with a more or less appropriate output of insulin. Thus, the challenge is to quantify the relationship between the glucose stimulus and the insulin secretion elicited by it.

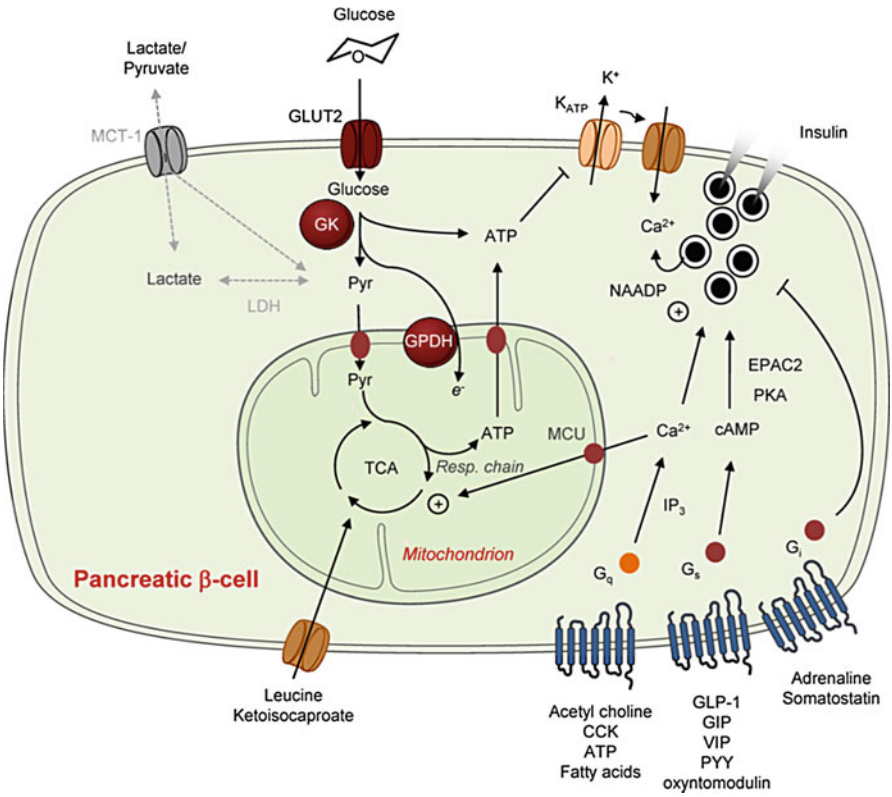


Fig. 10 Pancreatic beta cell glucose sensing, and response to secretory potentiators and inhibitors (From Rutter et al. (2015): publisher's permission requested)

From the experimental viewpoint, this asks the investigator to measure two time series during a test of beta cell function:

1. C-peptide curve
2. Glucose curve

These two sets of primary data must then coupled to each other, and this requires to elaborate a mathematical description (model) of the relationship between glucose and insulin secretion rate in vivo in humans.

In the last two decades, this challenge was met by the converging efforts of different laboratories (Breda et al. 2001; Mari et al. (2001b, 2002); Toffolo et al. 2001; Toschi et al. 2001), which have resulted into models with a very high degree of commonality. Indeed, these models share the following structure:

1. There is a basal rate of insulin secretion (ISR_{Basal}), which is detected in the fasting, unperturbed state.

2. After a glucose (carbohydrate) challenge, the beta cell, as a glucose sensor, responds with an insulin secretion rate which is the sum of two distinct components:
   (a) Derivative (or dynamic) control (DC): a secretory response which is proportional to the rate of increase of glucose concentration, i.e.,:

$$ISR_{DC} \propto \frac{dG}{dt} \rightarrow \text{if } \frac{dG}{dt} > 0; \text{otherwise } ISR_{DC} = 0 \tag{30}$$

   (b) Proportional (or static) control: a secretory response which, above a glucose threshold, is proportional to glucose concentration, i.e.,:

$$ISR_{PC} \propto G \tag{31}$$

In summary, at any moment, total insulin secretion rate by the beta cells is described by the following formula:

$$ISR = ISR_{Basal} + ISR_{DC} + ISR_{PC} \tag{32}$$

It should be noted that both derivative and proportional control are governed by plasma glucose concentration, i.e., they are sensitive to glucose. Hence, compact descriptors of beta cell function are the glucose sensitivity of derivative control and the glucose sensitivity of proportional control.

There is one additional detail which differentiates the models proposed by the two world leading labs. One group adds to the above described structure a "potentiation factor" which is suggested to be the mathematical counterpart of the influence exerted by incretins on the beta cells in vivo (Toschi et al. 2001). The other group introduces a "time constant," i.e., a delay, in the proportional (static) control, i.e., the secretory response which is proportional to glucose concentration (Breda et al. 2001). Independently of their differences, both models have enjoyed a widespread application and seem to be equivalent in describing beta cell behavior during meal challenges.

It should be noted, however, that the model with a time constant of proportional control can describe very well the i.v. glucose tests, such as the IVGTT (Basu et al. 2003) (Fig. 11) and the hyperglycemic clamp (Weiss et al. 2005) (Fig. 12), and the oral tests, such as the OGTT (Fig. 13) and the MTT: one could say that "one model fits all tests."

In our experience, in many instances, a better description of the hyperglycemic clamp can be achieved by adding also a component of insulin secretion proportional to the integral of the hyperglycemic stimulus (ISR_{IC}). Eq. 32 becomes:

$$ISR = ISR_{Basal} + ISR_{DC} + ISR_{PC} + ISR_{IC} \tag{32a}$$

In this format, the model describes the beta cell as a typical PID (proportional-integrative-derivative) controller (Steil and Grodsky 2013). A PID model also has

been proposed and, reportedly, is in use in some devices, for the closed loop of the artificial pancreas (Laxminarayan et al. 2012). These studies have also shown that the derivative control is the component which accounts for first phase insulin secretion during the IVGTT and the hyperglycemic clamp, whereas the proportional control is responsible of second phase insulin secretion (Figs. 11 and 12).

A huge body of papers shows that, when explored by the present tools, the beta cell functional mass shows a constant decline, going from normal glucose regulation to diabetes, through each of the intermediate stages of impaired glucose regulation (Weiss et al. 2005). Even within the normal glucose regulation stage, the higher is plasma glucose the lower is, for instance, the derivative control of beta cell function (Bonadonna et al. 2003). Furthermore, first degree relatives of people with type 2 diabetes show a reduction in the derivative control of beta cell function even in the stage of normal glucose regulation (Bonadonna et al. 2003).

The exact significance of the beta cell assessment provided by these models needs be detailed. What these models measure is the beta cell functional mass, which, as a first approximation, can be thought of as:

$$\begin{aligned} \textit{Beta Cell Functional Mass} = \textit{Beta Cell Mass} \\ \cdot \textit{Average Beta Cell Function} \end{aligned} \tag{33}$$

Thus, the assessment provided by the current tools is like measuring the area of a rectangle. In the absence of an independent measure of beta cell mass, it is impossible to dissect out the relative contribution of each of these two components to the total functional mass. In the last years, significant steps forward have been made towards in vivo quantitative imaging of beta cell mass, especially with the use of beta cell, or islet cell, specific radiotracers suitable for PET (Eriksson et al. 2016).

If and when successful, these attempts will provide tools, which, combined with the above described functional assessments, allow to investigate the natural history of beta cell mass and (dys)function in several disorders, as well as the impact of different therapies on them.

Through the decades, a number of proposals can be found which aim to introduce functional biomarkers of beta cells as proxies of beta cell mass. For instance, the superimposition of the arginine stimulus during a hyperglycemic clamp is considered by many to be a "maximal response" which reflects beta cell mass (Hannon et al. 2017; Shankar et al. 2016). The acute insulin response during a standardized IVGTT similarly is used to quantify residual functional mass after beta cell transplantation (Rickels et al. 2007) in patients with type 1 diabetes. In pancreatic diabetes, the acute C-peptide response during the OGTT has been reported to be a good biomarker of beta cell area (Meier et al. 2009). Similarly, C-peptide is considered the best biomarker of residual beta cell mass in people with type 1 diabetes (Greenbaum et al. 2012; Oram et al. 2014, 2015).

While these tools are likely to be helpful in very well characterized and limited situations, their validity cannot be generalized. In each of these cases, a relationship between the biomarker and the beta cell mass may be found, because in vivo tests always challenge beta cell functional mass, of which beta cell mass is a major, but

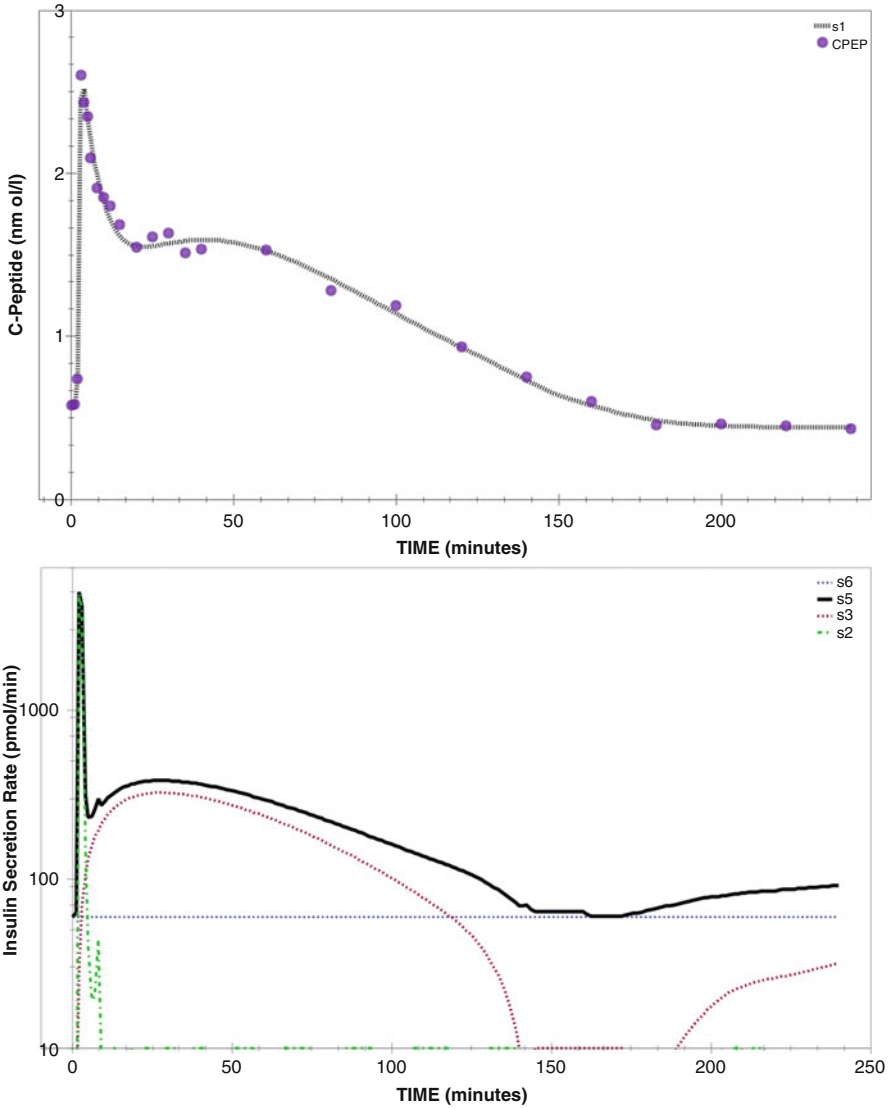


Fig. 11 Mathematical modeling of an IVGTT to assess beta cell function. Left panel. Minimal model fit (black dotted line) of plasma C-peptide concentration (y axis; violet circles) versus time (x axis) after an IVGTT of 12 $g \cdot m^2 BSA$ in a nonobese individual with normal glucose tolerance. Right panel. Minimal model computation of insulin secretion rate (y axis: please note the logarithmic scale; $pmoles \cdot min^{-1}$) versus time (x axis) during the IVGTT depicted in the left panel: total insulin secretion rate (s5; black continuous line), basal insulin secretion rate (s6; blue dotted line), insulin secretion rate due to derivative control (1st phase insulin secretion) (s2; green dotted line), insulin secretion rate due to proportional control (2nd phase insulin secretion) (s3; red dotted line) (unpublished data)

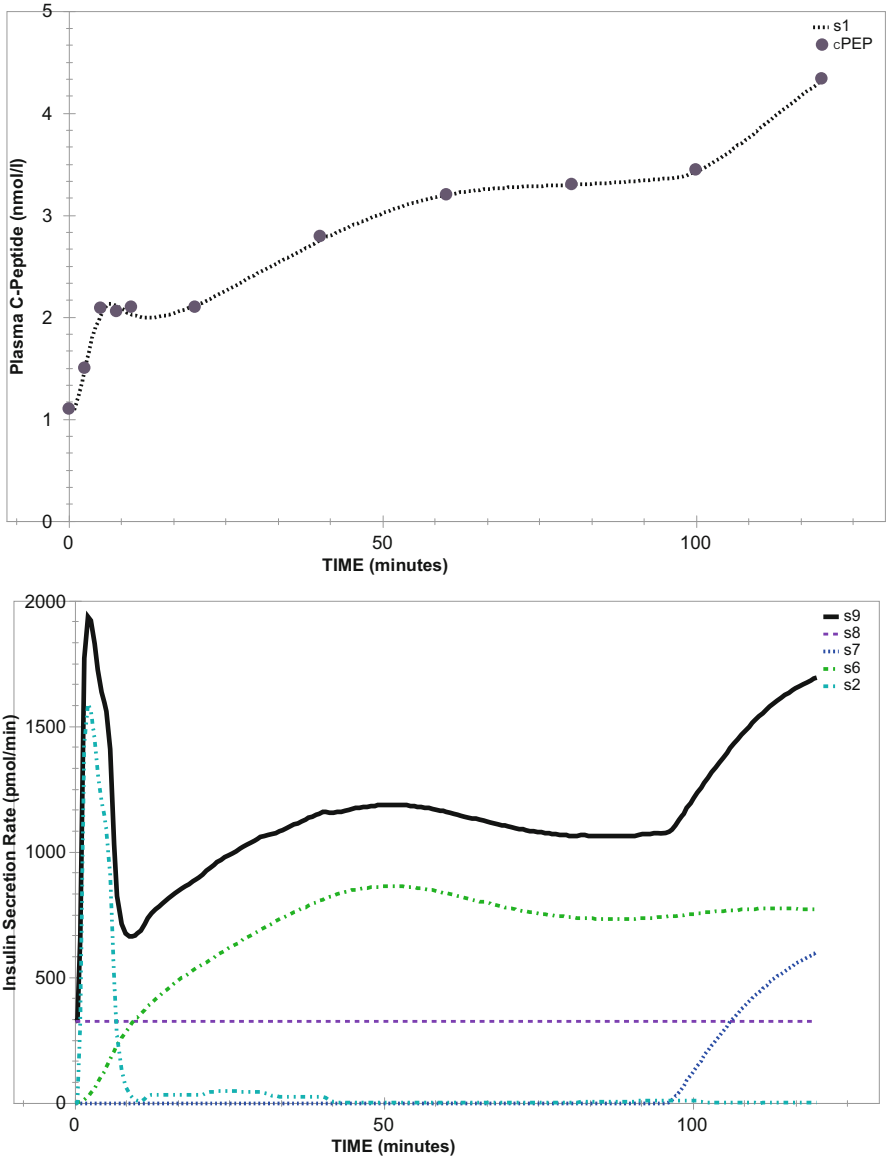


Fig. 12 Mathematical modeling of a hyperglycemic clamp to assess beta cell function. Left panel. Minimal model fit (black line) of plasma C-peptide concentration (y axis; violet circles) versus time (x axis) after a hyperglycemic clamp at 10 $mmol \cdot l^{-1}$ glucose concentration. Right panel. Minimal model computation of insulin secretion rate (y axis; $pmoles \cdot min^{-1}$) versus time (x axis) during the hyperglycemic clamp depicted in the left panel: total insulin secretion rate (s9; black continuous line), basal insulin secretin rate (s8; violet dotted line), insulin secretion rate due to derivative control (1st phase insulin secretion) (s2; light blue dotted line), insulin secretion rate due to proportional control (2nd phase insulin secretion) (s6; green dotted line), insulin secretion rate due to integrative control (s7; blue dotted line) (unpublished data)

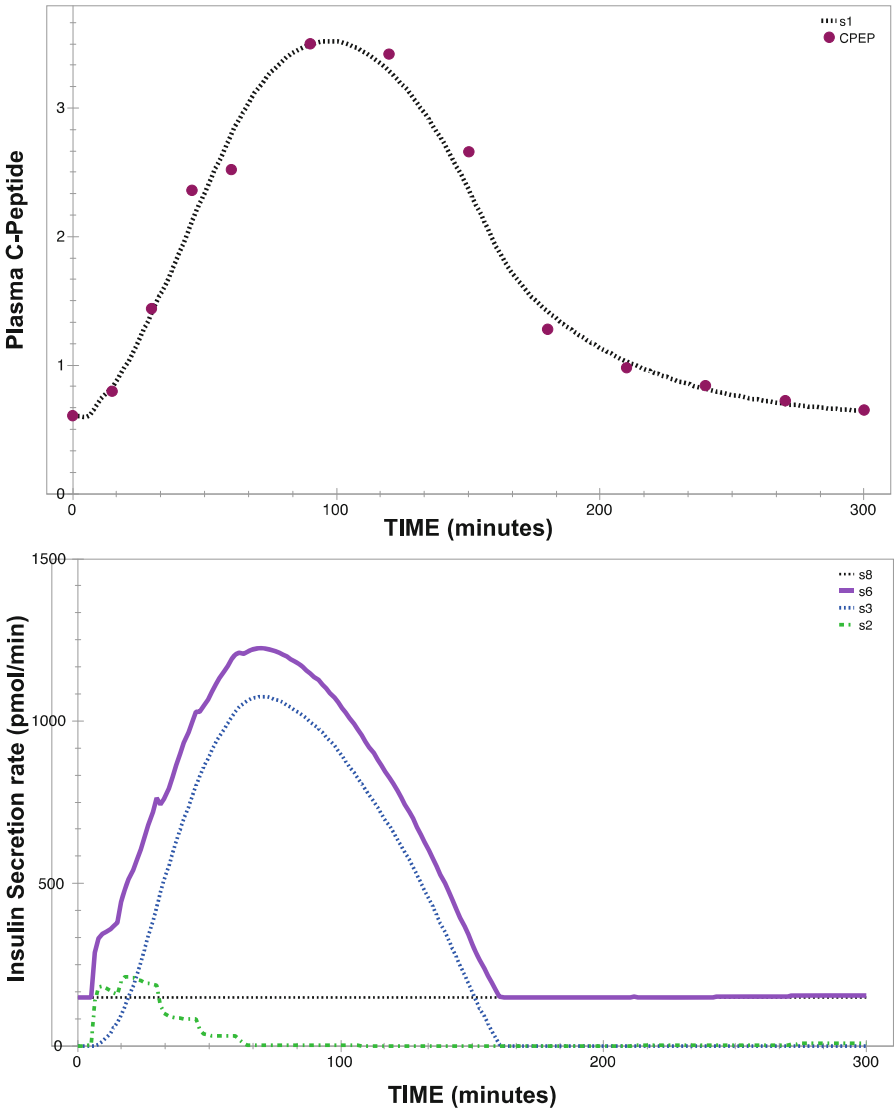


Fig. 13 Mathematical modeling of an OGTT to assess beta cell function. Left panel. Minimal model fit (black dotted line) of plasma C-peptide concentration (y axis; violet circles) versus time (x axis) after a standard OGTT in a subject individual with type 2 diabetes mellitus. Right panel. Minimal model computation of insulin secretion rate (y axis; $pmoles \cdot min^{-1}$) versus time (x axis) during the OGTT depicted in the left panel: total insulin secretion rate (s6; violet continuous line), basal insulin secretion rate (s8; black dotted line), insulin secretion rate due to derivative control (1st phase insulin secretion) (s2; green dotted line), insulin secretion rate due to proportional control (2nd phase insulin secretion) (s3; blue dotted line) (unpublished data)

not solitary, determinant. Even the claim of a "maximal response" cannot be endorsed, because it still is unclear how to elicit the maximal response in vivo by the beta cells. Earlier papers have shown that, by superimposing a GLP-1 stimulus over hyperglycemia+arginine, one further amplifies the beta cell secretory response (Fritsche et al. 2000), and it is unknown whether summing another secretory stimulus on top of the triplet hyperglycemia+arginine+GLP-1 can foster further increases in the beta cell secretory response.

The Pancreatic Beta Cell as the Guardian of Insulin Action on Glucose

As the only endocrine cell secreting insulin, the function of beta cell is intertwined with insulin action. In their seminal paper introducing the minimal model of the IVGTT (Bergman et al. 1981), Bergman and Cobelli postulated that in healthy humans there is an inverse curvilinear (hyperbolic) relationship between insulin sensitivity, assessed as insulin action on fractional net glucose clearance rate, and beta cell function, assessed by an index ϕ_2, which quantified the glucose sensitivity of second phase insulin secretion and can be considered an ancestor of proportional (or static) control of beta cell function (Bergman et al. 1981). However, it should be noted that ϕ_2 was the slope relating insulin concentration (not secretion rate) to glucose concentration, when the latter exceeds a threshold value. Bergman, Phillips, and Cobelli proposed to name the product of insulin sensitivity times ϕ_2 as "disposition factor." They postulated that a physiologic negative feedback underlined the hyperbolic relationship between ϕ_2 and insulin sensitivity and showed that the "disposition factor" was the best discriminator between people with normal and people with poor glucose tolerance (Bergman et al. 1981).

After a decade, and a number of studies which showed that in humans beta cell function physiologically adjusts itself to changes in insulin sensitivity, Kahn et al. reported that there is an inverse hyperbolic relationship between insulin sensitivity, as measured by the IVGTT, and acute insulin response (AIR), measured as the area under the curve of insulin concentration during the first 10 min following glucose injection (In the same paper, Kahn et al. show that the same type of relationship holds also for other tests of beta cell function. For the sake of brevity, these data are not discussed and the interested reader is referred to the original publication.) (Kahn et al. 1993).

Hence, since the general equation of a hyperbola is $xy = k$, the product AIR ($pmol \cdot l^{-1}$) [there are two ways to present AIR: one is the average insulin concentration during AIR, and this is the one we use in this chapter; the other is the area under the curve of AIR ($pmol \cdot min \cdot l^{-1}$)] times insulin sensitivity, again measured as insulin action on net fractional glucose clearance rate (see Eq. 15), should be a constant, physiological value which quantifies the capability of beta cell to cope with body insulin sensitivity. It was named "disposition index," because it quantifies the capability of beta cell to promote the decline in plasma glucose (Kahn et al. 1993).

Hence,

$$Disposition\ Index = AIR \cdot Insulin\ Sensitivity = AIR \cdot S_I \tag{34}$$

The units of the Disposition Index of (Kahn et al. 1993) and Eq. 34 and of the Disposition Index (Factor) of (Bergman et al. 1981) are different: for the former they are min^{-1}, for the latter they are $l \cdot mmol^{-1} \cdot min^{-1}$.

Since both AIR (Kahn et al. 1993) and ϕ_2 (Bergman et al. 1981) were presented as measures of beta cell function, a sort of general principle was derived (please note that this equation will be shown to be incorrect a few lines ahead):

$$\begin{aligned} Disposition\ Index &= Beta\ Cell\ Function \cdot Insulin\ Sensitivity \\ &= \frac{Beta\ Cell\ Function}{Insulin\ Resistance} \end{aligned} \tag{35}$$

In the footsteps of Eq. 35 a great number of papers have used almost any possible combination of indexes of beta cell function and insulin sensitivity or resistance to compute a "disposition index." Special attention should be devoted to their discussion and interpretation, but this is not always the case.

It should be noted that in Kahn et al. (1993) AIR, instead of ϕ_2 (Bergman et al. 1981), was selected because in a significant number of the IVGTTs of that study insulin secretion was boosted with an i.v. injection of tolbutamide 20′ after the glucose challenge. While this experimental maneuver is believed to improve the assessment of insulin sensitivity during the IVGTT, it makes it impossible to quantify ϕ_2.

According to the equation adopted, the physiological meaning of the disposition index varies. The original "disposition factor" (Bergman et al. 1981) quantifies the net hypoglycemic effect of the insulin concentration which the body reaches in response to a unitary (1 mg/dl or 1 mmol/l) increase in plasma glucose by virtue of second phase insulin secretion. The original "disposition index" by Kahn et al. quantifies the average net hypoglycemic effect of AIR, which in turn is mostly, but not entirely, due to first phase insulin secretion (Kahn et al. 1993). Thus, these two Disposition Indexes, and their pathophysiological meaning, are not identical to each other, and some caution should be exerted in interpreting them. Furthermore, in both cases the metrics of beta cell function is insulin concentration, not secretion rate.

Thus, the disposition index, as per Eq. 34, actually is the net result of insulin secretion rate, insulin catabolism, and insulin sensitivity, and hence, it sums the roles played by beta cells and insulin catabolism in matching body insulin sensitivity. On one hand, the disposition index is quite useful because it measures the amount of insulin action that the body is capable to put in motion. On the other hand, it is not a measure of the capability of beta cell functional mass to precisely match insulin sensitivity. Stated otherwise, Eq. 35 is an incorrect descriptor of the Disposition Index, and the correct relationship is the following one:

$$Disposition\ Index \propto \frac{Beta\ Cell\ Function \cdot Insulin\ Sensitivity}{Insulin\ Catabolism} \tag{36}$$

or

$$Disposition\ Index \propto \frac{Beta\ Cell\ Function}{(Insulin\ Catabolism \cdot Insulin\ Resistance)} \tag{37}$$

These equations are explanatory of the concept that the disposition index, as meant by the investigators who introduced it (Bergman et al. 1981; Kahn et al. 1993), requires to assess an insulin concentration based metrics, which physiologically is the net balance between insulin secretion rate and insulin catabolism. This class of parameters, e.g., AIR of Kahn's paper or ϕ_2 of Bergman's paper, is the one within which to select a metrics to compute the disposition index. Alternatively, one can use a descriptor of beta cell functional mass, e.g., the parameter quantifying proportional control of beta cell function (Eq. 28), a measure of insulin catabolism/clearance, and a measure of insulin sensitivity to compute a correct disposition index according to Eq. 36. In any case, the preliminary requirement is that, in healthy individuals, the insulin concentration-based metrics (e.g., the ratio *Beta Cell Function*/*Insulin Catabolism*) display a hyperbolic relationship with the measure of *Insulin Sensitivity.*

Equation 35, albeit an erroneous descriptor of the Disposition Index, expresses a quite sensible concept. Following the same line of reasoning in Bergman et al. (1981) and Kahn et al. (1993), one can envision to address selectively the individual role of beta cell function in matching insulin sensitivity, by dropping the insulin catabolism component and using only the metrics of beta cell functional mass, i.e., those developed to assess the beta cells as a glucose sensor/transducer. Ahren and Pacini did it in an earlier paper (Ahren and Pacini 1997), in which they reported both the disposition index, as defined by Kahn et al. (1993), and a novel index, the product of the glucose sensitivity of the derivative control of beta cell function times insulin sensitivity, which they named "adaptation index." The same concept can be extended also to the proportional control of beta cell function. Thus, according to Ahren and Pacini (1997):

$$Adaptation\ Index = Beta\ Cell\ Glucose\ Sensitivity \cdot Insulin\ Sensitivity \tag{38}$$

Ahrén and Pacini were quite correct in realizing that the physiologic significance of the disposition index and of the adaptation index is different, and that the latter is the one which actually gauges the exact role played by beta cell function in the face of insulin sensitivity (Ahren and Pacini 1997). On the other hand, the postulate that beta cell glucose sensitivity and insulin sensitivity are linked to each other by a hyperbolic function is less likely to be true than in the case of the disposition index. The latter, indeed, incorporates an insulin concentration based metrics, and it is reasonable to expect that, especially in the same individual, the product of insulin concentration and insulin sensitivity at any glucose level tends to stay constant (Bergman et al. 1981). For the adaptation index, it is less likely that the relationship is hyperbolic, because it does not include the often neglected, but relevant component of the disposition index: insulin catabolism (see Eqs. 36 and 37). Not surprisingly, in many cases the relationship between beta cell glucose sensitivity and insulin

sensitivity, although inverse and curvilinear, did not fit a hyperbola (Bonadonna et al. 2003).

To further complicate this issue, in many cases investigators, even the most renowned ones, compute an adaptation index as per Eq. 38, but they present it under the name of disposition index (Sharma et al. 2017).

No matter whether the inverse curvilinear relationship between measures or biomarkers of insulin sensitivity and measures or biomarkers of beta cell function insulin catabolism is hyperbolic or of a different nature, if sufficiently high numbers of individuals are collected, one can compare directly the curves of different groups of individuals in a Cartesian plot of beta cell function versus insulin sensitivity. Individuals with homogeneous glucose regulation lie on the same upward concave curve, whereas people with worse (better) glucose regulation lie on a concave curve shifted below (above) (Weiss et al. 2005; Bonadonna et al. 2003) (Fig. 14). Another application of the Cartesian plots of beta cell function versus insulin sensitivity is the "joint vector plots," which allow to detect the change over time of the main pathophysiologic determinants of glucose regulation and to appreciate whether the joint changes in beta cell function and insulin sensitivity over time, for instance, bring an individual (group) closer to or farther from the reference concave curve on which people with normal glucose regulation lie (Kahn et al. 2011; Weyer et al. 1999) (Fig. 15) or, after a therapeutic

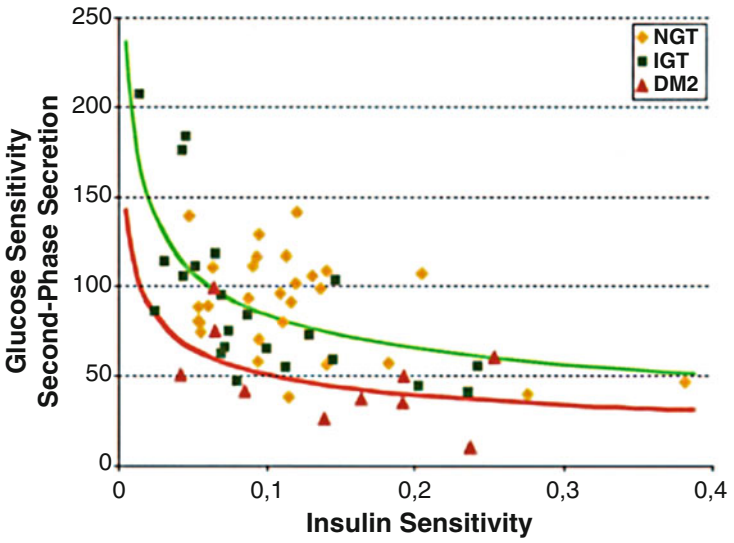


Fig. 14 Scatterplot and inverse curvilinear relationship of beta cell proportional control (y axis) versus insulin sensitivity (x axis) in adolescents with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM). Measures are provided by a hyperglycemic clamp. It can be appreciated that the curve of the adolescents with T2DM is shifted below the curve of the adolescents with NGT or IGT (From Weiss et al. (2005): publisher's permission requested)

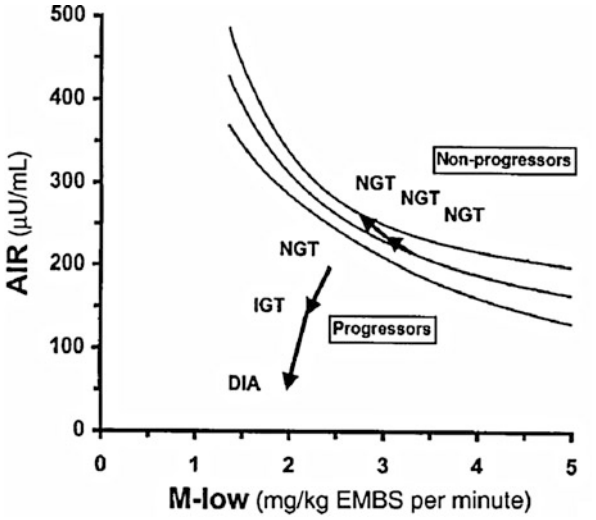


Fig. 15 Joint vector plot of acute insulin response (AIR; y axis) and insulin sensitivity (*M value* of the euglycemic clamp; x axis). The inverse curvilinear relationship found in a reference population with normal glucose tolerance is depicted with 95% confidence intervals. The vector plot of 11 individuals who progressed over time form normal glucose tolerance to type 2 diabetes and the vector plot of 23 individuals who retained normal glucose tolerance over time are depicted (From Weyer et al. (1999): publisher's permission requested)

intervention, move an individual (group) towards better (worse) beta cell function and better (worse) glucose regulation.

The Pancreatic Beta Cell as the Guardian of Glucose Homeostasis

Both adaptation index and disposition index often are used under the belief that both include all the relevant components determining glucose concentration and, ultimately, homeostasis. However, both, and the adaptation index more than the disposition index, are incomplete under this regard, because they neglect at least another component determining glucose regulation, i.e., glucose effectiveness (S_G). Glucose effectiveness is the action of glucose itself in restraining endogenous glucose output and stimulating glucose utilization (Del Prato et al. 1997). When no glucose tracers are used, S_G may be quantified as the algebraic sum of these two effects (e.g., in the IVGTT analyzed by a minimal model), but the two components can also be quantified with rather busy experiments employing also glucose tracers (Alzaid et al. 1994; Del Prato et al. 1997; Vicini et al. 1997; Trombetta et al. 2013; Vella et al. 2003). When the description of the glucose-insulin system is consistent with the definition of insulin sensitivity of Eqs. 10 and 14, the units of S_G are $ml \cdot min^{-1}$, i.e., those of a glucose clearance rate. It should be noted that in more recent elaborations of the minimal model of the glucose-insulin system, insulin-independent glucose metabolism is split in two components: glucose utilization by brain and red blood cells, which has a fixed, maximal

rate, and glucose metabolism due to glucose effectiveness (S_G) (Vicini et al. 1997; Trombetta et al. 2013). Furthermore, if in the test used to assess beta cell function carbohydrates or glucose are administered by the gastro-intestinal route, digestion/absorption and first pass splanchnic glucose extraction are additional determinants of glucose regulation.

If one's goal, therefore, is to compute a comprehensive descriptor of the capability of the beta cells to keep glucose at bay, glucose concentration itself, or a summary index of glucose homeostasis, such as e.g., glycated hemoglobin, are quantifiers of this ultimate performance of beta cells (and of the other determinants of glucose metabolism as well). A metrics proposed to fulfill this goal could be named "glucoregulatory index." Since the higher the glucose levels, the worse is the role played by beta cells in determining glucose homeostasis, one could write the following relationship:

$$Glucoregulatory\ Index \propto \frac{1}{Glycated\ Hemoglobin} \tag{39}$$

Of course, this view of the Glucoregulatory Index ends up in being a tautology of the classification of glucose regulation and/or average glucose concentration (American Diabetes Association 2017).

However, an experimentally assessed glucoregulatory index, independent of the obvious use of clinical parameters of glucose control (fasting glucose, 2-h glucose, glycated hemoglobin), would be needed. Thus far, no metrics has been proposed to fulfill this goal. However, such a metrics should stem from, and satisfy, the following relationship for an intravenous challenge test:

$$Glucoregulatory\ Index \propto \left(\frac{Beta\ Cell\ Function \cdot Insulin\ Sensitivity}{Insulin\ Catabolism}; S_G\right) \tag{40}$$

Further studies are needed to accelerate the pace of progress in this field.

A Special Case: The Intravenous Glucagon Test

The intravenous glucagon test, with blood sampling for C-peptide 6′ after injection, was proposed 40 years ago as a tool to evaluate residual insulin secretion in people with diabetes mellitus (Faber and Binder 1977) and quickly gained vast popularity, also because of its relative simplicity and convenience. Furthermore, it was reported that a threshold value of 0.6 nmol/l of C-peptide at 6′ could discriminate between patients with insulin requiring and patients with noninsulin requiring diabetes mellitus (Madsbad et al. 1981).

The glucagon test has been used in several studies as an assessment of beta cell functional mass. It should be noted that the simplicity of the outcome (C-peptide concentration at 6′) neglects any relationship of beta cell to ambient glucose, insulin

sensitivity. etc. Even as an assessment of the beta cell as a glucagon sensor, it would fall somewhat short of its goal because neither the glucose levels nor the glucagon levels are taken into account. The correlation between the glucagon test and the acute insulin response in the IVGTT is highly significant, but less than close (Yoneda et al. 1992). In a recent report, the glucagon test was found to be closely correlated with the relative area of insulin-positive cell in the pancreas of people undergoing surgery for pancreatic diseases, thereby supporting the concept that this test may be of value in estimating beta cell mass (Fujita et al. 2015).

Indexes of Beta Cell Function

Assessing beta cell function is cumbersome and expensive. Furthermore, none of the tests described in section "Measuring Insulin Secretion/Beta Cell Function" lends itself to be used in the clinical arena. These limitations have paved the way to the use of surrogate indexes. Schematically, they can be divided between fasting, steady-state indexes, and nonsteady-state indexes. On practical grounds, *HOMAbeta* is the reference index of the first class, and OGTT-derived indexes are the reference for the second class of indexes.

Biomarkers of Beta Cell Function Derived in the Fasting State

HOMAbeta is a fasting, steady-state index, which is computed with postabsorptive insulin and glucose values, and, therefore, owing to its simplicity and straightforwardness, it has been used in literally thousands of studies. Together with *HOMAIR*, it also was derived on the basis of a complex theoretic analysis of fasting glucose metabolism (Matthews et al. 1985). Its connections with the direct assessments of beta cell function can be appreciated by looking at Eq. 31, which describes the proportional component of insulin secretion rate, and by extending the same relationship to basal insulin secretion rate. The simplest relationship one can envision from Eq. 31 is:

$$ISR_{Basal} = k \cdot G \tag{41}$$

by adding a physiologic assumption that at $G \leq 3.5\ mmol \cdot l^{-1} ISR_{Basal}$ goes to 0 (to protect against the risk of hypoglycemia), one can write:

$$k = \frac{ISR_{Basal}}{G - 3.5} \tag{42}$$

in which k is a metrics of beta cell function in the basal state. Equation is very similar to the equation of *HOMAbeta*, with the key difference that in the latter ISR_{Basal} is substituted for by fasting I. Thus, all the considerations (see Eq. 28) regarding the caution to be exerted when insulin concentration replaces insulin secretion rate in the assessment of beta cell function need be reiterated also in this case.

Awareness of these caveats has prompted a number of investigators to substitute fasting C-peptide concentration for fasting insulin concentration in the formula of *HOMAbeta*, a move which clearly mitigates the concerns raised by the use of *I*.

At the basis of the use of *HOMAbeta* is the assumption that basal insulin secretion rate is closely related also to insulin secretion rate after nutrient challenges. In some very specified conditions, e.g., type 1 diabetes, this may be a reasonable assumption, but it can hardly be generalized. Not surprisingly, *HOMAbeta*, even in its C-peptide based form, is not highly correlated to bona fide tests of beta cell function, but its simplicity is such that it has been used in a great number of studies. The improved HOMA2 model (Hill et al. 2013) has resulted into a *HOMA2beta*, which can be easily calculated with the aid of an available software (iHOMA2) (Oxford DTU-Uo).

Many investigators have thought to use *HOMAIR* and *HOMAbeta* to compute a disposition index, in analogy to Eq. 37:

$$DI_{(HOMA)} = \frac{HOMAbeta}{HOMAIR} \tag{43}$$

However, simplicity is highly deceptive:

$$DI_{(HOMA)} = \frac{\frac{I}{G-3.5}}{\frac{G \cdot I}{22.4}} = \frac{I}{G-3.5} \cdot \frac{22.4}{G \cdot I} = \frac{22.4}{G \cdot (G-3.5)} \tag{44}$$

Equation 44 shows that the disposition index computed with the *HOMAbeta* and *HOMAIR* is related only to the inverse of the square of fasting glucose, i.e., it belongs to the class of those "glucoregulatory indexes" (Eq. 39) which are nothing else but glucose concentration measures or its proxies, and which have been criticized in a previous paragraph, because they carry no additional information.

OGTT-Derived, or MTT-Derived, Indexes of Beta Cell Function

Over the years, huge efforts have been devoted to the development of novel, simple indexes based on hormone and glucose values during oral challenges, such as the OGTT and the standardized MTT. Two indexes have enjoyed wide use in the research community: the insulinogenic index (IG) (Seltzer et al. 1967) and the Sluiter's index (or corrected insulin release index, CIR) (Sluiter et al. 1976).

Both are conceptually linked to Eq. 31, which describes the proportional component of beta cell function and suggests that the ratio between insulin secretion rate and glucose concentration is a quantifier of beta cell function. In both indexes, however, insulin concentration takes the place of insulin secretion rate; hence, the usual caveats (see Eqs. 27 and 28) apply:

$$Insulinogenic\ Index = \frac{I_{30'} - I_{0'}}{G_{30'} - G_{0'}} \tag{45}$$

$$CIR_{120'} = \frac{I_{120'}}{G_{120'} \cdot (G_{120'} - 3.0)} \tag{46}$$

The two formulas above are the most widely used ones to compute insulinogenic index and CIR. The latter may be considered more practical because it requires no additional blood sampling (the 120′ of the OGTT is mandatory to assess glucose tolerance). On the other hand, many researchers have relied on the insulinogenic index, because it is believed to capture an equivalent of first phase insulin secretion during the OGTT (and also during the MTT). At that moment of OGTT, however, insulin secretion rate already is the sum of the derivative component, which accounts for first phase insulin secretion, and of the proportional component, which accounts for second phase insulin secretion during intravenous challenges (Fig. 13).

Both indexes have been quite useful in huge epidemiological studies.

One further index was developed by Cretti et al. (2001). It differentiates from the others, because it is based on the analysis of only four time points of glucose/C-peptide between 0′ and 120′ of the OGTT by a further simplified minimal model of glucose stimulated C-peptide secretion to provide a model based global index of beta cell function, the OGTT β-index.

The attractive feature of this approach is that it allows to exploit modeling of insulin secretion in OGTTs with a limited number of samples ($n = 4$), which should not be analyzed by the full models we have described above. The limitation is that, owing to oversimplification of the model structure, its ability to accurately predict C-peptide time course in the first 30′ of the OGTTs is clearly inferior to the full sized models. The OGTT β-index was carefully validated in the original paper (Cretti et al. 2001), it was shown to be an inheritable trait (Lehtovirta et al. 2005) and to be able to detect even subtle changes in beta cell function (Bonadonna et al. 2003; Lencioni et al. 2006; Santilli et al. 2017). In spite of these advantages, it has been employed quite rarely.

Conclusions

The brave reader who has been journeying so far through a possibly quite tedious list of tests, methods, pros and cons, to say nothing about the 46 equations, may wonder whether there are key take-home messages and which ones they are.

First, and this stems from a bird's eye view of this field, there still is the unmet need of simple and accurate tests for both insulin sensitivity and beta cell function for both clinical and research purposes. Thus far, a good measure is by no means simple, convenient and cheap, and vice versa.

Secondly, intravenous challenge tests are to be preferred to measure net insulin sensitivity.

Third, both intravenous and oral challenge tests can be used to measure insulin secretion and beta cell function, with the caveat that the role played by the incretinergic system is the most prominent difference between the two routes.

Fourth, beta cell function currently can be described by using a widely shared conceptual frame, which then is declined in slightly different mathematical models.

Fifth, without the use of glucose tracers, only two tests can simultaneously provide measures of insulin sensitivity and of beta cell function: the hyperglycemic clamp and the IVGTT.

Sixth, many surrogate indexes are available to estimate insulin sensitivity and beta cell function, which display specific advantages and limitations. Their use can be of great help in the hands of the investigator or of the clinician who is well aware of their exact contribution and significance.

As a final, repetitious reminder, this chapter has focused only on the first level of complexity in testing insulin sensitivity and beta cell function. Much more can be achieved with the use of other tools, which, however, are available only to few top notch research laboratories. But, even at this elementary level of sophistication, it was felt that an effort was needed to make our concepts ("perceptiones") vivid and clear ("clara et distincta") (DesCartes 1644a, b). If this attempt has fallen short of its target and/or has bored the reader, the miss is unintentional.

Acknowledgments We thank prof. Stefano Del Prato, prof. Anna Solini, prof. Enzo Bonora and prof. Ivana Zavaroni for useful discussions about the topics of this chapter. This work was supported in part with funds of the Italian Ministry of Education, University and Research (MIUR) PRIN 2015 2015373Z39_004 and with University of Parma research funds, both to RCB, and with University of Verona research funds to MT, and with University of Parma research funds to ADC.

References

Ahren B, Pacini G. Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. Am J Phys. 1997;273(4 Pt 1):E701–7.

Alzaid AA, Dinneen SF, Turk DJ, Caumo A, Cobelli C, Rizza RA. Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans. J Clin Invest. 1994;94(6): 2341–8.

American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2017;40 (Suppl 1):S11–24.

Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes. 2003;52(7):1738–48.

Beard JC, Bergman RN, Ward WK, Porte D Jr. The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. Diabetes. 1986;35(3):362–9.

Bergman RN, Iyer MS. Indirect regulation of endogenous glucose production by insulin: the single gateway hypothesis revisited. Diabetes. 2017;66(7):1742–7.

Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest. 1981;68(6):1456–67.

Bergman RN, Stefanovski D, Kim SP. Systems analysis and the prediction and prevention of Type 2 diabetes mellitus. Curr Opin Biotechnol. 2014;28:165–70.

Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, et al. Effects of nonglucose nutrients on insulin secretion and action in people with pre-diabetes. Diabetes. 2007;56 (4):1113–9.

Bonadonna RC, Groop LC, Zych K, Shank M, DeFronzo RA. Dose-dependent effect of insulin on plasma free fatty acid turnover and oxidation in humans. Am J Phys. 1990a;259(5 Pt 1): E736–50.

Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: a dose-response study. Metabolism. 1990b;39(5):452–9.

Bonadonna RC, Saccomani MP, Seely L, Zych KS, Ferrannini E, Cobelli C, et al. Glucose transport in human skeletal muscle. The in vivo response to insulin. Diabetes. 1993a;42(1):191–8.

Bonadonna RC, del Prato S, Bonora E, Gulli G, Solini A, DeFronzo RA. Effects of physiological hyperinsulinemia on the intracellular metabolic partition of plasma glucose. Am J Phys. 1993b;265(6 Pt 1):E943–53.

Bonadonna RC, Saccomani MP, Del Prato S, Bonora E, DeFronzo RA, Cobelli C. Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. Circulation. 1998;98(3):234–41.

Bonadonna RC, Stumvoll M, Fritsche A, Muggeo M, Haring H, Bonora E, et al. Altered homeostatic adaptation of first- and second-phase beta-cell secretion in the offspring of patients with Type 2 diabetes: studies with a minimal model to assess beta-cell function. Diabetes. 2003;52 (2):470–80.

Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, et al. Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. J Clin Endocrinol Metab. 1989;68(2):374–8.

Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care. 2000;23(1):57–63.

Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. Diabetes. 2001;50(1):150–8.

Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA. Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. Role of substrate availability on estimates of whole body protein synthesis. J Clin Invest. 1987;80(6):1784–93.

Caumo A, Bergman RN, Cobelli C. Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. J Clin Endocrinol Metab. 2000;85(11):4396–402.

Cobb J, Gall W, Adam KP, Nakhle P, Button E, Hathorn J, et al. A novel fasting blood test for insulin resistance and prediabetes. J Diabetes Sci Technol. 2013;7(1):100–10.

Cretti A, Lehtovirta M, Bonora E, Brunato B, Zenti MG, Tosi F, et al. Assessment of beta-cell function during the oral glucose tolerance test by a minimal model of insulin secretion. Eur J Clin Investig. 2001;31(5):405–16.

Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: estimation of insulin sensitivity from a meal test. IEEE Trans Biomed Eng. 2002;49(5):419–29.

Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. Am J Physiol Endocrinol Metab. 2004;287(4):E637–43.

Dalla Man C, Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G, et al. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. Diabetes. 2005a;54(11): 3265–73.

Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. Am J Physiol Endocrinol Metab. 2005b;289(5):E909–14.

Dalla Man C, Yarasheski KE, Caumo A, Robertson H, Toffolo G, Polonsky KS, et al. Insulin sensitivity by oral glucose minimal models: validation against clamp. Am J Physiol Endocrinol Metab. 2005c;289(6):E954–9.

DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1988;37(6):667–87.

DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Phys. 1979;237(3):E214–23.
Del Prato S, Bonadonna RC, Bonora E, Gulli G, Solini A, Shank M, et al. Characterization of cellular defects of insulin action in Type 2 (non-insulin-dependent) diabetes mellitus. J Clin Invest. 1993;91(2):484–94.
Del Prato S, Matsuda M, Simonson DC, Groop LC, Sheehan P, Leonetti F, et al. Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. Diabetologia. 1997;40(6):687–97.
DesCartes R. Principia philosophiae. Frankfurt am Main: Knoch, F. & Sons; 1644a. p. 1722.
Descartes R. Principles of phylosophy. 1644b. Available from: http://www.earlymoderntexts.com/assets/pdfs/descartes1644part1.pdf.
Dodd GT, Tiganis T. Insulin action in the brain: roles in energy and glucose homeostasis. J Neuroendocrinol. 2017;29e:12513.
Dube S, Errazuriz I, Cobelli C, Basu R, Basu A. Assessment of insulin action on carbohydrate metabolism: physiological and non-physiological methods. Diabet Med. 2013;30(6):664–70.
Engin A. The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol. 2017;960:1–17.
Eriksson O, Laughlin M, Brom M, Nuutila P, Roden M, Hwa A, et al. In vivo imaging of beta cells with radiotracers: state of the art, prospects and recommendations for development and use. Diabetologia. 2016;59(7):1340–9.
Faber OK, Binder C. C-peptide response to glucagon. A test for the residual beta-cell function in diabetes mellitus. Diabetes. 1977;26(7):605–10.
Ferrannini E, Santoro D, Bonadonna R, Natali A, Parodi O, Camici PG. Metabolic and hemodynamic effects of insulin on human hearts. Am J Phys. 1993;264(2 Pt 1):E308–15.
Finegood DT, Pacini G, Bergman RN. The insulin sensitivity index. Correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. Diabetes. 1984;33(4):362–8.
Fritsche A, Stefan N, Hardt E, Schutzenauer S, Haring H, Stumvoll M. A novel hyperglycaemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. Eur J Clin Investig. 2000;30(5):411–8.
Fujita Y, Kozawa J, Iwahashi H, Yoneda S, Uno S, Yoshikawa A, et al. Increment of serum C-peptide measured by glucagon test closely correlates with human relative beta-cell area. Endocr J. 2015;62(4):329–37.
Graci S, Baratta R, Degano C, Luppa A, Vigneri R, Frittitta L, et al. The intravenous insulin tolerance test is an accurate method for screening a general population for insulin resistance and related abnormalities. J Endocrinol Investig. 1999;22(6):472–5.
Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite type 1 diabetes TrialNet data. Diabetes. 2012;61(8):2066–73.
Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G. Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. Diabetes. 1981;30(5):387–92.
Groop LC, Saloranta C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA. The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1991;72(1):96–107.
Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab. 2015;19(1):160–4.
Hannon TS, Kahn SE, Utzschneider KM, Buchanan TA, Nadeau KJ, Zeitler PS, et al. Review of methods for measuring beta-cell function: design considerations from the Restoring Insulin Secretion (RISE) Consortium. Diabetes Obes Metab. 2017;20(1):14–24.
Hill NR, Levy JC, Matthews DR. Expansion of the homeostasis model assessment of beta-cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: iHOMA2. Diabetes Care. 2013;36(8):2324–30.
Himsworth HP. Insulin deficiency and insulin inefficiency. Br Med J. 1940;1(4139):719–22.

James HA, O'Neill BT, Nair KS. Insulin regulation of proteostasis and clinical implications. Cell Metab. 2017;26(2):310–23.

Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes. 1993;42(11):1663–72.

Kahn SE, Lachin JM, Zinman B, Haffner SM, Aftring RP, Paul G, et al. Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. Diabetes. 2011;60(5):1552–60.

Lassen NA, Perl W. Tracer kinetic methods in medical physiology. New York: Raven Press; 1979.

Laxminarayan S, Reifman J, Steil GM. Use of a food and drug administration-approved type 1 diabetes mellitus simulator to evaluate and optimize a proportional-integral-derivative controller. J Diabetes Sci Technol. 2012;6(6):1401–12.

Lehtovirta M, Kaprio J, Groop L, Trombetta M, Bonadonna RC. Heritability of model-derived parameters of beta cell secretion during intravenous and oral glucose tolerance tests: a study of twins. Diabetologia. 2005;48(8):1604–13.

Lencioni C, Volpe L, Miccoli R, Cuccuru I, Chatzianagnostou K, Ghio A, et al. Early impairment of beta-cell function and insulin sensitivity characterizes normotolerant Caucasian women with previous gestational diabetes. Nutr Metab Cardiovasc Dis. 2006;16(7):485–93.

Madsbad S, Krarup T, McNair P, Christiansen C, Faber OK, Transbol I, et al. Practical clinical value of the C-peptide response to glucagon stimulation in the choice of treatment in diabetes mellitus. Acta Med Scand. 1981;210(3):153–6.

Mandarino L, Bonadonna RC, McGuinness OP, Halseth AE, Wassermann DH. Regulation of muscle glucose uptake in vivo. In: Jefferson LS, Cherrington AD, editors. Handbook of physiology. Vol. II: The endocrine pancreas and regulaiton of metabolism. American Physiological Society, Oxford University Press, Oxford, UK; 2001. p. 803–45.

Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care. 2001a;24(3):539–48.

Mari A, Camastra S, Toschi E, Giancaterini A, Gastaldelli A, Mingrone G, et al. A model for glucose control of insulin secretion during 24 h of free living. Diabetes. 2001b;50(Suppl 1):S164–8.

Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta -cell function: modeling analysis in normal subjects. Am J Physiol Endocrinol Metab. 2002;283(6):E1159–66.

Mari A, Pacini G, Brazzale AR, Ahren B. Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test. Diabetologia. 2005;48(4):748–51.

Mather KJ, Hunt AE, Steinberg HO, Paradisi G, Hook G, Katz A, et al. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. J Clin Endocrinol Metab. 2001;86(11):5457–64.

Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22(9):1462–70.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.

Meier JJ, Menge BA, Breuer TG, Muller CA, Tannapfel A, Uhl W, et al. Functional assessment of pancreatic beta-cell area in humans. Diabetes. 2009;58(7):1595–603.

Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and Type 2 diabetic patients. Diabetes. 2008;57(5):1340–8.

Navalesi R, Pilo A, Ferrannini E. Kinetic analysis of plasma insulin disappearance in nonketotic diabetic patients and in normal subjects. A tracer study with 125I-insulin. J Clin Invest. 1978;61(1):197–208.

Oram RA, Jones AG, Besser RE, Knight BA, Shields BM, Brown RJ, et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia. 2014;57(1):187–91.

Oram RA, McDonald TJ, Shields BM, Hudson MM, Shepherd MH, Hammersley S, et al. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. Diabetes Care. 2015;38(2):323–8.

Oxford DTU-Uo. HOMA calculator. 2017. Available from: http://www.dtu.ox.ac.uk/homacalculator/download.php.

Polonsky K, Jaspan J, Pugh W, Cohen D, Schneider M, Schwartz T, et al. Metabolism of C-peptide in the dog. In vivo demonstration of the absence of hepatic extraction. J Clin Invest. 1983;72(3):1114–23.

Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, et al. Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. J Clin Invest. 1986a;77(1):98–105.

Polonsky KS, Given BD, Pugh W, Licinio-Paixao J, Thompson JE, Karrison T, et al. Calculation of the systemic delivery rate of insulin in normal man. J Clin Endocrinol Metab. 1986b;63(1):113–8.

Pye S, Watarai T, Davies G, Radziuk J. Comparison of the continuously calculated fractional splanchnic extraction of insulin with its fractional disappearance using a new double-tracer technique. Metabolism. 1993;42(2):145–53.

Quon MJ. QUICKI is a useful and accurate index of insulin sensitivity. J Clin Endocrinol Metab. 2002;87(2):949–51.

Radziuk J. Insulin sensitivity and its measurement: structural commonalities among the methods. J Clin Endocrinol Metab. 2000;85(12):4426–33.

Rickels MR, Naji A, Teff KL. Acute insulin responses to glucose and arginine as predictors of beta-cell secretory capacity in human islet transplantation. Transplantation. 2007;84(10):1357–60.

Rubenstein AH, Clark JL, Melani F, Steiner DF. Secretion of pro-insulin C-peptide by pancreatic beta cells and its circulation in blood. Nature. 1969;224:697–9.

Rutter GA, Pullen TJ, Hodson DJ, Martinez-Sanchez A. Pancreatic beta-cell identity, glucose sensing and the control of insulin secretion. Biochem J. 2015;466(2):203–18.

Salinari S, Bertuzzi A, Mingrone G. Intestinal transit of a glucose bolus and incretin kinetics: a mathematical model with application to the oral glucose tolerance test. Am J Physiol Endocrinol Metab. 2011;300(6):E955–65.

Santilli F, Simeone PG, Guagnano MT, Leo M, Maccarone MT, Di Castelnuovo A, et al. Effects of liraglutide on weight loss, fat distribution, and beta-cell function in obese subjects with prediabetes or early Type 2 diabetes. Diabetes Care. 2017;40(11):1556–64.

Seltzer HS, Allen EW, Herron AL Jr, Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest. 1967;46(3):323–35.

Seufert J. Incretins and their endocrine and metabolic functions. Endocr Dev. 2017;32:38–48.

Shankar SS, Vella A, Raymond RH, Staten MA, Calle RA, Bergman RN, et al. Standardized mixed-meal tolerance and arginine stimulation tests provide reproducible and complementary measures of beta-cell function: results from the Foundation for the National Institutes of Health Biomarkers Consortium Investigative Series. Diabetes Care. 2016;39(9):1602–13.

Sharma A, Laurenti MC, Dalla Man C, Varghese RT, Cobelli C, Rizza RA, et al. Glucose metabolism during rotational shift-work in healthcare workers. Diabetologia. 2017;60(8):1483–90.

Shen SW, Reaven GM, Farquhar JW. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J Clin Invest. 1970;49(12):2151–60.

Sluiter WJ, Erkelens DW, Reitsma WD, Doorenbos H. Glucose tolerance and insulin release, a mathematical approach I. Assay of the beta-cell response after oral glucose loading. Diabetes. 1976;25(4):241–4.

Steil GM, Grodsky GM. The artificial pancreas: is it important to understand how the beta cell controls blood glucose? J Diabetes Sci Technol. 2013;7(5):1359–69.

Steil GM, Hwu CM, Janowski R, Hariri F, Jinagouda S, Darwin C, et al. Evaluation of insulin sensitivity and beta-cell function indexes obtained from minimal model analysis of a meal tolerance test. Diabetes. 2004;53(5):1201–7.

Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295–301.

Stumvoll M, Haring H, Fritsche A. For debate: starling's curve of the pancreas–overuse of a concept? Horm Metab Res. 2003;35(7):391–5.

Toffolo G, Breda E, Cavaghan MK, Ehrmann DA, Polonsky KS, Cobelli C. Quantitative indexes of beta-cell function during graded up&down glucose infusion from C-peptide minimal models. Am J Physiol Endocrinol Metab. 2001;280(1):E2–10.

Toschi E, Camastra S, Mari A, Gastaldelli A, Baldi S, Masoni A, et al. A model for assessing insulin secretion and its control under free-living conditions. Diabetes. 2001;50(Suppl 1):S178–9.

Trombetta M, Boselli L, Cretti A, Cali A, Vettore M, Caruso B, et al. Type 2 diabetes mellitus: a disease of the governance of the glucose-insulin system: an experimental metabolic control analysis study. Nutr Metab Cardiovasc Dis. 2013;23(1):23–30.

Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes. 1992;41(3):368–77.

Varghese RT, Dalla Man C, Laurenti MC, Piccinini F, Sharma A, Shah M, et al. Performance of individually-measured vs population-based C-peptide kinetics to assess beta-cell function in presence and absence of acute insulin resistance. Diabetes Obes Metab. 2017;20:549–555.

Vella A, Reed AS, Charkoudian N, Shah P, Basu R, Basu A, et al. Glucose-induced suppression of endogenous glucose production: dynamic response to differing glucose profiles. Am J Physiol Endocrinol Metab. 2003;285(1):E25–30.

Vicini P, Caumo A, Cobelli C. The hot IVGTT two-compartment minimal model: indexes of glucose effectiveness and insulin sensitivity. Am J Phys. 1997;273(5 Pt 1):E1024–32.

Visentin R, Dalla Man C, Basu R, Basu A, Rizza RA, Cobelli C. Hepatic insulin sensitivity in healthy and prediabetic subjects: from a dual- to a single-tracer oral minimal model. Am J Physiol Endocrinol Metab. 2015;309(2):E161–7.

Weiss R, Caprio S, Trombetta M, Taksali SE, Tamborlane WV, Bonadonna R. Beta-cell function across the spectrum of glucose tolerance in obese youth. Diabetes. 2005;54(6):1735–43.

Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of Type 2 diabetes mellitus. J Clin Invest. 1999;104 (6):787–94.

Yoneda H, Ikegami H, Yamamoto Y, Yamato E, Cha T, Kawaguchi Y, et al. Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. Diabetes Care. 1992;15 (11):1517–21.

Screening for Diabetes and Prediabetes

11

Laura J. Gray, Andrew Willis, David Webb, Melanie J. Davies, and Kamlesh Khunti

Contents

Abstract

Type 2 Diabetes and Prediabetes are serious conditions which can lead to early morbidity and mortality. The numbers of people with Type 2 Diabetes and Prediabetes are increasing and by 2040 it is estimated that there will be

L. J. Gray
Department of Health Sciences, University of Leicester, Leicester, UK
e-mail: Lg48@le.ac.uk

A. Willis · D. Webb · M. J. Davies · K. Khunti (✉)
Diabetes Research Centre, University of Leicester, Leicester, UK
e-mail: aw187@le.ac.uk; david.webb@uhl-tr.nhs.uk; melanie.davies@uhl-tr.nhs.uk; kk22@le.ac.uk

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_12

642 million cases of diabetes alone worldwide, with many more people having Prediabetes. Type 2 diabetes can remain undiagnosed for many years and therefore screening can bring forward diagnosis, allowing treatment to be started earlier which may reduce diabetes related complications. Type 2 Diabetes is preventable in those with Prediabetes and therefore screening can be used to identify those with Prediabetes for inclusion in prevention programmes. In this chapter we consider the evidence for screening for both Type 2 Diabetes and Prediabetes, using case studies throughout to highlight issues.

Keywords
Type 2 Diabetes Mellitus · Prediabetes · Screening · Prevention

Introduction

This chapter gives an overview of the issues and challenges of screening for Type 2 Diabetes Mellitus (T2DM) and Prediabetes (PDM). We give a brief introduction to screening and approaches to screening for T2DM and PDM followed by an assessment of whether screening for T2DM and PDM should be advocated. Finally we assess whether screening for T2DM and PDM is cost effective. Throughout the chapter we give examples and case studies from the literature to demonstrate our points.

Type 2 Diabetes and Prediabetes

T2DM is a serious chronic condition caused by either insulin resistance and/or deficiency leading to high levels of blood glucose. T2DM is associated with reduced quality of life and increased risk of serious complications, such as blindness, kidney failure and cardiovascular disease (CVD). Life expectancy of individuals with T2DM may be shortened by as much as 10 years, with most dying of CVD (Roper et al. 2001). The prevalence of diabetes has been rising for a number of years. In 2015, the International Diabetes Federation estimated that 415 million people worldwide had diabetes, with this expected to increase to 642 million by 2040 (International Diabetes Federation 2015). This equates to one in 11 adults rising to one in ten. It is estimated that approximately 90% of all adults with diabetes have T2DM (International Diabetes Federation 2015).

The management of T2DM consumes around 10–12% of health care expenditure, which is also expected to rise to 17% by 2035/2036 (Hex et al. 2012; International Diabetes Federation 2015). T2DM has a long asymptomatic phase which means it can remain undiagnosed for many years. It has been estimated that this preclinical phase can last between 9–12 years (Harris et al. 1992). Studies suggest that screening for undiagnosed T2DM can bring diagnosis forward by up to 6 years compared to

diagnoses made as part of routine care (Harris et al. 2003). It is estimated that 193 million people worldwide have undiagnosed T2DM, which equates to 46.5% of adults with diabetes being undiagnosed, or one in two adults with T2DM (International Diabetes Federation 2015). Those with undiagnosed and therefore untreated T2DM may be at higher risk of complications, and studies have shown that around 20–30% of new diagnoses already have evidence of diabetic complications.

It is estimated that 90% of cases of T2DM are either preventable or the onset can be significantly delayed (Mozaffarian et al. 2009), and the prevention of T2DM has been highlighted by most developed countries as a health care priority. PDM is a high risk state where glucose levels are elevated above normal but do not reach the agreed threshold for diagnosis of T2DM. There is no consistent terminology for this high risk group and controversy surrounds the use of hyperglycaemia cut-points, but identifying high risk groups is useful for planning and implementing diabetes prevention programmes. PDM has been termed impaired glucose regulation, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), non-diabetic hyperglycaemia, borderline diabetes and at high risk of diabetes. Comparable to T2DM, PDM has become increasingly prevalent over the last two decades. It is estimated that 318 million adults worldwide have PDM, which is also expected to rise to 481 million by 2040 – this represents 6.7% of the adult population rising to 7.8% (International Diabetes Federation 2015). Those with PDM are at increased risk of developing T2DM and have increased morbidity and mortality compared to those with normal glucose levels (Stokes and Mehta 2013). Furthermore, those with PDM are significantly more likely to develop T2DM than those with normal blood glucose levels (Gerstein et al. 2007). A meta-analysis of progression rates from different PDM definitions to T2DM estimated that when PDM was defined as HbA1c between 6.0–6.4% (42–46 mmol/mol), IFG defined by ADA, IFG defined by WHO, or IGT then the incidence rates of progression to T2DM were 35.6, 35.5, 47.4, and 45.5 per 1000 person-years respectively (Morris et al. 2013). Progression rates are slightly higher in people who have both IFG and IGT (approximately 70 per 1000 person-years)(Morris et al. 2013).

Paradoxically, approximately half of those with PDM may revert to normal glucose levels naturally or due to measurement variability (Meigs et al. 2003; Engberg et al. 2009; Bodicoat et al. 2017). There is also robust evidence that T2DM can be prevented or delayed in those with PDM through lifestyle modification programmes that encourage dietary change and increased physical activity (Gillies et al. 2007), or through pharmacotherapy (Gillies et al. 2007; Phung et al. 2011). However, real-world replications of these results are challenging with pragmatic lifestyle modification programmes typically achieving 2–3% weight loss after 1 year, compared with 9–10% in randomised controlled trials (Dunkley et al. 2014). However, evidence suggests that this level of weight loss might be sufficient to increase the chances of reverting to normal glucose levels, thereby decreasing the risk of developing T2DM and associated complications in the future (Bodicoat et al. 2017).

Introduction to Screening

Screening aims to identify healthy people who may be at increased risk of a disease or condition. In a health care context it is defined as "*a process of identifying apparently healthy people who may be at increased risk of a disease or condition. They can then be offered information, further tests and appropriate treatment to reduce their risk and/or any complications arising from the disease or condition* (National Screening Committee 2003)." Screening is distinct to diagnosis; in a screening programme individuals may receive a diagnostic test, the key difference is why the individual receives the test. In a screening programme individuals are asymptomatic as opposed to an individual who presents to a clinician with symptoms which are then investigated. In terms of T2DM and PDM, the aim would be to identify people who either have PDM or are in a preclinical asymptomatic phase of T2DM. The UK National Screening Committee (NSC) describe screening as a sieve, where most people pass through, meaning that they are at low risk of having the condition being screened for. "Captured" individuals are at high risk and should receive further investigations or a confirmatory test (National Screening Committee 2016). In theory screening should identify disease earlier than it would have been without screening or it should identify 'high risk' individuals who can be offered an intervention to reduce the risk of developing a particular disease. Most screening tests are not perfect and therefore there is a risk that people will be either falsely reassured and told they are low risk when actually they have the disease or will develop it in the future, or people can receive a false positive result, i.e. told they are high risk when they don't have the disease or are at low risk of developing it in the future (see Fig. 1).

There are statistical measures which can be used to assess the performance of a screening programme (Fig. 2). To assess a screening programme, the results of the programme need to be compared to a gold standard (sometimes called a reference standard). This could be the test routinely used to diagnose the condition in clinical practice (for example OGTT or HbA1c for this setting) or it could be based on clinical assessment where no test exists. Sensitivity measures the ability of a screening programme to correctly identify people with the condition of interest and is calculated by dividing the number of true positives by the number of people with the condition. A screening programme with 100% sensitivity would correctly identify all individuals with the condition. A screening programme with 80% sensitivity would detect 80% of individuals with the condition (true positives) but 20% with the condition go undetected (false negatives) (Lalkhen and McCluskey 2008). A screening programme with a 100% sensitivity may not be perfect, as many people without the condition may have been given a positive screening test result. Therefore we also need to assess the ability of the screening test to identify people without the disease/condition correctly. Specificity is the ability of the screening programme to correctly identify those who do not have the condition (number of true negatives divided by the number of individuals without the condition). A perfect test would have a 100% sensitivity and a 100% specificity, but this is not usually seen and therefore we need to consider what constitutes an acceptable performance of a

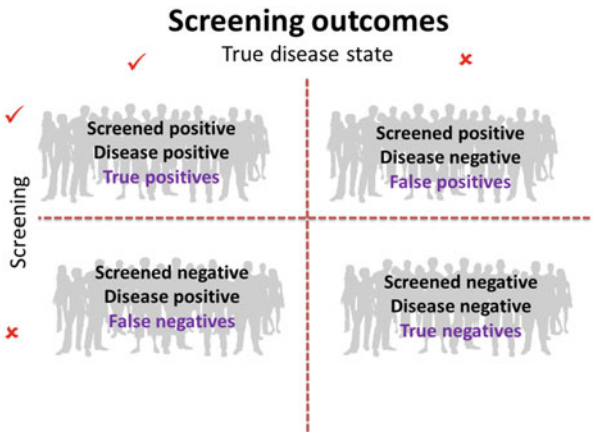


Fig. 1 Screening outcomes

Fig. 2 Statistical measures of the performance of a screening test

		Diagnostic test		
		Positive	Negative	
Screening test	Positive	*a*	*b*	*a+b*
	Negative	*c*	*d*	*c+d*
		a+c	*b+d*	*a+b+c+d*

Sensitivity = a / (a+c) PPV = a / (a+b)

Specificity = d / (b+d) NPV = d / (c+d)

screening test. The level of sensitivity and specificity which are acceptable for a particular programme will depend on the nature of the condition being detected (Bland 2015). The positive predictive value is a clinically useful measure as it gives information on how likely an individual with a positive screening result is to have the condition (number of true positives divided by the total number of positive results). The negative predictive value of a screening programme answers the question: "How likely is it that this individual does not have the condition given that the result is negative?" (number of true negatives divided by the total number of negatives).

There are internationally recognised criteria for critiquing screening programmes and assessing whether they should be implemented. Box 1 shows those criteria adopted by the World Health Organisation (WHO) to establish which medical conditions justify screening programmes (Wilson and Jungner 1968).

An example of a national criteria for screening is that used by the National Screening Committee (NSC) in the UK. They used the WHO criteria as a basis for a more extensive set of guidelines published in 2003 and updated and significantly extended these in 2015 (National Screening Committee 2003; Committee 2015). The updated criteria are shown in Box 2. It is important to note that these criteria do

not only relate to the condition and screening test, but also to the intervention offered to those found with the disease/condition or at high risk of developing the disease/condition. It is not ethical to identify through screening a condition for which there is no intervention or where giving an intervention earlier does not improve outcomes. The criteria also assess whether evidence exists that the screening programme (including interventions) is effective in reducing morbidity and mortality. Ideally this evidence should be from randomised controlled trials. The NSC also state a number of criteria around the implementation of the programme. These criteria have been used to assess whether screening for T2DM and/or PDM should be advocated in the UK, this will be discussed in the section "Should We Screen for T2DM with or Without PDM?" of this chapter.

Approaches to Screening for T2DM and PDM

Screening programmes can take many different forms. In mass screening a whole population is offered screening irrespective of risk, selective or targeted screening offers screening to only those identified at high risk. Screening programmes can be multi-staged, i.e. comprise more than one test and filter individuals out between tests. In multiphasic screening two or more screening tests are applied at the same time, an example of this is the UK NHS health checks programme which offers a midlife health check to individuals aged 40–74 years without a pre-existing condition and comprises screening tests for diabetes, heart disease, kidney disease and dementia. In terms of screening for diabetes related outcomes – programmes can screen either for undiagnosed T2DM only or for both T2DM and PDM. It is not practical or logical to screen for PDM alone as any PDM screening programme will also pick up those with undiagnosed T2DM. Modelling studies suggest that screening for T2DM and PDM and providing prevention interventions to those with PDM may be cost-effective (Gillies et al. 2008). Below we discuss in more detail single and multi-stage programmes in relation to screening for T2DM with or without PDM. These staged programmes can be applied to a whole population (mass) or targeted.

One Stage

In a one stage screening programme for T2DM with or without PDM all those screened would receive the diagnostic test. It's important here not to confuse a one stage screening programme with a clinical diagnosis, although the test strategy is the same. In screening the test is delivered to detect early disease or those at risk of disease in people without (reported) symptoms of the disease, here the health care professional invites the participant to come forward for screening. In a clinical diagnosis the patient approaches the health care professional and the diagnostic test is used to confirm a suspected diagnosis.

Table 1 Diagnostic criteria for T2DM and PDM

	HbA1c	Fasting plasma glucose	Two hour glucose
T2DM	≥ 6.5% (48 mmol/Mol)	≥7.0 mmol/l (126 mg/dl)	≥11.1 mmol/l (200 mg/dl)
PDM			
IGT	–	<7.0 mmol/l (126 mg/dl)[a]	≥7.8 and <11.1 mmol/l[a] (140 mg/dl and 200 mg/dl)
IFG	–	6.1 to 6.9 mmol/l (110 mg/dl to 125 mg/dl)	<7.8 mmol/l (140 mg/dl)[b]
ADA criteria	5.7–6.4% (42–47 mmol/Mol)	–	–
WHO criteria	6.0–6.4% (39–47 mmol/Mol)	–	–

[a]Requires both measures
[b]Only if measured

The current recommended diagnostic criterion for T2DM incorporates two tests, either the 75 g oral glucose tolerance test (OGTT) or HbA1c (see Table 1). An OGTT involves an overnight fast, a fasting glucose measure, followed by a standard glucose load, a 2 h wait and a repeat assessment of the blood glucose level (2-h post-challenge glucose). Although, until recently, this was viewed as the 'gold standard' test for diabetes diagnosis it is inconvenient, expensive and impractical when large numbers of people are being screened. The advantage for conducting an OGTT over other tests was the 2 h post challenge result, which has been shown to be a risk factor for CVD (Erlinger and Brancati 2001) and the diagnostic cut off for the 2 h test (≥11.1 mmol.l) was chosen due to the increased risk of diabetic complications seen beyond this point (Davidson et al. 1995). However similar data now exist for HbA1c which weakens this argument for the use of the OGTT (Davidson 2002). In addition, due to its lack of reproducibility a second confirmatory OGTT is required to diagnose T2DM. OGTT has also been reported as a barrier to the uptake of screening (Eborall et al. 2012). A review of screening studies reported an overall response rate to an invitation for an OGTT as part of a screening study of 65.5% (Khunti et al. 2015).

In 2011, WHO recommended that HbA1c can be used as a diagnostic test for T2DM, with a cut-off value of ≥6.5% (World Health Organisation 2011). Before this recommendation, HbA1c had been used in many large scale screening studies as the initial screening test, followed by a confirmatory OGTT. HbA1c is a good indicator of chronic hyperglycaemia and long term complications and is less affected by concurrent physical and emotional stress levels than plasma glucose levels. Measurement of HbA1c is standardised and has low inter-test variability. The HbA1c test can be done in a non-fasted state and is therefore convenient (Gholap et al. 2013). Over the last few years there has been a number of HbA1c point of care devices developed which allow HbA1c to be assessed using capillary blood rather than venipuncture. Point of care devices which allow a quick turnaround of the result and are relatively easily operated and transportable are obviously desirable for screening

for T2DM, it should be noted though that the ADA do not recommend their use for T2DM diagnosis with many devices having coefficients of variations above acceptable levels (Lenters-Westra and Slingerland 2010; ADA 2016). There are disadvantages associated with the use of HbA1c which include misleading results in those with various hemoglobinopathies, iron deficiency, hemolytic anaemias, and severe hepatic and renal disease which makes HbA1c unsuitable for screening in these patient groups (Gallagher et al. 2009). There are also data to suggest that HbA1c is systematically higher in particular ethnicities and that it can increase with age which may also affect its interpretation (Herman et al. 2007). For example, HbA1c has been shown to be 0.2% higher in South Asians compared to White Europeans independent of age and sex (Mostafa et al. 2012).

For both the OGTT and HbA1c a confirmatory test is required to diagnose T2DM in those with initial test results in the diagnostic range. This is required regarded of the number of stages used in a screening programme. For a clinical diagnosis where symptoms are present, only one test in the diagnostic category is required.

In terms of PDM, different definitions of exist. Traditionally, PDM was diagnosed using OGTTs as IFG, IGT, or both (World Health Organisation 1999). The World Health Organisation 1999 criteria define IGT as 2-h glucose between 7.8–11.0 mmol/l (World Health Organisation 1999). Two definitions of IFG are commonly used; the World Health Organisation 1999 criteria define IFG as fasting blood glucose between 6.1–6.9 mmol/l, and the American Diabetes Association 2003 criteria as fasting blood glucose between 5.6–6.9 mmol/l (American Diabetes Association 2004). In 2011, the World Health Organisation added HbA1c $\geq$6.5% (48 mmol/mol) to their T2DM diagnostic criteria. This created interest in PDM defined using HbA1c, instead of an OGTT. The World Health Organisation concluded that there was insufficient evidence to define an HbA1c PDM range (World Health Organisation 2011). Conversely, others have updated their PDM definitions to include HbA1c. The American Diabetes Association define PDM as HbA1c between 5.7–6.4% (39–46 mmol/mol)(American Diabetes Association 2010), while the International Expert Committee (The International Expert Committee 2009) and the UK NHS National Institute for Health and Clinical Excellence (NICE)(Chatterton et al. 2012) define it as HbA1c between 6.0–6.4% (42–46 mmol/mol). The test and cut points used to determine PDM have implications on prevalence. Using either HbA1c 6.0–6.4% or HbA1c 5.7–6.4% increases the prevalence of PDM by 1.1 and 2.8 fold respectively compared to OGTT defined PDM (Mostafa et al. 2010b). In the UK using the 5.7–6.4% range would lead to 50% of the population being defined as PDM (Gray et al. 2012b). The potential increase in prevalence seen when using different cut points for HbA1c has important consequences for prevention planning, in particular in terms of health care resources.

Since 2011 and the increased use of HbA1c for the identification of PDM and diagnosis of T2DM, there has been interest in individuals identified with T2DM and PDM from HbA1c and OGTT and whether these two routes lead to different diagnoses. An analyses conducted using data from completed screening studies found that in terms of undiagnosed T2DM, 3.3% of the 8696 individuals screened

were found to have T2DM using an OGTT compared to 5.8% if using HbA1c to define T2DM. 1.2% of those diagnosed using OGTT had an HbA1c not in the T2DM diagnostic range (i.e. <6.5%) and using HbA1c instead of an OGTT resulted in 304 additional cases of T2DM (Mostafa et al. 2010a). These results have been replicated in other populations (Rathmann et al. 2012). Similar results were shown when assessing the identification of PDM using both tests which can have major implications for the provision of prevention programmes (Mostafa et al. 2010b). This discrepancy between the tests in terms of the number of people identified and the different individuals identified creates a dilemma in clinical practice. Additionally most data on the natural history of T2DM and PDM are based on those identified using OGTT, future research should compare this to those identified using HbA1c.

Two Stage

In a two stage screening programme, individuals are pre-screened so only those at the highest risk of PDM/T2DM are given the diagnostic test. The pre-screen test can either be non-invasive using a risk score questionnaire approach or invasive using a blood test. Both are discussed in the following sections.

First Stage: Risk Scores

In a two stage screening programme individuals could be pre-screened using a non-invasive assessment of their risk factors – a risk score. Risk scores offer a quick and simple way of identifying those at high risk for invitation to screening programmes. A plethora of risk scores have been developed and validated for use in specific populations over the past 10 years which aim to identify those at risk of diabetes related outcomes (Buijsse et al. 2011; Collins et al. 2011; Noble et al. 2011). These scores generally follow one of two approaches, either being applied as questionnaires to the individual being assessed – "self-assessment" or as a query to a clinical database where all those at risk are identified using routinely stored data – i.e. a population based approach using general practice data.

Diabetes risk scores also differ in the outcome for which they were developed to identify. Risk scores developed using longitudinal data generally identify those at risk of developing diabetes in the future – for example the Finnish Diabetes Risk Score (FINDRISC) score which was developed for use in Finnish adults. The FINDRISC score was developed using data from a random sample of adults (n = 4746) aged 35–64 years old who were diabetes free (Lindström and Tuomilehto 2003). These adults were followed up for 10 years, incident T2DM during this time was identified by prescriptions of anti-diabetes medications from the National Drug Registry; 196 people had developed drug treated diabetes during the 10 year follow up. These data were used to model the associations between risk factors at baseline with developing drug treated diabetes over 10 years. The final model included age, BMI, waist circumference, high blood pressure medication, history of high blood glucose, physical activity, and daily consumption of vegetables, fruits and berries. From this final model a crude risk score was developed which allocated points to

categories of these risk factors. Using crude scoring system means the FINDRISC can be completed by members of the public using a paper-based questionnaire. The risk score was externally validated using a separate data set of 4615 individuals with five years follow up. Using a cut point of greater than or equal to nine gave sensitivity of 81%, specificity of 76%, a positive predictive value of 5% and a negative predictive value of 99% (Lindström and Tuomilehto 2003). Even though the FINDRISC was designed to predict 10 year diabetes risk, it has also proved to be a reasonably reliable method in identifying current undiagnosed T2DM and PDM (Saaristo et al. 2005), insulin resistance and progression from PDM to T2DM (Schwarz et al. 2009).

Risk scores developed using cross-sectional data detect those at risk of current prevalent conditions, such as undiagnosed T2DM and PDM. One such score is the Leicester Risk Assessment score (see Fig. 3) which was developed for use in a multi-ethnic population in the UK. This score, like the FINDRISC, was designed as a questionnaire to be completed by members of the public without intervention from a heath care professional or the results of medical tests (Gray et al. 2010a). The score was developed using data from diabetes free adults aged 40–75 years old from a population-based screening study (ADDITION-Leicester, n = 6186). The score was developed using similar methodology to the FINDRISC and a crude scoring system was produced which includes the following risk factors: age, sex, ethnicity, BMI, waist circumference, family history of diabetes and hypertension. Based on the total score completers are placed into one of four risk categories (low, medium, high or very high). Those in the high and very high categories (i.e. a score of greater than or equal to 16) are advised to visit their GP for a blood test. The risk score was externally validated using cross-sectional data from another screening study (STAR study, n = 3171) (Gray et al. 2010b).

This cut point has been shown to reliably detect those at high risk of having undiagnosed PDM/T2DM, with sensitivity of 81% and specificity of 45%. This score has also been shown to be a good predictor of future diabetes risk (Barber et al. 2016), and was the first diabetes risk score to be validated in a population with intellectual disabilities (Dunkley et al. 2016). The score has also been translated into a number of Indian languages to increase use in minority ethnic populations (Patel et al. 2016). The Leicester Practice Risk Score is similar to the Leicester Risk Assessment score but for use within primary care databases to rank those listed by risk, this score does not therefore include waist circumference as this is not routinely stored in primary care in the UK (Gray et al. 2012a).

The risk scores developed to date tend to be for a specific population as studies have found that scores which have been developed elsewhere and used on a different population tend to have low validity (Rathmann et al. 2005; Witte et al. 2010). Although a large number of risk scores have been developed for T2DM related outcomes these tend to be for use in developed countries and the majority are for use in white populations. This is probably because population based data are required for the development and such infrastructure may not be established in developing countries. The DETECT-2 study has developed a globally applicable screening tool but this is yet to be tested in other countries (Vistisen et al. 2012).

QUESTIONNAIRE: Do you want to know your risk of Type 2 diabetes?

For each question, tick one box.

1. Which age group are you in?					
49 years and younger		0	60 - 69 years		9
50 - 59 years		5	70 years and older		13
2. Are you male or female?					
Male		1	Female		0
3. How would you describe your ethnicity?					
White European		0	Any other ethnic group		6
4. Do you have a parent, brother, sister and/or child with Type 1 or Type 2 diabetes? (Do not count step-relatives)					
Yes		5	No		0
5. Which waist size group are you in? (See instructions)					
Less than 90 cm (35 inches)		0	100 -109 cm (39 - 42 inches)		6
90 - 99 cm (35 - 38 inches)		4	110 cm (43 inches) & above		9
6. Which Body Mass Index (BMI) group are you in? (See explanation and instructions)					
Less than 25		0	30 - 34		5
25 - 29		3	35+		8
7. Have you ever been told by a doctor or nurse that you have high blood pressure?					
Yes		5	No		0
To get your risk score add up the numbers in the blue boxes next to the seven boxes that you have ticked. Write the total number here – **This is your risk score:** To find out what this means go to page 6					

Fig. 3 Leicester risk assessment score (Gray et al. 2010a)

First Stage: Blood Tests

Invasive tests can also be used in the first stage of a two-stage screening programme. Blood tests which have been shown to have reasonable performance when evaluated against OGTT include random glucose and fasting glucose. Random glucose testing usually involves a capillary measure and is attractive as it can be carried out opportunistically and does not require an overnight fast, which may increase uptake.

However it is not widely used as it has high variability and poor sensitivity, particularly in low risk groups [7]. Very high results are a good indicator of PDM, but lower ranges of 6–10 mmol/l may need to be rescreened using a fasting test. Fasting glucose requires an overnight fast so is not as appealing or practical as the random glucose but these disadvantages are outweighed by its stability and sensitivity. Although this test may miss people whose hyperglycaemia is only manifested after a carbohydrate load. Furthermore, it is advocated that plasma glucose samples should be placed on ice immediately (to stop actively living cells within the blood samples utilising glucose supplies) and should be processed within 1 h, which may be logistically challenging (World Health Organization 2003). A systematic review showed no difference in response rates and diagnostic yields in studies of two stage screening programmes using invasive tests for the first stage compared to non-invasive tests (Khunti et al. 2015).

Risk scores have also been developed to include novel circulating and genetic biomarkers as risk factors. These novel markers require blood tests for their measurement and therefore risk scores including such risk factors may not be as convenient to use as a score containing only non-invasive factors. A review completed in 2012 assessed the incremental improvement in risk prediction when biomarkers were added to traditional risk factors (Echouffo-Tcheugui et al. 2013). In total they found 25 studies which assessed the incremental predictive ability of genetic markers using 106 different single nucleotide polymorphisms (SNPs) from 62 different loci of known gene variants and eight studies assessed non-genetic circulating biomarkers. Overall they reported minimal or no improvement in discrimination and calibration over traditional risk factors. Therefore given the added expense and inconvenience of this approach, little work implementing such risk scores has been conducted.

Second Stage

The second stage of a two-stage screening programme would use the same diagnostic tests as described previously under the one stage section.

Multiple Stages

A systematic review of response rates and diagnostic yields from screening studies for PDM and T2DM was conducted in 2015. They compared studies using different numbers of screening steps, i.e. in a one stage screening programme participants were invited directly for an OGTT and two, three/four stage if participants were screened at one or more levels prior to invitation to OGTT. Forty seven studies were identified which included over 400,000 participants (Khunti et al. 2015). Three studies used a three-step approach and four used a four step strategy. The intermediate steps in these multi-step strategies included risk score, fasting or random glucose and HbA1c. For example the study by Christensen conducted in Denmark included the following screening steps: (1) risk score; (2) random blood glucose or HbA1c; (3) fasting blood glucose; and (4) OGTT (Christensen et al. 2004; Dalsgaard

et al. 2010). Response rates to the initial screening invitation were higher in screening programmes using fewer steps (i.e. one or two step strategies) with no difference in the final screening yields between 1/2 step and 3/4 step programmes. Although as the number of steps increased the number needed to screen by OGTT decreased.

Opportunistic Screening

The approaches to screening described above are all organised screening programmes, i.e. people are invited to participate in a screening programme which has been well defined. Another approach for identifying T2DM with/without PDM is opportunistic screening. WHO define opportunistic screening as screening which "*carried out at a time when people are seen, by health care professionals, for a reason other than the disorder in question* (World Health Organization 2003)," opportunistic screening can also happen outside of a health care setting. For example, risk scores – in theory – could be in super markets or within the local media. Opportunistic risk identification methods provide a possibility to engage with populations who do not usually respond to invitation-based screening. Below we highlight a number of specific case studies demonstrating how opportunistic screening can be used within this context. These case studies are grouped into two broad categories. Firstly we highlight a number of opportunistic screening programmes using a multi-stage approach conducted in Leicester UK, secondly we focus on two key examples of opportunistic screening conducted in alternative medical settings.

Multi-Stage Opportunistic Screening Programmes in Practice: Leicester Experience

Leicester is one of the most diverse cities within the UK, with only 45% of residents describing themselves as white British compared to 80% nationally. The predominate non-white group is Indian. Screening studies have shown that uptake to screening is significantly lower in south Asians compared to White Europeans (Webb et al. 2011). The following sections summarise three studies conducted in Leicester which used an opportunistic approach to recruitment to provide screening for undiagnosed T2DM and PDM within primary care and community settings. These screening pathways can be used to support primary care based screening methods in order to improve uptake.

Risk screening programmes have been previously exclusively undertaken within primary care sites. It has been suggested that such an approach may widen health inequalities by excluding those who do not routinely access organised health care. Community pharmacists have been described as "the biggest untapped resource for health improvement"(Department of Health 2003) and successful screening programmes for other conditions have been provided by them. The PRISM (Pharmacy based screening of high risk individuals using stepwise methods) study used a randomised controlled trial design to

assess the effectiveness of two opportunistic community pharmacist initiated screening strategies for T2DM (Willis 2015). Participants (40% south Asian) were randomised to be offered either; a self-assessment risk score with a referral to their GP if found to be at high risk, or a self-assessment risk score followed by a near patient test for HbA1c and referral to their GP if their HbA1c was in the range 6–6.4% (42–46 mmol/mol). The study found similar rates of attendance to a confirmatory blood test at the GP surgery and similar screening yields for both methods of screening. Overall 3.5% were found to have undiagnosed T2DM and 6.6% had PDM. The study demonstrates the benefit of providing opportunistic screening for diabetes in a community setting but further work is required to maximise uptake to confirmatory testing.

Opportunistic screening can also take place in primary care when people visit their general practitioner for another reason. The ATTEND (Assessment of response rates and yields for two opportunistic tools for early detection of non-diabetic hyperglycaemia and diabetes) study used a randomised controlled trial design to assess the opportunistic use in primary care of a computer based risk score versus a self-assessment risk score for identifying undiagnosed T2DM (Khunti et al. 2016). The study found a significantly higher rate of uptake to a confirmatory blood test in those found to be at 'high risk' using the computer based risk score compared to the self-assessment risk score. Screening yields for PDM and T2DM were similar between the two arms (self-assessment PDM-5.4% T2DM- 0.9%; computer risk score PDM: 6.2% T2DM:3.3%). The data on uptake and screening yield were used to perform a cost-effectiveness analysis. For the base case scenario the cost per new case of T2DM diagnosed was lower for the computer risk score compared to the self-assessment, £168 (~$218), and £352 (~$457) respectively. In conclusion, compared to a self-assessment risk score, a computer based risk score resulted in greater attendance to an initial blood test and is potentially a more cost-effective method of identifying those with undiagnosed diabetes.

Due to the high number of South Asians living in Leicester city a screening pathway was developed to be delivered in local faith and community centres. The CRAFT (Community faith centre based screening and educational intervention to reduce the risk of T2DM) study tested the feasibility of delivering screening for T2DM in the form of a self-assessment risk score and near patient test for HbA1c followed by a group education intervention aimed at increasing step count for those at high diabetes risk (Willis et al. 2016). Almost two thirds of the population screened were found to have a high risk score. Thirty two participants (15.8%) had an HbA1c result in the PDM range and eight (4.0%) had HbA1c over the T2DM threshold. Of those eligible for the diabetes prevention education programme, 18 participants (56.3%) attended. The study found that that screening followed by group education within faith centre settings is feasible and acceptable to participants. In particular, when compared to other pathways based on opportunistic recruitment methods the pathway identified a relatively high screening yield for T2DM and PDM. In addition to this, the group education achieved a high attendance rate when compared to other educational interventions which use an invite via primary care.

Results from these studies demonstrate the effectiveness of carrying out screening activities outside of a GP surgery. This is demonstrated by the high screening yields found. Providing opportunistic screening within the community has the potential to engage with members of the community who may experience barriers to accessing more mainstream screening routes through primary care, and may be from hard to reach groups. All of these studies included 'uptake to screening' or 'uptake to a confirmatory test' as an outcome measure. Although all three studies used an opportunistic approach to recruitment, individual participant consent was required in addition to baseline questionnaire measures being taken prior to screening. It is important to acknowledge that the screening took place as part of a clinical trial and that may affect the validity of the results. Many of the previous barriers to screening including time and resources may actually be exacerbated by study procedures such as completing consent and related study documentation.

Opportunistic Screening in Alternative Medical Settings

The rise of non-invasive risk scores and near patient testing of HbA1c has led to an increased interest in opportunistic screening for T2DM in novel settings. Below two examples of such initiatives are discussed: (1) screening in dental settings, and (2) screening in emergency department settings.

In terms of dental settings, recently there has been increased attention paid to the relationship between periodontal disease and diabetes (Vernillo 2001). Studies have shown that inflammatory periodontal disease is worse in patients with poorly controlled diabetes and among those with PDM. Those with hyperglycaemia are also more susceptible to other oral inflammatory conditions compared to those with normal glycaemia. As those with periodontal conditions may represent a high risk group, dental settings may offer another route for identifying those with undiagnosed T2DM or PDM. To date a limited number of studies have addressed whether screening in dental settings is feasible, which are summarised in Table 2. All three studies show fairly high yields of PDM and T2DM, ranging from 15.8% up to 31.8% for PDM and 4.2% up to 16.4% for T2DM. In line with these studies, data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 were used to compare the prevalence of periodontal disease against the ADA's screening criteria (Strauss et al. 2010). They found a total of 62.9% of those without periodontitis and 93.4% of those with periodontal disease met ADA guidelines for diabetes screening, suggesting that the dental visit may provide an important potential venue for this screening. NHANES data has also been used to develop and validate a risk score for use in dental settings which takes into account periodontal status as well as established diabetes risk factors (Li et al. 2011).

Research into opportunistic screening programmes for PDM and T2DM has also been conducted in emergency departments. The rationale for undertaking such screening is that many people living in inner city areas have limited access to health care where high proportions of the population are not registered with a general practice. In addition inner city areas also have higher populations from black and minority ethnic groups who have a higher prevalence of T2DM and are also less

Table 2 Examples of screening programmes for T2DM/PDM conducted in dental settings

Study	Reference	Eligibility	Targeted	Glucose test	Number screened	PDM	T2DM
Columbia University College of dental medicine, 2011	(Lalla et al. 2011)	Aged over 40 years old if non-Hispanic white and over 30 years old if Hispanic or non-white	At least one risk factor	HbA1c	535	31.8%	4.2%
Saudi Arabia, 2013	(AlGhamdi et al. 2013)	Aged 40 years and over	Random blood glucose test	OGTT	153	15.8%	16.4%
USA, 2014	(Genco et al. 2014)	Aged 45 years and over	American Diabetes Association diabetes risk test	HbA1c	1022	23.3%	12.3%

Table 3 Examples of screening programmes for T2DM/PDM conducted in emergency settings

Study	Reference	Eligibility	Targeted	Glucose test	Number screened	PDM	T2DM
UK, 2005	(George et al. 2005)	Over the age of 40 years	Random blood glucose	Fasting glucose	500	1.6%	2.6%
Spain, 2016	(Gomez-Peralta et al. 2016)	Aged 18 years and over	Consecutive patients	HbA1c	187	NR	5.9%
Australia, 2016	(Hng et al. 2016)	All patients undergoing blood sampling	Random blood glucose	HbA1c	2652	27.4%	32.2%

likely to access routine screening services. Table 3 summarizes the results from a sample of such studies.

This sample of studies assessing the feasibility of screening in emergency room settings show that this is feasible and is a good opportunity for detecting T2DM and potentially PDM in individuals who would not usually seek routine medical care, and who may otherwise go undetected.

Although we have focused on two specific examples of opportunistic screening in health care settings here, there are many other setting where screening for T2DM/PDM has been assessed. Other examples include opticians, inpatient post heart attack, people utilising mental health services and those with previous gestational diabetes or polycystic ovary syndrome. Future work in this area should assess the cost effectiveness of these approaches compared to current screening programmes in primary care.

Should We Screen for T2DM with or Without PDM?

In this section we assess the evidence for and against screening for T2DM against the UK NSC criteria. A number of key published reports (World Health Organization 2003; Waugh et al. 2007, 2013; Simmons et al. 2010; Selph et al. 2015) have previously assessed T2DM screening against the previous versions of these criteria and we make use of their findings throughout. We also draw on the wider literature which has been discussed and summarised within this chapter.

The Condition

There is no doubt that T2DM is an important health problem. The epidemiology, incidence, prevalence and natural history of the condition are well understood. There is a known pre-clinical phase in which T2DM exists but is symptomless in which screening could bring forward diagnosis. Also we know those with T2DM have an increased risk of CVD and early mortality compared to those without the disease. We also know that those with PDM are also at an increased risk of morbidity and mortality. The NSC criteria states that *"all the cost-effective primary prevention interventions should have been implemented as far as practicable."* In terms of primary prevention for T2DM, there is robust evidence from randomised controlled trials that T2DM can be prevented in those with PDM through lifestyle change (Gillies et al. 2007). To date it has been challenging to replicate the findings of the pivotal trials in a real world setting (Dunkley et al. 2014). In 2016, England became the first country to implement an evidence based nationwide diabetes prevention programme. Primary prevention of PDM has not been evaluated. Simmons et al. highlight that alongside these relatively resource intensive programmes for those at high risk of developing T2DM, programmes for population level approaches which aim to reduce glucose levels by a small amount across the whole population are also needed (Simmons et al. 2010). Such strategies may include reformulation of foods to reduce sugar content or new infrastructure for active commuting, such as cycle lanes (White 2016). An example of an actual population level change is the implementation of the excise tax on sugar sweetened beverages in Mexico. One year post implementation of the 'sugar tax' was associated with reductions in purchases of such beverages and increases in purchases of un-taxed beverages (Colchero et al. 2016). To date, although some population-level interventions have been implemented they generally have not been fully evaluated and there are still many areas which have not be exploited. The fourth criteria under 'condition' is not applicable to this area.

The Test

As outlined in previous sections, all of the criteria in terms of the test are met in this case for T2DM with or without PDM (criteria 8 is not applicable). Simple, safe,

precise and validated screening tests are available and many recommend using a non-invasive risk score as a first stage, followed by a blood test in those found to be at high risk. For both validated risk scores and the blood tests the distribution of test values in the target population are known and a suitable cut-off levels have been defined and agreed (although these may differ by country for PDM) and there is agreed policies for further investigation. One such policy included in the UK NICE guidance for identifying those at risk of T2DM (National Institute for Health and Clinical Excellence 2012). It is difficult to assess acceptability of the various available screening programme options. Previous studies have shown that the OGTT was a barrier to screening and therefore the use of HbA1c and near patient testing of HbA1c should be preferable and significantly more convenient for the person being screened. Additionally the use of a non-invasive test to filter for those at highest risk means that blood testing is only used in those who really require it.

The Intervention

The UK Diabetes Prospective Study showed that intensive glucose lowering treatment can reduce cardiovascular risk (UK Prospective Diabetes Study (UKPDS) Group 1998). Although this trial was in people with newly diagnosed diabetes, it could be hypothesised that the same would stand for screen-detected T2DM. In 2011 the results from the ADDITION-Europe Study showed that intensive management of people with screen-detected T2DM did not reduce cardiovascular outcomes (Griffin et al. 2011). This study is discussed in detail in the subsequent sections. In terms of PDM, as discussed previously there is robust evidence that T2DM can be delayed or prevented through lifestyle change (Gillies et al. 2007). For example, the Finnish Diabetes Prevention Study (DPS) found that the risk of T2DM was reduced by 58% in those undertaking an intensive lifestyle intervention compared to usual care over a 3 year period (Tuomilehto et al. 2001). Identical findings were reported for Diabetes Prevention Program (DPP) conducted in the USA (Knowler et al. 2002). Similar and consistent results have been observed in many different and diverse countries including India (Ramachandran et al. 2006) Japan (Kosaka et al. 2005) and China (Pan et al. 1997). Successful lifestyle change programmes have also been shown to have so-called legacy effects whereby the effect persists well after the active intervention has ceased. DPS, DPP and the Chinese Da Qing diabetes prevention study, all found sustained reductions in the incidence of T2DM relative to the control group after seven to 20 years of follow-up (Lindström et al. 2006; Li et al. 2008; Diabetes Prevention Program Research Group 2009). The Da Qing study also showed a 47% reduction in the incidence of severe, vision-threatening retinopathy and significant reductions in CVD events over a 20 year interval (Li et al. 2008, 2014; Gong et al. 2011). These findings suggest that once individuals are enabled to successfully change and self-manage their lifestyle behaviours, benefits can be sustained long after active lifestyle interventions have ceased. Therefore in terms of the intervention offered to those screened the case for screening for T2DM with PDM is stronger than T2DM alone.

The Screening Programme

A Health Technology Assessment (HTA) programme in the UK compared current evidence around screening for T2DM to the NSC screening criteria (Waugh et al. 2007; Waugh et al. 2013). The report concluded that population based screening for T2DM did not meet all of the NSC criteria and therefore should not be recommended. In particular it highlighted that data from two studies showed no overall benefit of screening – the Ely cohort and ADDITION-Europe study, these two pivotal studies are discussed in detail below (Waugh et al. 2013). Finally we give an example of a screening programme with an intervention to identify and treat those with PDM – The Let's prevent Diabetes study.

The Ely Cohort Study

The Ely cohort study was initiated in one general practice in 1900 in Ely in the UK (Williams et al. 1995). In 1990 a third of the practice aged 40–65 years who were free from diabetes were invited for T2DM screening using an OGTT (n = 1705). This cohort were invited for re-screening for diabetes in 1994 (~4.5 years follow up) and 2000 (~10 years follow up). Alongside this, of those not invited for screening in 1990 around half were randomly selected and invited for diabetes screening in 2000 to assess the potential impact of screening on self-rated health and cardiovascular risk measures (Rahman et al. 2012a). Overall no difference between the originally screened and unscreened cohorts were found for self-rated functional health, health utility, most clinical measures (BMI, blood pressure, total cholesterol and LDL cholesterol), self-reported medication use (anti-hypertensive, lipid-lowering, anti-platelet, antidepressant or anxiolytic drugs) and cardiovascular morbidity (self-reported myocardial infarction, stroke, angina and hypertension). Mortality was also assessed finding a non-significant reduction in mortality in those invited to screening between 1990–1999 compared to those not invited (Hazard ratio 0.79, 95% CI 0.63, 1.00) and no difference in mortality when assessed between 2000–2008 (Simmons et al. 2011). Data from this study were also used to assess the duration of diabetes and health outcomes in those diagnosed with T2DM in the screened and unscreened cohorts (Rahman et al. 2012b). They found that on average those in the screened cohort had diabetes durations 3.3 years longer than those in the unscreened cohort, suggesting that screening brought the diagnosis forward by 3.3 years. Interestingly this is shorter than suggested in previous studies (Harris et al. 1992). Clinical measures, prescribed medication and functional status were similar between screened and unscreened populations, suggesting that earlier diagnosis did not lead to improvements in health outcomes.

The ADDITION-Europe Study

The ADDITION-Europe study was a pivotal study in terms of the evidence base for screening for T2DM. The study was conducted across UK, Denmark and the Netherlands and enrolled people without known diabetes from 343 general practices.

The aims of the study were to determine the feasibility, yield, risks, and benefits of primary care–based screening for T2DM and to determine whether early, intensive, multifactorial treatment of hyperglycemia and cardiovascular risk factors reduced the composite cardiovascular outcome of stroke, myocardial infarction (MI), revascularization, amputation, and cardiovascular mortality compared with routine care (Griffin et al. 2011). To identify people with screen-detected T2DM centres used a variety of screening methods including risk scores, capillary blood tests or OGTT.

Overall, 76,308 people were screened, resulting in 3057 people with screen-detected T2DM being recruited into the trial phase of the study (Sandbaek et al. 2008), showing that a stepwise strategy for screening for T2DM is feasible in primary care. In the Cambridge site an embedded sub study assessed whether receiving a negative test result resulted in false reassurance, from the data gathered this did not seem to be the case. Therefore suggesting that implementing a stepwise screening programme for T2DM in primary care is unlikely to cause an increase in unhealthy behaviours arising from false reassurance among people who screen negative (Paddison et al. 2009).

The trial cluster randomised general practices to either provide multifactorial intensive risk factor management to those identified with screen-detected T2DM or usual care (Griffin et al. 2011). The intervention involved target and guideline driven management of hyperglycaemia, blood pressure, and cholesterol levels by medical treatment and promotion of healthy lifestyles in addition to routine care. After an average follow up of 5.3 years, the incidence of the composite cardiovascular outcome was 7.2% in the intensively managed group and 8.5% in the routine care group, this difference was not statistically significant (hazard ratio 0.83, 95% CI 0.65–1.05) (Griffin et al. 2011). Additionally, intensively treating individuals with screen detected T2DM was not cost effective compared to standard care in the UK (Tao et al. 2015). In both groups the number of participants meeting targets for hyperglycaemia, blood pressure, and cholesterol levels increased over the study period. There was also no benefit in terms of microvascular complications at five years (Sandbæk et al. 2014).

It is difficult to estimate the direct benefit of screening, in an ideal situation the ADDITION-Europe study would have randomised practices to either intensive management versus delayed intensive management or screening versus no screening. These studies would not be ethically feasible. Therefore the ADDITION group used the results from the ADDITION-Europe study to simulate what would have happened in such a hypothetical trial. Using the Michigan Model for T2DM to simulate the progression of diabetes and its complications, comorbidities, quality of life, and costs, they estimated the absolute risk of cardiovascular outcomes and the relative risk reduction associated with screening and intensive treatment, screening and routine treatment, and no screening with a 3- or 6-year delay in the diagnosis and routine treatment of diabetes and cardiovascular risk factors. This modelling study found major benefits associated with early diagnosis and treatment of T2DM (Herman et al. 2015).

The Let's Prevent Diabetes Study

The programmes used in the pivotal prevention trials were intensive, for example, in the first year of the United States DPP, participants received 16 1 h one-to-one counselling sessions followed by an average of eight additional contacts and two telephone consultations (Knowler et al. 2002, The Diabetes Prevention Program Research Group 2003). Participants were also offered supervised exercise classes. The difficulty, therefore, has been translating such programmes in the real world setting – with much lower levels of effectiveness seen for programmes conducted in a real world setting (Dunkley et al. 2014).

A pragmatic study which aimed to translate the findings seen in the intensive lifestyle programmes described above into a programme suitable for delivery in the NHS UK was the Let's Prevent Diabetes study (Gray et al. 2012c). The Let's Prevent Diabetes study had two stages: (1) screening for PDM in primary care; (2) cluster randomised trial of the Let's Prevent Diabetes prevention programme in those found to have PDM in (1). In the first stage a non-invasive risk score was used to screen 44 general practices for those at high risk of PDM; 17,972 individuals were invited for screening, of which 3449 (19.2%) attended. All received a 75 g OGTT. PDM was detected in 880 (25.5%) of those screened showing that using a risk score approach in primary care for the identification of those suitable for inclusion in a diabetes prevention programme was feasible (Gray et al. 2012b). Those with PDM were included in the trial. The trial cluster randomised general practices to either provide standard care to those found with PDM or the Let's Prevent Diabetes programme. The Let's Prevent Diabetes programme consisted of a 6-h group structured-education programme, aimed at promoting increased physical activity, a healthy diet and weight regulation, followed by an annual refresher course, and phone contact every three months to increase motivation (Troughton et al. 2016). The aim of the programme was to increase knowledge and promote realistic perceptions of PDM, and to promote health behaviour, with the aims of reducing body weight by 5%, limiting total and saturated fat intake to 30% and 10% of total energy intake respectively, increasing fibre intake and promoting physical activity. Of those included in the trial, 131 participants developed T2DM over the three year follow up period. There was a non-significant 26% reduced risk of developing T2DM in the intervention arm compared to standard care (Davies et al. 2016). A dose response relationship between attendance and outcome was seen, with an 88% reduction in T2DM in those who attended all sessions compared to standard care (Gray et al. 2016). There were also statistically significant improvements in HbA1c, LDL cholesterol, psychosocial wellbeing, sedentary time and step count. The intervention was found to result in a net gain of 0.046 QALYs over three years at an overall cost of £168 per patient, with an incremental cost effectiveness ratio of £3643 and a probability of 0.86 of being cost-effective at a willingness to pay threshold of £20,000 (Leal et al. 2015).

This study showed that a relatively low resource, pragmatic programme fit for implementation in the UK NHS may lead to a reduction in T2DM and improved biomedical and psychosocial outcomes and is cost effective. Future studies should

focus on how to increase engagement and retention in diabetes prevention programmes.

The Implementation Criteria

To date there are no population based T2DM only screening programmes internationally. T2DM screening is incorporated into a wider multifaceted vascular risk assessment (NHS Health Checks programme) for 40–75 year olds in the UK (Dalton et al. 2011). Therefore it is difficult to critique the implementation criteria. Criteria 15 states that "*Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme."* For both T2DM and PDM we know that this is currently not the case. Substantial clinical inertia exists for T2DM, a study of primary care records showed that two years after diagnosis 22% of patients still have poor glycaemic control, with 26% having no intensification of treatment during that time. Delaying intensification of treatment has been shown to increase the risks of CVD (Paul et al. 2015).

Cost Effectiveness of Screening T2DM/PDM

The argument supporting the various methods of screening for T2DM are based on the assumption that costs attributed to conducting the screening and providing ongoing disease management to those diagnosed are outweighed by the cost saving of preventing future diabetic complications, mortality and associated treatment costs.

Developments in point of care testing technology, treatment for T2DM and increasing prevalence rates make direct up to date cost effectiveness analysis difficult. When considering the screening pathway as a whole, by including prevention, there have been no studies presenting direct evidence reporting on cost effectiveness due to the practicalities of collecting long term follow up data on such a large cohorts and appropriate data to act as a comparator group. Evidence in this area has been based on economic modelling using current data on progression rates from PDM to T2DM and assumptions on treating T2DM in its early preclinical phase (Waugh et al. 2007, 2013). Recent evidence from the UK suggests that screening for T2DM and PDM, followed by appropriate intervention is cost effective. Estimated costs for each quality adjusted life year (QALY) gained (discounted at 3.5% a year for both costs and benefits) were £14,150 (€17,560; $27860) for screening for T2DM, £6242 for screening for both T2DM and PDM followed by lifestyle interventions, and £7023 for screening for both T2DM and PDM followed by pharmacological interventions, all compared with no screening (Gillies et al. 2008). Furthermore, a European study concluded that screening for T2DM allows diagnosis 3.8 years earlier and a gain of 0.8 life-years. Intervention proved cost-effective and reduced the average cost of illness by £605 (€807) per case of T2DM detected in the screening programme when compared with no screening. Intervention for PDM produced average savings per detected case of £4861 (€6481). Costs per QALY

showed that screening was cost-effective for T2DM (€563 per QALY with lifestyle) and could be cost-saving in PDM (Schaufler and Wolff 2010).

In terms of preventing T2DM, a simulation study which aimed to estimate the costs and benefits of a nationwide community-based lifestyle intervention programme for preventing T2DM in the USA found that over 25 years such a programme would prevent or delay about 885,000 cases of T2DM and produce savings of $5.7 billion (Zhuo et al. 2012). Further modelling has been carried out to establish the most cost effective screening strategy for identification of T2DM and PDM. Khunti et al. carried out modelling on a cost per case detected basis using a number of different one and two stage screening strategies (Khunti et al. 2012). This study concluded that the lowest cost screening strategies ranged from £457 to £523 (€526–€601) and involved a two-stage screening strategy, a non-invasive risk stratifying tool followed by a blood test.

Much of the research undertaken in terms of assessing the cost effectiveness of screening for T2DM has been conducted from a developed country perspective. In 2001 the Brazilian Ministry of Health conducted a one-off mass population based screening programme for T2DM and between March and April 2001, 22.1 million capillary blood glucose tests were performed (Toscano et al. 2008, 2015). During this period it was estimated that 346,168 new cases of T2DM were diagnosed, with a number needed to screen of 64 to detect one new case of T2DM. Using data from this one-off programme a cost effectiveness model was developed to estimate the long terms costs and benefits of such a programme compared to no screening. Compared to no screening, screen detection of T2DM was associated with US$ 31,147 per QALY gained. This was reduced if targeting high risk hypertensive individuals. Interestingly they found that the cost of the screening programme impacted little on the overall cost-effectiveness and therefore population-based approach which would have a larger participation rate maybe justifiable given the lower screening costs in developing countries. This study only considered T2DM, other studies have shown that screening and prevention in those with PDM could be cost saving and therefore the Brazilian government may wish to consider widening such programmes to also focus on those with PDM.

Summary

There is robust evidence that screening for PDM and providing prevention interventions can reduce progression to T2DM, the challenge here is in the prevalence of PDM and therefore the resources required to provide such programmes for all those who may be identified. The pivotal prevention trials have used very resource intensive interventions and there has been difficulty in replicating these findings in clinical practice. The argument for screening for T2DM is less clear and is still a hot topic which is debated. The key trials have failed to show long term benefits of screening. Therefore currently screening for T2DM tends to be offered as part of a multifaceted vascular check rather than as a standalone programme.

Box 1 World Health Organisation Criteria for Screening (Wilson and Jungner 1968)

1. The condition should be an important health problem.
2. There should be a treatment for the condition.
3. Facilities for diagnosis and treatment should be available.
4. There should be a latent stage of the disease.
5. There should be a test or examination for the condition.
6. The test should be acceptable to the population.
7. The natural history of the disease should be adequately understood.
8. There should be an agreed policy on whom to treat.
9. The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
10. Case-finding should be a continuous process, not just a "once and for all" project

Box 2 Summary of National Screening Committee Programme Appraisal Criteria (National Screening Committee 2003)

The Condition

1. The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease.
2. All the cost-effective primary prevention interventions should have been implemented as far as practicable.
3. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

The Test

4. There should be a simple, safe, precise and validated screening test.
5. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.
6. The test, from sample collection to delivery of results, should be acceptable to the target population.

(*continued*)

Box 2 (continued)

7. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.
8. If the test is for a particular mutation or set of genetic variants the method for their selection and the means through which these will be kept under review in the programme should be clearly set out.

The Intervention

9. There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered.
10. There should be agreed evidence based policies covering which individuals should be offered interventions and the appropriate intervention to be offered.

The Screening Programme

11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.
12. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.
13. The benefit gained by individuals from the screening programme should outweigh any harms for example from over diagnosis, overtreatment, false positives, false reassurance, uncertain findings and complications.
14. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.

(*continued*)

Box 2 (continued)

The Implementation Criteria

15. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.
16. All other options for managing the condition should have been considered (such as improving treatment or providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.
17. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.
18. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.
19. Evidence-based information, explaining the purpose and potential consequences of screening, investigation and preventative intervention or treatment, should be made available to potential participants to assist them in making an informed choice.
20. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.

References

ADA. 2. Classification and diagnosis of diabetes. Diabetes Care. 2016;39(Suppl 1):S13–22.

AlGhamdi AST, Merdad K, Sonbul H, Bukhari SM, Elias WY. Dental clinics as potent sources for screening undiagnosed diabetes and prediabetes. Am J Med Sci. 2013;345(4):331–4.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2004;27:s5–s10.

American Diabetes Association. Summary of revisions for the 2010 clinical practice recommendations. Diabetes Care. 2010;33:S3.

Barber SR, Dhalwani NN, Davies MJ, Khunti K, Gray LJ.External national validation of the Leicester Self-Assessment score for Type 2 diabetes using data from the English Longitudinal Study of Ageing. Diabet Med. 2017 Nov;34(11):1575–1583. https://doi.org/10.1111/dme.13433. Epub 2017 Aug 20.

Bland M. An introduction to medical statistics. Oxford: Oxford University Press; 2015.

Bodicoat DH, Khunti K, Srinivasan BT, Mostafa S, Gray LJ, Davies MJ, Webb DR. Incident Type 2 diabetes and the effect of early regression to normoglycaemia in a population with impaired glucose regulation. Diabet Med. 2017 Mar;34(3):396–404.

Buijsse B, Simmons RK, Griffin SJ, Schulze MB. Risk assessment tools for identifying individuals at risk of developing type 2 diabetes. Epidemiol Rev. 2011;33(1):46–62.

Chatterton H, Younger T, Fischer A, Khunti K, on behalf of the Programme Development Group. Risk identification and interventions to prevent type 2 diabetes in adults at high risk: summary of NICE guidance. BMJ. 2012;12(345):e4624.

Christensen J, Sandbæk A, Lauritzen T, Borch-Johnsen K. Population-based stepwise screening for unrecognised Type 2 diabetes is ineffective in general practice despite reliable algorithms. Diabetologia. 2004;47(9):1566–73.

Colchero MA, Popkin BM, Rivera JA, Ng SW. Beverage purchases from stores in Mexico under the excise tax on sugar sweetened beverages: observational study. BMJ. 2016;352:h6704.

Collins GS, Mallett S, Omar O, Yu L. Developing risk prediction models for type 2 diabetes: a systematic review of methodology and reporting. BMC Med. 2011;9:103.

Committee NS. Criteria for appraising the viability, effectiveness and appropriateness of a screening programme. 2015. https://www.gov.uk/government/publications/evidence-review-criteria-national-screening-programmes/criteria-for-appraising-the-viability-effectiveness-and-appropriateness-of-a-screening-programme#the-condition. Retrieved 4 Aug 2016.

Dalsgaard E-M, Christensen JO, Skriver MV, Borch-Johnsen K, Lauritzen T, Sandbaek A. Comparison of different stepwise screening strategies for type 2 diabetes: finding from Danish general practice, addition-DK. Prim Care Diabetes. 2010;4(4):223–9.

Dalton ARH, Bottle A, Okoro C, Majeed A, Millett C. Uptake of the NHS health checks programme in a deprived, culturally diverse setting: cross-sectional study. J Public Health. 2011. https://doi.org/10.1093/pubmed/fdr034.

Davidson MB. Counterpoint: the oral glucose tolerance test is superfluous. Diabetes Care. 2002;25(10):1883–5.

Davidson MB, Peters AL, Schriger DL. An alternative approach to the diagnosis of diabetes with a review of the literature. Diabetes Care. 1995;18(7):1065–71.

Davies MJ, Gray LJ, Troughton J, Gray A, Tuomilehto J, Farooqi A, Khunti K, Yates T. A community based primary prevention programme for type 2 diabetes integrating identification and lifestyle intervention for prevention: the Let's prevent diabetes cluster randomised controlled trial. Prev Med. 2016;84:48–56.

Department of Health. A vision for pharmacy in the new NHS. 2003. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4068356.pdf. Retrieved 10 Jan 2013.

Diabetes Prevention Program Research Group. 10-year follow-up of diabetes incidence and weight loss in the diabetes prevention program outcomes study. Lancet. 2009;374(9702):1677–86.

Dunkley AJ, Bodicoat DH, Greaves CJ, Russell C, Yates T, Davies MJ, Khunti K. Diabetes prevention in the real world: effectiveness of pragmatic lifestyle interventions for the prevention of type 2 diabetes and of the impact of adherence to guideline recommendations: a systematic review and meta-analysis. Diabetes Care. 2014;37:922–33. Diabetes Care 37(6):1775–1776

Dunkley AJ, Tyrer F, Spong R, Gray LJ, Gillett M, Doherty Y, Martin-Stacey L, Patel N, Yates T, Bhaumik S, Chalk T, Chudasama Y, Thomas C, Sadler S, Cooper S, Gangadharan SK, Davies MJ, Khunti K. Screening for glucose intolerance and development of a lifestyle education programme for prevention of Type 2 diabetes in a population with intellectual disabilities. Programme Grants for Applied Research. Southampton (UK), In press; 2016.

Eborall H, Stone M, Aujla N, Taub N, Davies M, Khunti K. Influences on the uptake of diabetes screening: a qualitative study in primary care. Br J Gen Pract. 2012;62(596):e204–11.

Echouffo-Tcheugui JB, Dieffenbach SD, Kengne AP. Added value of novel circulating and genetic biomarkers in type 2 diabetes prediction: a systematic review. Diabetes Res Clin Pract. 2013;101(3):255–69.

Engberg S, Vistisen D, Lau C, Glümer C, Jørgensen T, Pedersen O, Borch-Johnsen K. Progression to impaired glucose regulation and diabetes in the population-based Inter99 study. Diabetes Care. 2009;32(4):606–11.

Erlinger TP, Brancati FL. Postchallenge hyperglycemia in a National Sample of U.S. adults with type 2 diabetes. Diabetes Care. 2001;24(10):1734–8.

Gallagher EJ, Bloomgarden ZT, Roith D. Review of hemoglobin A1c in the management of diabetes. J Diabetes. 2009;1:9–17.

Genco RJ, Schifferle RE, Dunford RG, Falkner KL, Hsu WC, Balukjian J. Screening for diabetes mellitus in dental practices: a field trial. J Am Dent Assoc. 2014;145(1):57–64.

George PM, Valabhji J, Dawood M, Henry JA. Screening for type 2 diabetes in the accident and emergency department. Diabet Med. 2005;22(12):1766–9.

Gerstein H, Santaguida P, Raina P, Morrison K, Balion C, Hunt D. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract. 2007;78(3):305–12.

Gholap NN, Davies MJ, Mostafa SA, Khunti K. Diagnosing type 2 diabetes and identifying high-risk individuals using the new glycated haemoglobin (HbA1c) criteria. Br J Gen Pract. 2013;63(607):e165–7.

Gillies CL, Abrams KR, Lambert PC, Cooper NJ, Sutton AJ, Hsu RT, Khunti K. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. BMJ. 2007;334(7588):299.

Gillies CL, Lambert PC, Abrams KR, Sutton AJ, Cooper NJ, Hsu RT, Davies MJ, Khunti K. Different strategies for screening and prevention of type 2 diabetes in adults: cost effectiveness analysis. BMJ. 2008;336(7654):1180–5.

Gomez-Peralta F, Abreu C, Andreu-Urioste L, Antolí AC, Rico-Fontsaré C, Martín-Fernández D, Resina-Rufes R, Pérez-García JJ, Negrete-Muñoz Á, Muñoz-Álvarez D, Umpierrez GE. Point-of-care capillary HbA1c measurement in the emergency department: a useful tool to detect unrecognized and uncontrolled diabetes. Int J Emerg Med. 2016;9(1):1–6.

Gong Q, Gregg EW, Wang J, An Y, Zhang P, Yang W, Li H, Jiang Y, Shuai Y, Zhang B, Zhang J, Gerzoff RB, Roglic G, Hu Y, Li G, Bennett PH. Long-term effects of a randomised trial of a 6-year lifestyle intervention in impaired glucose tolerance on diabetes-related microvascular complications: the China Da Qing diabetes prevention outcome study. Diabetologia. 2011; 54(2):300–7.

Gray LJ, Taub N, Khunti K, Gardiner E, Hiles S, Webb DR, Srinivasan B, Davies MJ. The Leicester risk assessment score for detecting undiagnosed type 2 diabetes and impaired glucose regulation for use in a multiethnic UK setting. Diabet Med. 2010a;27(8):887–95.

Gray LJ, Tringham J, Davies MJ, Webb DR, Jarvis J, Skinner TC, Farooqi A, Khunti K. Screening for type 2 diabetes in a multiethnic setting using known risk factors to identify those at high risk: a cross-sectional study. J Vasc Health Risk Manag. 2010b;6:837–42.

Gray LJ, Davies MJ, Hiles S, Taub NA, Webb DR, Srinivasan BT, K. K. Detection of impaired glucose regulation and/or type 2 diabetes mellitus, using primary care electronic data, in a multiethnic UK community setting. Diabetologia. 2012a;55(4):959–66.

Gray LJ, Khunti K, Edwardson C, Goldby S, Henson J, Morris DH, Sheppard D, Webb D, Williams S, Yates T, Davies MJ. Implementation of the automated Leicester practice risk score in two diabetes prevention trials provides a high yield of people with abnormal glucose tolerance. Diabetologia. 2012b;55(12):3238–44.

Gray LJ, Khunti K, Williams S, Goldby S, Troughton J, Yates T, Gray A, Davies MJ. Let's prevent diabetes: study protocol for a cluster randomised controlled trial of an educational intervention in a multi-ethnic UK population with screen detected impaired glucose regulation. Cardiovasc Diabetol. 2012c;11:56.

Gray LJ, Yates T, Troughton J, Khunti K, Davies MJ. Engagement, retention, and progression to type 2 diabetes: a retrospective analysis of the cluster-randomized "Let's Prevent Diabetes" trial. PLoS Med. 2016;13(7):e1002078. https://doi.org/10.1371/journal.pmed.1002078.

Griffin SJ, Borch-Johnsen K, Davies MJ, Khunti K, Rutten GE, Sandbæk A, Sharp SJ, Simmons RK, van den Donk M, Wareham NJ, Lauritzen T. Effect of early intensive multifactorial therapy on 5-year cardiovascular outcomes in individuals with type 2 diabetes detected by screening (ADDITION-Europe): a cluster-randomised trial. Lancet. 2011;378(9786):156–67.

Harris MI, Klein R, Welborn TA, Knuiman MW. Onset of NIDDM occurs at least 4-7 yr before clinical diagnosis. Diabetes Care. 1992;15(7):815–9.

Harris R, Donahue K, Rathore SS, Frame P, Woolf SH, Lohr KN. Screening adults for type 2 diabetes: a review of the evidence for the U.S. preventive services task force. Ann Intern Med. 2003;138:215–29.

Herman WH, Ma Y, Uwaifo G. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care. 2007;30:2453–7.
Herman WH, Ye W, Griffin SJ, Simmons RK, Davies MJ, Khunti K, Rutten GEHM, Sandbaek A, Lauritzen T, Borch-Johnsen K, Brown MB, Wareham NJ. Early detection and treatment of type 2 diabetes reduce cardiovascular morbidity and mortality: a simulation of the results of the Anglo-Danish-Dutch study of intensive treatment in people with screen-detected diabetes in primary care (ADDITION-Europe). Diabetes Care. 2015;38(8):1449–55.
Hex N, Bartlett C, Wright D, Taylor M, Varley D. Estimating the current and future costs of type 1 and type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. Diabet Med. 2012;29(7):855–62.
Hng T-M, Hor A, Ravi S, Feng X, Lin J, Astell-Burt T, Chipps D, McLean M, Maberly G. Diabetes case finding in the emergency department, using HbA1c: an opportunity to improve diabetes detection, prevention, and care. BMJ Open Diabetes Res Care. 2016;4(1).
International Diabetes Federation. IDF diabetes atlas. 7th ed. International Diabetes Federation, Brussels, Belgium. 2015.
Khunti K, Taub N, Gillies C, Abrams K, Hiles S, Webb D, Srinivasan B, Davies MJ. A comparison of screening strategies for impaired glucose tolerance and type 2 diabetes mellitus in a UK community setting: a cost per case analysis. Diabetes Res Clin Pract. 2012;97(3):505–13.
Khunti K, Mani H, Achana F, Cooper N, Gray LJ, Davies MJ. Systematic review and meta-analysis of response rates and diagnostic yield of screening for type 2 diabetes and those at high risk of diabetes. PLoS ONE. 2015;10(9):e0135702. https://doi.org/10.1371/journal.pone.0135702.
Khunti K, Gillies CL, Dallosso H, Brady EM, Gray LJ, Kilgallen G, Willis A, Zafar A, Davies MJ. Assessment of response rates and yields for two opportunistic tools for early detection of non-diabetic hyperglycaemia and diabetes (ATTEND). A randomised controlled trial and cost-effectiveness analysis. Diabetes Res Clin Pract. 2016;118:12–20.
Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.
Kosaka K, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. Diabetes Res Clin Pract. 2005;67(2):152–62.
Lalkhen AG, McCluskey A. Clinical tests: sensitivity and specificity. Contin Educ Anaesth Crit Care Pain. 2008;8(6):221–3.
Lalla E, Kunzel C, Burkett S, Cheng B, Lamster IB. Identification of unrecognized diabetes and pre-diabetes in a dental setting. J Dent Res. 2011;90(7):855–60.
Leal J, Ahrabian D, Gray AM. Cost effectiveness of a pragmatic structured education intervention for type 2 diabetes: economic evaluation of data from the Let's Prevent trial. BMJ Open. 2015;7:e013592. https://doi.org/10.1136/bmjopen-2016-013592.
Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. Clin Chem. 2010;56(1):44–52.
Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, e. al. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing diabetes prevention study: a 20-year follow-up study. Lancet. 2008;371(9626):1783–9.
Li S, Williams PL, Douglass CW. Development of a clinical guideline to predict undiagnosed diabetes in dental patients. J Am Dent Assoc. 2011;142(1):28–37.
Li G, Zhang P, Wang J, An Y, Gong Q, Gregg EW, Yang W, Zhang B, Shuai Y, Hong J, Engelgau MM, Li H, Roglic G, Hu Y, Bennett PH. Cardiovascular mortality, all-cause mortality, and diabetes incidence after lifestyle intervention for people with impaired glucose tolerance in the Da Qing diabetes prevention study: a 23-year follow-up study. Lancet Diabetes Endocrinol. 2014;2(6):474–80.
Lindström J, Tuomilehto J. The diabetes risk score. Diabetes Care. 2003;26(3):725–31.
Lindström J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson J, Hemiö K, Hämäläinen H, Härkönen P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Paturi M, Sundvall J, Valle T, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention Study Group.

Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish diabetes prevention study. Lancet. 2006;368(9548):1673–9.
Meigs JB, Muller DC, Nathan DM, Blake DR, Andres R. The natural history of progression from normal glucose tolerance to type 2 diabetes in the Baltimore longitudinal study of aging. Diabetes. 2003;52(6):1475–84.
Morris DH, Khunti K, Achana F, Srinivasan B, Gray LJ, Davies MJ, Webb D. Progression rates from HbA1c 6.0–6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. Diabetologia. 2013;56(7):1489–93.
Mostafa SA, Davies MJ, Webb D, Gray LJ, Srinivasan BT, Jarvis J, Khunti K. The potential impact of using glycated haemoglobin, HbA1c, as the preferred diagnostic tool for type 2 diabetes mellitus. Diabet Med. 2010a;72:762–9.
Mostafa SA, Khunti K, Srinivasan BT, Webb D, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a UK multi-ethnic cohort. Diabetes Res Clin Pract. 2010b;90(1):100–8.
Mostafa SA, Davies MJ, Webb DR, Srinivasan BT, Gray LJ, Khunti K. Independent effect of ethnicity on glycemia in south Asians and white Europeans. Diabetes Care. 2012;35(8):1746–8.
Mozaffarian D, Kamineni A, Carnethon M, Djoussé L, Mukamal KJ, Siscovick D. Lifestyle risk factors and new-onset diabetes mellitus in older adults: the cardiovascular health study. Arch Intern Med. 2009;169(8):798–807.
National Institute for Health and Clinical Excellence. Public health Guidence 38, preventing type 2 diabetes: risk identification and interventions for individuals at high risk. London: National Institute for Health and Clinical Excellence; 2012.
National Institute of Health and Clinical Excellence. Preventing type 2 diabetes: risk identification and interventions for individuals at high risk. London: NICE; 2012.
National Screening Committee. Criteria for appraising the viability, effectiveness and appropriateness of a screening programme. UK. http://www.screening.nhs.uk/criteria. 2003. Retrieved 1 June 2014.
National Screening Committee. https://www.gov.uk/guidance/nhs-population-screening-explained. 2016. Retrieved 21 Mar 2016.
Noble D, Mathur R, Dent T, Meads C, Greenhalgh T. Risk models and scores for type 2 diabetes: systematic review. BMJ. 2011;343:d7163.
Paddison CAM, Eborall HC, Sutton S, French DP, Vasconcelos J, Prevost AT, Kinmonth A-L, Griffin SJ. Are people with negative diabetes screening tests falsely reassured? Parallel group cohort study embedded in the ADDITION (Cambridge) randomised controlled trial. BMJ. 2009;339:b4535.
Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: the Da Qing IGT and diabetes study. Diabetes Care. 1997;20(4):537–44.
Patel N, Willis A, Stone M, Barber S, Gray L, Davies M, Khunti K. Developing a conceptually equivalent type 2 diabetes risk score for Indian Gujaratis in the UK. J Diabetes Res. 2016;2016:9.
Paul SK, Klein K, Thorsted BL, Wolden ML, Khunti K. Delay in treatment intensification increases the risks of cardiovascular events in patients with type 2 diabetes. Cardiovasc Diabetol. 2015;14(1):1.
Phung OJ, Sood NA, Sill BE, Coleman CI. Oral anti-diabetic drugs for the prevention of type 2 diabetes. Diabet Med. 2011;28(8):948–64.
Rahman M, Simmons RK, Hennings SH, Wareham NJ, Griffin SJ. Effect of screening for type 2 diabetes on population-level self-rated health outcomes and measures of cardiovascular risk: 13-year follow-up of the Ely cohort. Diabet Med. 2012a;29(7):886–92.
Rahman M, Simmons RK, Hennings SH, Wareham NJ, Griffin SJ. How much does screening bring forward the diagnosis of type 2 diabetes and reduce complications? Twelve year follow-up of the Ely cohort. Diabetologia. 2012b;55:1651–9.
Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V, Indian Diabetes Prevention Programme (IDPP). The Indian diabetes prevention programme shows that lifestyle

modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49(2):289–97.
Rathmann W, Martin S, Haastert B, Icks A, Holle R, Lowel H, Giani G, for the Kora Study Group. Performance of screening questionnaires and risk scores for undiagnosed diabetes: the KORA survey 2000. Arch Intern Med. 2005;165(4):436–41.
Rathmann W, Kowall B, Tamayo T, Giani G, Holle R, Thorand B, Heier M, Huth C, Meisinger C. Hemoglobin A1c and glucose criteria identify different subjects as having type 2 diabetes in middle-aged and older populations: the KORA S4/F4 study. Ann Med. 2012;44(2):170–7.
Roper NA, Bilous RW, Kelly WF, Unwin NC, Connolly VM. Excess mortality in a population with diabetes and the impact of material deprivation: longitudinal, population based study. BMJ. 2001;322(7299):1389–93.
Saaristo T, Peltonen M, Lindstrom J, Saarikoski L, Sundvall J, Eriksson JG, Tuomilehto J. Cross-sectional evaluation of the Finnish diabetes risk score: a tool to identify undetected type 2 diabetes, abnormal glucose tolerance and metabolic syndrome. Diab Vasc Dis Res. 2005;2(2):67–72.
Sandbaek A, Griffin SJ, Rutten G, Davies M, Stolk R, Khunti K, Borch-Johnsen K, Wareham NJ, T L. Stepwise screening for diabetes identifies people with high but modifiable coronary heart disease risk. The ADDITION study. Diabetologia. 2008;51(7):1127–34.
Sandbæk A, Griffin SJ, Sharp SJ, Simmons RK, Borch-Johnsen K, Rutten GEHM, van den Donk M, Wareham NJ, Lauritzen T, Davies MJ, Khunti K. Effect of early multifactorial therapy compared with routine care on microvascular outcomes at 5 years in people with screen-detected diabetes: a randomized controlled trial. The ADDITION-Europe study. Diabetes Care. 2014;37 (7):2015–23.
Schaufler TM, Wolff M. Cost effectiveness of preventive screening programmes for type 2 diabetes mellitus in Germany. Appl Health Econ Health Policy. 2010;8(3):191–202.
Schwarz PE, Li J, Reimann M, Schutte AE, Bergmann A, Hanefeld M, Bornstein SR, Schulze J, Tuomilehto J, Lindström J. The Finnish diabetes risk score is associated with insulin resistance and progression towards type 2 diabetes. J Clin Endocrinol Metab. 2009;94(3):920–6.
Selph S, Dana T, Blazina I, Bougatsos C, Patel H, Chou R. Screening for type 2 diabetes mellitus: a systematic review for the U.S. preventive services task ForceScreening for type 2 diabetes mellitus. Ann Intern Med. 2015;162(11):765–76.
Simmons R, Echouffo-Tcheugui J, Griffin S. Screening for type 2 diabetes: an update of the evidence. Diabetes Obes Metab. 2010;12(10):838–44.
Simmons RK, Rahman M, Jakes RW, Yuyun MF, Niggebrugge AR, Hennings SH, Williams DRR, Wareham NJ, Griffin SJ. Effect of population screening for type 2 diabetes on mortality: long-term follow-up of the Ely cohort. Diabetologia. 2011;54(2):312–9.
Stokes A, Mehta NK. Mortality and excess risk in US adults with pre-diabetes and diabetes: a comparison of two nationally representative cohorts, 1988–2006. Popul Health Metrics. 2013;11(1):1–7.
Strauss SM, Russell S, Wheeler A, Norman R, Borrell LN, Rindskopf D. The dental office visit as a potential opportunity for diabetes screening: an analysis using NHANES 2003-2004 data. J Public Health Dent. 2010;70(2):156–62.
Tao L, Wilson ECF, Wareham NJ, Sandbæk A, Rutten GEHM, Lauritzen T, Khunti K, Davies MJ, Borch-Johnsen K, Griffin SJ, Simmons RK. Cost-effectiveness of intensive multifactorial treatment compared with routine care for individuals with screen-detected type 2 diabetes: analysis of the ADDITION-UK cluster-randomized controlled trial. Diabet Med. 2015;32(7): 907–19.
The Diabetes Prevention Program Research Group. Costs associated with the primary prevention of type 2 diabetes mellitus in the diabetes prevention program. Diabetes Care. 2003;26(1):36–47.
The International Expert Committee. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care. 2009;32:1327–34.
Toscano CM, Duncan BB, Mengue SS, Polanczyk CA, Nucci LB, e Forti AC, Fonseca CD, Schmidt MI. Initial impact and cost of a nationwide population screening campaign for diabetes in Brazil: a follow up study. BMC Health Serv Res. 2008;8(1):1.

Toscano CM, Zhuo X, Imai K, Duncan BB, Polanczyk CA, Zhang P, Engelgau M, Schmidt MI. Cost-effectiveness of a national population-based screening program for type 2 diabetes: the Brazil experience. Diabetol Metab Syndr. 2015;7(1):1–11.

Troughton J, Chatterjee S, Hill SE, Daly H, Stacey LM, Stone MA, Patel N, Khunti K, Yates T, Gray LJ. Development of a lifestyle intervention using the MRC framework for diabetes prevention in people with impaired glucose regulation. J Public Health. 2016 Sep;38 (3):493–501.

Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Aunola S, Cepaitis Z, Moltchanov V, Hakumaki M, Mannelin M, Martikkala V, Sundvall J, Uusitupa M, the Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344(18):1343–50.

UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837–53.

Vernillo AT. Diabetes mellitus: relevance to dental treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91(3):263–70.

Vistisen D, Lee CM, Colagiuri S, Borch-Johnsen K, Glümer C. A globally applicable screening model for detecting individuals with undiagnosed diabetes. Diabetes Res Clin Pract. 2012;95 (3):432–8.

Waugh N, Scotland G, McNamee P, Gillett M, Brennan A, Goyder E, Williams R, John A. Screening for type 2 diabetes: literature review and economic modelling. Health Technol Assess. 2007;11(17):1–125.

Waugh N, Shyangdan D, Taylor-Phillips S, Suri G, Hall B. Screening for type 2 diabetes: a short report for the National Screening Committee. 2013.

Webb DR, Gray LJ, Khunti K, Srinivasan B, Taub N, Campbell S, Barnett J, Farooqi A, Echouffo-Tcheugui J, Griffin S, Wareham N, Davies M. Screening for diabetes using an oral glucose tolerance test within a western multi-ethnic population identifies modifiable cardiovascular risk: the ADDITION-Leicester study. Diabetologia. 2011;54(9):2237–46.

White M. Population approaches to prevention of type 2 diabetes. PLoS Med. 2016;13(7): e1002080.

Williams DRR, Wareham NJ, Brown DC, Byrne CD, Clark PMS, Cox BD, Cox LJ, Day NE, Hales CN, Palmer CR, Shackleton JR, Wang TWM. Undiagnosed glucose intolerance in the community: the Isle of Ely diabetes project. Diabet Med. 1995;12(1):30–5.

Willis AW. The effectiveness of screening for type 2 diabetes within a community pharmacy setting. PhD, University of Leicester. 2015.

Willis A, Roshan M, Patel N, Gray L, Yates T, Davies M, Khunti K. A community faith centre based screening and educational intervention to reduce the risk of type 2 diabetes: a feasibility study. Diabetes Res Clin Pract. 2016;120:73–80.

Wilson JMG, Jungner G. Principles and practice of screening for disease, Public health paper, vol. 34. Geneve: World Health Organization; 1968.

Witte DR, Shipley MJ, Marmot MG, Brunner EJ. Performance of existing risk scores in screening for undiagnosed diabetes: an external validation study. Diabet Med. 2010;27(1):46.

World Health Organisation. Definition, diagnosis, and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva: World Health Organisation; 1999.

World Health Organisation. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Geneva: World Health Organisation; 2011.

World Health Organization. Screening for type 2 diabetes. Report of a World Health Organization and International Diabetes Federation meeting. 2003.

Zhuo X, Zhang P, Gregg EW, Barker L, Hoerger TJ, Pearson-Clarke T, Albright A. A nationwide community-based lifestyle program could delay or prevent type 2 diabetes cases and save $5.7 billion in 25 years. Health Aff. 2012;31(1):50–60.

Home Blood Glucose Monitoring and Digital-Health in Diabetes

12

Andrew Farmer and Kingshuk Pal

Contents

A. Farmer (✉)
Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK
e-mail: andrew.farmer@phc.ox.ac.uk

K. Pal
Research Department of Primary Care and Population Health, University College London, London, UK

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_13

Abstract

Diabetes is a disorder of glucose metabolism and a major cause of death and disability. It currently affects 387 million people worldwide and is expected to affect 592 million by 2035. Monitoring of glucose levels is an essential component of treatment - providing feedback to clinician and patient on management through lifestyle and pharmacotherapy. This chapter provides an overview of the evidence that monitoring levels of glycaemia leads to improved outcomes for diabetes; a brief history of the technologies used for monitoring; and an update on recent research into ways in which people can be supported with use of their medication. Clinical support systems are now available and have been refined to improve their effectiveness, and combined with systems that enable personal support for self-monitoring can help make better use of the data available. The chapter includes a brief overview of recent developments with continuous glucose monitoring, flash monitoring and closed loop systems.

Keywords

Diabetes · Glucose monitoring · Digital technologies · Insulin treatment · Self-management support · Adherence

Introduction

Diabetes is a disorder of glucose metabolism and a major cause of death and disability. It currently affects 387 million people worldwide and is expected to affect 592 million by 2035 (Guariguata et al. 2014). It is responsible for five million deaths a year, and $673 billion is spent on healthcare for diabetes (12% of global health expenditure) (International Diabetes Federation n.d.). A detailed analysis of costs highlighted the importance of both direct and indirect costs, with a marked impact on employment potential (Seuring et al. 2015). Detailed within country analysis in the UK has identified the contribution to costs arising from hospital admission, which can arise either from either the consequences of poor glycemic control or arising from complications of the disease, including cardiovascular disease, cerebrovascular disease, renal disease, and amputations (Hex et al. 2012). These amount to ten per cent of the healthcare budget. Although the complexity of the homeostatic mechanisms underpinning glucose metabolism is increasingly understood, this knowledge still remains to be effectively applied to deliver glucose levels constrained toward physiological levels in the range 4–6 mmol/L (80–110 mg/dL).

Technological progress, alongside pharmacological advances, has revolutionized the management of, and outcomes for, people with diabetes. Urine testing has now been largely replaced by self-monitoring of blood glucose. Accurate measurement of blood glucose levels using finger-prick devices allows targeting of therapy and can provide feedback on the impact of physical activity and food intake on glycemia. The impact of continuous monitoring, or monitoring using devices that avoid repeated fingertip sampling, is yet to be fully assessed in clinical practice.

This chapter describes the way in which blood glucose self-monitoring is currently being used to support the care of people with diabetes, the potential impact of linking glucose monitoring to digital health devices, and the potential for such devices to also provide better self-management of other aspects of care, including management of therapeutic regimens, diet, and physical activity. In providing an overview of these issues, this chapter highlights the way that digital technologies can be used to ensure that the benefits of monitoring are fully delivered for patients and for health services.

Background

Blood glucose levels vary throughout the day, and for many individuals with type 1 diabetes, awareness of these variations and adjustment of insulin dose is a means to avoid both the immediate consequences of symptoms arising from hyper- or hypoglycemia and a means to deliver an overall average glucose level that is associated with a lower risk of long-term complications. The average overall level of glucose control, however, can be judged by the level of HbA_{1c}, a glycated protein that reflects levels of control over the previous 120 days and has been shown to be closely linked to long-term morbidity and mortality.

Achieving Optimal Glycemic Control with Monitoring

For individuals with both type 1 and type 2 diabetes, maintenance of long-term levels of glycemia contributes to a lower risk of long-term complications. In the diabetes control and complications trial (type 1 diabetes) (the Diabetes Control and Complications Trial Epidemiology of Diabetes Interventions and Complications DCCT/EDIC Study Research Group 2005), the risk of cardiovascular events was reduced by 42% alongside substantial reductions in renal disease and eye complications for those with better glycemic control. For individuals with type 2 diabetes, long-term follow-up of the UKPDS study where glucose levels were lower in the intervention compared to a control group observed reductions of 24% for microvascular complications and 15% for myocardial infarction (Holman et al. 2008).

Tight control of other risk factors, including blood pressure, cholesterol, and smoking are also major contributors to reduction in complications, but the management of glycemia presents unique challenges, as well as providing an exemplar for management of other risk factors including blood pressure and cholesterol levels.

Poor glycemic control among people with diabetes remains a major public health problem. A recent prospective cohort study of European patients with type 2 diabetes identifies over 37% with an $HbA_{1c} \geq 7\%$ (53 mmol/mol), while the UK National Diabetes Audit identified 66% meeting a HbA1c target of ≤ 58 mmol/mol in 2014/2015. In the same UK audit, control of both blood pressure ($\leq 140/80$) and cholesterol (<5 mmol/L) were better at 74.2% and 77.5%, respectively (Health and Social Care Information Centre 2016).

There is therefore an unmet need for improved glucose control for people with diabetes in the context of maintaining quality of life and reducing the burden of self-care. Utilizing data about levels of glucose control to bring glycemic control for people with diabetes back to physiological levels requires pharmacological and lifestyle measures. Measurement of blood glucose or HbA_{1c} is often considered in the context of a diagnostic test, with a reason for an abnormal measurement considered and an action prescribed. However, it is not just used as a single test, but as a test repeated over time with the aim of identifying excursions beyond a defined range of normal values or to modify an intervention intended to reestablish the parameter within a defined range.

Theoretical Approaches to Monitoring

The concept of a cycle of events in which the response of a system is measured and adjustment made to maintain a constant state is taken from engineering control theory (Del Toro and Parker 1960). For people with type 1 diabetes, short term and within day, measurement of glucose levels and adjustment of short-acting insulin dose is used to maintain glycemic control. The same control-cycle principles apply to adjustment of long-acting or basal insulin in response to glucose levels, although a more gradual adjustment of dose over a period of days reflects the longer period required to achieve a steady state of insulin levels for a change in insulin dose. Similar principles can be considered for people with type 2 diabetes and gestational diabetes where insulin treatment is used. The concept of a cycle of events can be applied to the use of HbA_{1c} to monitor long-term control for people with type 2 diabetes, where adjustment of oral medication can be carried out on the basis of knowledge of the average glucose control over a preceding period of weeks, with subsequent retesting to judge the need for further adjustment of medication.

The concept of a control cycle is more specifically referenced in behavior change theories such as control theory (Carver and Scheier 2002). This theory postulates that there is a synergistic association between receiving information about one's behavior (via "self-monitoring" or "feedback") and having a strategy for acting on this information ("action planning" or "information on where and when to perform the behavior"). The former provides a cue and motivation for the latter. Education that supports patients understanding associations between patterns of behavior (e.g., eating, physical activity, and medication adherence) and outcomes (blood glucose levels) has the potential to be more effective than education or blood glucose testing on their own.

For some people, the experience of self-monitoring extends to a greater understanding of the physiological processes and thus enables adjustment of lifestyle and pharmacological treatment to avoid the development of hyperglycemia, particularly during periods of illness. It can also allow recognition of low levels of blood glucose that could lead to hypoglycemia. The impact of self-monitoring on illness understanding can be difficult to interpret, particularly as the changes in beliefs and perceptions can be very personal, varies widely between individuals, and is not consistently linked with changes in behavior (French et al. 2008).

Increasing the Impact of Monitoring with Education and Technology

Diabetes self-management education and support (DSMES) is an important element of diabetes healthcare provision that has been shown to reduce the risks of developing diabetes-related complications and improve glycemic control, at least in the short term (Norris et al. 2002; Powers et al. 2015). However there are significant challenges in providing DSMES and uptake rates are often low (Coonrod et al. 1994; Centre 2016). Barriers to attendance at self-management education sessions (whether individual or in a group) include inconvenience, fear of stigma, and a lack of knowledge about the potential benefits (Winkley et al. 2015). Digital DSMES programs have the potential for delivery at multiple locations at convenient times, can be used anonymously, and present content in an attractive and tailored format (Pal et al. 2013). Delivering DSMES online can improve glycemic control and diabetes-related knowledge (Pereira et al. 2015; Arambepola et al. 2016).

Adherence with a recommended regimen for taking diabetes medication is needed to obtain maximal benefit from treatment (Farmer et al. 2015). A systematic review of medication adherence studies found that retrospective analyses showed adherence with oral hypoglycemic agents ranged from 36% to 93% and prospective analysis showed adherence between 67% and 85% (Cramer 2004). Around one-third of patients with type 2 diabetes stop their medication within 1 year of starting treatment, and this leads to poorer clinical outcomes and higher healthcare costs (Egede et al. 2012; Hertz et al. 2005; Pladevall et al. 2004). Recent studies of adherence to medication in type 2 diabetes report up to 30% primary nonadherence (Karter et al. 2009) with up to 13% of those continuing to use medication taking less than 80% of their prescribed medication (Farmer et al. 2015).

Interventions using the Internet and digital devices have a growing evidence base. For example, using short messaging service (SMS) text messages to deliver behavioral support focused on medication adherence has been shown to be an effective way of improving medication adherence (Bobrow et al. 2016). The majority of the population in most countries could use such potentially low-cost scalable digital interventions. Reminders and online portals to track medication refills have also been shown to improve medication adherence in people living with type 2 diabetes (Misono et al. 2010; Sarkar et al. 2014). However, for such interventions to have a meaningful impact, they would need to become part of standard care, and, as with DSMES interventions above, providing patients with links between blood glucose levels and their use to diabetes medication would be a potent feedback mechanism to motivate and support adherence to treatment.

The Artificial Pancreas

The technological culmination of successful management of glucose homeostasis is the "artificial pancreas." The extent to which technology has been able to deliver a functioning system that replicates the physiological functions of the pancreas is discussed at the end of this chapter. However, the technology underpinning

monitoring, including glucose sensors, computer algorithms, patient education and advice, and insulin pumps, has all undergone transformations over recent years, offering potential for patient benefit.

Urine Testing to Home Blood Glucose Monitoring

The characteristic sweet urine of diabetes, described by Thomas Willis in 1674 in differentiating diabetes mellitus from diabetes insipidus, has been recognized for thousands of years. In 1776 Matthew Dobson established that the smell arose from sugar. In 1838 George Owen Rees showed how sugar could be isolated from the blood of people with diabetes. The development of the copper reduction test by Benedict in the early twentieth century gained widespread acceptance as a means of testing for glucose levels in urine. In the 1940s, Clinitest, a self-heating alkaline copper reduction test, gained widespread acceptance, to be superseded in 1957 by a urine test stick: the glucose oxidase-based Clinistix (Free et al. 1957).

In 1963, Ernie Adams developed Dextrostix, in which a glucose oxidase/peroxidase reaction was used with a semipermeable membrane through which glucose, but not red blood cells, could pass. The reaction led to a color change; the blue color produced was proportional to blood glucose levels. Although the method allowed an estimate of blood glucose levels, accurate results depended on having experience of the methods and being able to judge the intermediate color changes. In 1970, Anton Clemens was the first person to develop a blood glucose meter, the Ames Reflectance Meter. The meter used the light reflected from a Dextrostix to provide a more accurate estimate of blood glucose level. Despite its size and weight, and originally intended for physician office use, the meter was rapidly adopted by many clinicians and their patients for home use with the first case reports of use of a meter for home glucose monitoring dating from 1975, rapidly followed by detailed reports of its use (Tattersall 1979). Other meters were then developed using other chemicals to react with a dye and, depending on glucose levels, produce a color change.

In 1982 Hill and colleagues developed a ferrocene electrode in which the glucose oxidase reaction led to a change in electrical conductivity rather than a color change and thus opened the way to development of more accurate estimates of blood glucose levels using portable and convenient meters.

Current blood glucose meters are compliant with international standards for accuracy, but have lower levels of accuracy compared to laboratory methods, with a coefficient of variance/variation of around 4% to 6% compared to laboratory standards of less than 2%. Most meters are now factory calibrated, use very small quantities of blood, are quick, and record readings in an internal memory. Many also allow download of their readings to a computer for further review and interpretation. Current standards date from 2013 (ISO: 15197:2013) with the aim that 95% of blood glucose results should be within $\pm$ 0.83 mmol/L of laboratory results at concentrations of under 5.6 mmol/L (within $\pm$ 15 mg/dl of laboratory results at concentrations of under 100 mg/dL) and within $\pm$ 20% of laboratory results at concentrations of

5.6 mmol/L (100 mg/dL) or more. The guidance also requires 99% of readings to fall within zones A and B of the consensus error grid for type 1 diabetes.

Careful handling of test strips and attention to standardized procedures are important for accurate testing. Exposure of test strips to air reduces accuracy, and some reagents are affected by altitude and humidity. Contamination of strips from handling without hand-washing is also a potential problem.

There is a wide range of other equipment available for glucose and glycated hemoglobin. These also range from laboratory-based analyzers to small point of care analyzers and self-monitoring devices that can be used for finger-prick measurement. In addition, the measurement of finger-prick blood samples is now supplemented with technology for intermittent or continuous monitoring using implantable sensors, considered later in this chapter.

Using Blood Glucose Testing for Home Management

Regardless of the indication for using self-monitoring of blood glucose (SMBG), careful instructions in technique and knowledge and skills in using the data acquired to adjust therapy are needed. Regular review is needed to ensure that skills are maintained and that the type of monitoring carried out is relevant and contributing to maintaining health. Obtaining blood samples from finger tips is best done by using a lancet on the side of the finger rather than directly on the finger pad.

Most blood glucose meters in current use allow measurements to be stored and tagged to indicate whether the readings are made before or after food. Detailed records of blood glucose levels alongside changes in treatment, physical activity, and food intake are needed for self-management and adjustment of treatment. Increasingly blood glucose meters include the facility for charting or displaying data in graphical form and downloading data to computer or other digital devices. These technological developments are considered later in the chapter.

Use of Home Blood Glucose Monitoring Type 1 Diabetes

With the potential for enabling people with type 1 diabetes to adjust their insulin dose and check for hypoglycemia, self-blood glucose monitoring is widely accepted on the basis of early case studies showing a clear impact on diabetes control (Walford et al. 1978), although randomized studies of insulin treatment in type 1 diabetes have SMBG as part of the treatment and not separately evaluated. Self-monitoring, along with education and experience in adjustment of insulin levels to reflect lifestyle, provides the tool with which desired blood glucose levels can be accurately targeted.

Adults with type 1 diabetes are recommended to aim for a fasting plasma glucose level of 5–7 mmol/liter on waking and a level of 4–7 mmol/liter before meals at other times of the day. For those individuals testing after meals, a target of 5–9 mmol/liter 90 min after eating is recommended (National Institute for Health and Clinical Excellence 2015). People should be supported to aim for an HbA1c of 48 mmol/

mol (6.5%), but an individualized target should take into account a wide range of factors. To achieve these levels, testing is recommended four times a day including before each meal and before bed. Testing up to ten times a day can be needed if the agreed target for blood glucose control measured by HbA_{1c} is not met; hypoglycemia becomes a problem, during illness, when taking part in a sport, during pregnancy, and if there are legal reasons for doing so (e.g., when driving) (National Institute for Health and Clinical Excellence 2015).

Use of Home Blood Glucose Monitoring for Insulin Treated Type 2 Diabetes

Evidence from a small number of trials does not provide convincing evidence that intensive monitoring of individuals with type 2 diabetes using insulin leads to clinically significant benefits from HbA_{1c} reduction. Never the less, the use of regular blood glucose testing is needed to safely achieve control of glycemia in a timely manner without leading to hypoglycemia. Incremental increases in insulin required to reach an acceptable level of control without testing would be unsafe and risk hypoglycemia. Individuals with type 2 diabetes starting insulin using a basal (once daily long acting) regimen can titrate insulin requirements straightforwardly using once daily testing (Holman et al. 2007); as fasting glucose levels fall, additional tests may be needed where hypoglycemia is a possibility (e.g., with physical activity or changing meal patterns). If basal insulin treatment fails to reduce HbA_{1c} to an acceptable level, then additional testing may be needed to adjust insulin dose with introduction of prandial insulin or mixed insulin regimens.

Use of Home Blood Glucose Monitoring for Non-insulin-Treated Type 2 Diabetes

For many people with type 2 diabetes, measurements of HbA_{1c} are sufficient to guide any necessary changes in non-insulin glucose-lowering treatments. Many treatments for type 2 diabetes now available do not lead to hypoglycemia and do not, therefore, need routine monitoring. However there remain concerns that some drugs, for example, sulfonylurea drugs, may increase risk of hypoglycemia, and therefore SMBG should be available. The circumstances through which SMBG for non-insulin treated type 2 diabetes came into widespread use, and then following careful examination of the evidence moved to a more restricted role, highlights the need for careful evaluation of technology intended to improve outcomes.

Following the development and wider use of blood glucose meters for self-monitoring for people with type 1 diabetes, the potential for blood glucose meters to be used by people with type 2 diabetes to control their blood glucose levels was suggested. This was followed by much research intended to evaluate the extent of benefit of using self-monitoring of blood glucose to support self-management by people with non-insulin-treated type 2 diabetes. The first reported randomized trial

was in 1986 (Wing et al. 1986), and a series of subsequent trials were reported up to 2000.

Two important reports published at that time raised concerns about the use of current strategies for SMBG. A systematic review identified that the pooled data from randomized trials to date comparing the effectiveness of people using SMBG, to those not using SMBG, did not show any additional benefit in reducing blood glucose levels (Coster et al. 2000). A cohort study also showed that people treated with insulin using SMBG showed improvements in blood glucose control, but no benefit was observed for people treated with diet or with oral glucose-lowering drugs. In addition, the possibility of increased distress, worry, and depressive symptoms for those using SMBG was raised (Franciosi et al. 2001). A number of well-designed trials were established to establish whether structured education in the use of medication, closer attention to medication titration, use soon after diagnosis, or other factors might improve the impact of the technology when routinely for non-insulin treated people (Farmer et al. 2007; Davidson et al. 2005; O'Kane et al. 2008; Schwedes et al. 2002). In addition, the largest of these trials included an integral cost-effectiveness analysis (Simon et al. 2008).

These trials and a number of others have been examined for evidence of benefit from using SMBG. Pooling of composite data (Clar et al. 2010; Malanda and Welschen 2012) did not provide evidence for a clinically important effect, and pooling of individual data from six trials using a prespecified protocol and intention to treat analysis confirmed a benefit of 1–2 mmol/mol (0.2%) in $HbA_{1c}$and did not identify any subgroups in which there might be more benefit from using SMBG. Further studies have identified and carried out proof-of-principal studies to establish whether focusing on further structuring of the delivery of SMBG might improve effectiveness, but trials have not identified a clinically important benefit (Polonsky and Fisher 2013; Franciosi et al. 2011).

In 2015 the UK National Institute for Health and Clinical Excellence reviewed the evidence for use of blood glucose self-monitoring for people with type 2 diabetes. The guideline development group examined a range of trials that might have identified a potential benefit. Of the 17 trials comparing SMBG with no SMBG, there was only a small, clinically unimportant reduction in HbA_{1c} levels, although hypoglycemic events were increased. However, the extent to which this might have been due to increased awareness of low blood glucose levels is unclear. Different forms of SMBG were examined, including SMBG plus education versus conventional SMBG in three studies. Overall differences between the groups were not significant. SMBG plus telecare versus conventional SMBG was tested in five trials, but the only trials reporting benefit did not report the types of glucose-lowering treatment being used. Trials looking at frequency of monitoring did not find any differences in HbA_{1c} when comparing less frequent with more frequent monitoring. The health economic evidence suggested that use of SMBG resulted in a lower benefit in terms of quality of life year estimates, as well as being more costly.

Although measurement of blood glucose levels may provide some insight into the impact of lifestyle on glucose control, the extent to which this can be achieved when used routinely at a wide-scale is therefore unproven. Similarly, although the extent to

which SMBG might be used for people with type 2 diabetes to support self-management and improve communication with clinicians is often discussed, evidence of benefit remains limited.

Following the widespread introduction and standardization of HbA_{1c} measurement, along with the observation that for many individuals, HbA_{1c} levels remain relatively stable over time, regular measurement of blood glucose levels has been replaced with HbA_{1c} testing. Two to three monthly measurements allow titration of medication, and annual tests allow maintenance of control to be confirmed in those who have a stable treatment regimen. The potential for self-monitoring to provide information about the pattern of blood glucose levels throughout the day and the extent to which the measurements can provide additional motivation and support remain a matter of debate.

NICE has therefore recommended that SMBG should not be used routinely, although it may still have a place where there is an increased risk of hypoglycemia, for example in sulfonylurea drug treatment.

Digital Health and Glucose Control for Type 2 Diabetes

Progress in technology has greatly expanded the potential for supporting better blood glucose control through multiple channels, not only those directly relating to glucose measurement. Over the past 20 years, connectivity between devices and computing power has evolved rapidly. Systems used by health professionals to maintain electronic medical records have developed to allow patient access to their data through the Internet. Mobile phones now support wearable smart devices allowing for increasingly sophisticated information processing and sharing (van Rooij and Marsh 2016). Digital health interventions based on these technologies (often referred to as mHealth) offer a range of functions that support self-monitoring and self-management including distance-based care, education, support for medication adherence, clinical decision support, and personal applications and devices. This section will look at digital interventions to support better self-management of type 2 diabetes, focusing on blood glucose monitoring and control.

Distance-Based Care

Blood glucose self-monitoring solutions involve data recording and displays. The use of these facilities on blood glucose meters has been described earlier in this chapter. These facilities can be complemented by software that allows logging and visual displays of the information stored on the meters. Early telehealth interventions added the ability to share this data with healthcare professionals and support distance-based care. An early systematic review of telehealth interventions to support self-blood glucose monitoring in patients with diabetes did not find evidence of improvements in HbA_{1c} (Farmer et al. 2005). Simply keeping a record of blood glucose readings does not improve long-term outcomes, and transferring data

collection from paper to computer, using technology to record data, or sharing data remotely do not influence this. However, a systematic review of the use of information technology to manage diabetes found that more sophisticated interventions that included computerized insulin dose adjustment, remote case-management, or distance learning were more likely to show improvements in outcomes like glycemic control (Riazi et al. 2015).

Clinical Decision Support

Clinical decision support systems (CDSS) are computer programs that offer patient-specific, actionable recommendations or management options to improve clinical decisions (Hunt et al. 1998; Kawamoto et al. 2005; Roshanov et al. 2013). Older reviews suggested that the features that lead to improved outcomes were automatic decision support as part of clinician workflow, provision of recommendations rather than assessments, and provision of decision support at the time and location of decision-making. However recent reviews suggest that there is potential for clinicians to be at risk of "alert fatigue" and that integration of support with workflow risks generating too many alerts that are subsequently overridden or ignored (Roshanov et al. 2013; van der Sijs et al. 2006). However, ensuring that patients are also informed of outputs from the CDSS appears to be an effective strategy. Thus, there are opportunities for systems that have traditionally been clinician focused to be improved by taking a more patient-centered approach through collection of patient-reported data and using suitable user-interfaces (O'Connor et al. 2016). Self-reported blood glucose monitoring data feeding into such systems could be used to guide clinical decision-making and be analyzed to provide suggestions for changes to lifestyle and medication. This data would be uploaded automatically without manual input of values. This integrated and holistic approach to digital health could target individuals who might benefit from the regular use blood glucose monitoring or highlight circumstances when it could be valuable in people who would not otherwise need regular SMBG.

Personal Self-Monitoring and Self-Management Support

Mobile phone-based applications (apps) are becoming increasingly popular with over half of adults in the United States owning a smartphone (Eng and Lee 2013). However even though there are more than 1000 publically available smartphone apps for diabetes, a recent review found only 20 peer-reviewed evaluations of these apps (Garabedian et al. 2015). Most apps do not adhere to evidence-based guidance and lack an empirical or theoretical basis for development, and there are no universal standards to help users judge apps by such criteria (Breland et al. 2013; Boulos et al. 2014). In spite of the large volume of apps for diabetes, the majority offer similar functionalities and combine only one or two functions, usually manual blood glucose recording (Arnhold et al. 2014). Given the evidence discussed previously

in this chapter, these apps are unlikely to have any impact on outcomes, yet they are marketed and sold to users. However most apps score quite highly on assessments of usability and acceptability, even for adults aged over 50, and the fewer the functions, the higher the usability (Arnhold et al. 2014; Payne et al. 2015). The challenge that lies ahead is work to combine the usability of commercially created apps with a theoretical and empirical basis that can create usable and effective interventions, within a regulated framework, that can link glucose meters, personal records, electronic medical records, and CDSS (Klonoff 2013).

However, it is also worth noting that not all mHealth is high-tech or smartphone based. SMS text messaging is a cheap and widely available technology that can be used in most parts of the world and is another popular area of research (Bin Abbas et al. 2015; Capozza et al. 2015). It has also been shown to be an effective way of improving outcomes in a range of long-term conditions (Free et al. 2013; Leon et al. 2015; Lester et al. 2010). Algorithm-driven SMS-advice based on patient-entered blood glucose data has been shown to reduce HbA_{1c}, and although it is not widely used, it can be effective (Liang et al. 2011). A systematic review of computerized diabetes support trials suggests mobile phone-based interventions that provided tailored feedback and advice based on blood glucose reading have significantly larger improvements in HbA_{1c} than other digital self-management interventions (Pal et al. 2014).

A wide range of behavioral approaches has been combined with monitoring to provide smartphone-based health coaching: health-related education, behavior change, and support for patients (Sherifali et al. 2016). Health professionals or peers can lead these interventions, and they have been shown to help reduce HbA_{1c} and improve patient outcomes (Quinn et al. 2011; Thom et al. 2013; van der Wulp et al. 2012; Wayne and Ritvo 2006).

Potential Challenges with Digital Interventions

Although digital health interventions have potential to improve care through the wide range of functions described above, they also have a number of barriers to their effectiveness that need to be overcome. These include engaging people with their use, inequity in provision, facilitating their adoption by health systems, security, and rapid changes in systems as technology evolves.

Disengagement with digital interventions is a significant concern as the usage of digital interventions is associated with their effectiveness (Couper 2010; Donkin 2011). It is particularly important for digital interventions to have active strategies to facilitate uptake and engagement with users (van Vugt et al. 2016). Technology-based prompts can help with this (Alkhaldi et al. 2016), and human input and support in using digital interventions by facilitators and peers have also been shown to increase exposure (Brouwer 2011).

One of the biggest concerns with new technologies is equity and access, often referred to as the digital divide. The digital divide can be defined as an economic and social inequality arising from lack of access or impact from information and

communications technology (ICT) (US Department of Commerce, National Telecommunications and Information Administration (NTIA) 1995) – and it has been noted that use of the Internet and access is highly associated with income, age, education, and occupation (van Dijk 2006). However access to ICT is improving rapidly – for example, in the UK in 2015, 86% of adults had used the Internet within the last 3 months and that number is increasing (Statistics 2015). Digital interventions increasingly have the potential to be a channel through which health outcomes can be improved across society and used to reach those with the greatest need and the most potential to benefit.

Effective digital interventions consist of multiple components, often referred to as complex interventions, and are not always widely adopted in healthcare settings, and when they are, the process is often much slower than other sectors of business and society (Chaudoir et al. 2013; Cresswell and Sheikh 2013). The process of implementing such complex interventions into routine clinical practice faces a number of challenges (Murray et al. 2010). There is a general difficulty perceived in making the transition "from clinical studies to everyday clinical practice and health decision making" (Woolf 2008). Therefore approaches to implanting digital technologies for diabetes need to adapt to address the likely barriers (Grol 1997) and address the interdisciplinary nature of the problem. A review of the implementation literature identified more than ten different academic disciplines that contribute to the uptake of innovations in health services (Greenhalgh et al. 2005). There are more than 60 theories and frameworks that have been developed to guide the process of implementation (Tabak 2012). Although there is no simple solution to the challenges of the implementing digital health technologies, taking a theoretically informed approach to anticipating barriers and generating possible solutions as part of the conception, development, and evaluation of the technologies is likely to be key to success.

Increasing dependence on technology for care for people with diabetes carries with it a number of risks. These include risks for patients from unauthorized access to their data and loss of data. These could arise unintentionally through human error, power failure, or malicious tampering. Security standards required for devices, for example, insulin pumps, are rapidly developing and are addressing such threats.

Technical obsolescence is a major risk facing all IT systems (Samy et al. 2010). Hardware and software systems are rapidly evolving: Moore's law predicts that processing power of computers doubles every 2 years, and this has remained true for nearly five decades (Roberts 2000; Schaller n.d.); Lehman's laws predict that the software size and complexity will increase with time and system quality will decline with time unless the system is rigorously monitored and adapted to these changes (Yu and Mishra 2013). The digital landscape has further evolved with the advent of multiple computing devices that now include tablets, smartphones, and wearable technology. IT systems now need to be compatible with multiple ecosystems (e.g., Windows, Android, IOS) with different interfaces and devices sizes – and also factor in planned obsolescence with annual iterations of many hardware and software platforms. Achieving sustainability by gaining sufficient adoption and use, while keeping up with an evolving environment, is an increasingly complex task. The time

taken to evaluate and implement healthcare services puts digital health interventions as risk of obsolescence before they have a chance to be widely adopted.

Developing Technology Around Home Glucose Monitoring

Continuous Blood Glucose Monitoring

The possibility of using more frequent glucose measurements than feasible with blood glucose meters to guide insulin therapy has stimulated the development of increasingly practical technologies for continuous glucose monitoring (CGM), capable of providing up to 300 measurements a day.

All the currently available systems require calibration using capillary blood glucose measurement and use a subcutaneously implanted sensor that can remain in place for up to 7 days. This sensor usually transmits data wirelessly to a monitor. CGM systems are intended for intermittent use to identify periods of hyperglycemia that can be corrected by changing therapy (e.g., increasing the dose of insulin or changing timing of injections) or detecting periods of biochemical hypoglycemia that may be too brief to cause symptoms but may nevertheless cause some impairment in cognitive function. These devices are not as accurate as conventional blood glucose meters, so blood glucose levels need to be confirmed before a change in treatment.

Evidence for effectiveness of CGM in selected people with T1DM aged over 25 years using intensive insulin therapy comes from a randomized trial with 322 people with T1DM (Juvenile Diabetes Research Foundation 2008). Those allocated to the CGM arm experienced a 0.5% (6 mmol/mol) reduction in HbA1c from 7.6% to 7.1% (60 to 54 mmol/mol) compared to conventional therapy. Evidence for HbA1c lowering is less strong in children, teenagers, and younger adults, although there may be specific clinical circumstances in which CGM might be helpful. Success correlates with adherence to the ongoing use of the device. Many people with type 1 diabetes indicate that CGM is a valued addition to diabetes care with a perceived improvement in HbA1c and reduction in hypoglycemia (Pickup et al. 2015).

In the UK, the National Institute for Health and Care Excellence (NICE) does not currently recommend routinely offering real-time CGM to adults with type 1 diabetes. However it can be considered for use where individuals are willing to use the systems and treatment is otherwise optimized, in those with severe hypoglycemia, loss of awareness of hypoglycemia, frequent asymptomatic hypoglycemia, or HbA_{1c} levels above 9% (77 mmol/mol) (National Institute for Health and Clinical Excellence 2015).

Continuous glucose monitoring during pregnancy is also an area where periods of intermittent continuous monitoring may offer benefit. To date, studies have not shown an improvement in glycemic control or clinical outcomes (Secher et al. 2013), but further work on the abnormalities detected by monitoring may be needed to better target glycemia. For example, using closed-loop systems in a proof-of-concept setting shows the potential for improved glycemic control (Law et al. 2015).

Flash Monitoring Systems

Innovative approaches to interstitial glucose measurement have now been developed, building on the experience of continuous glucose monitoring. These are referred to as flash monitoring – using an implantable sensor that is scanned to read the current glucose level, rather than providing a continuous stream of data. For example, the FreeStyle® Libre device uses a sensor worn for up to 2 weeks. It is designed for continuous use and does not require calibration, but is scanned, giving readings over the previous 8 hours, using a handheld device that avoids the need for a direct connection between a sensor and the recording device.

Insulin Bolus Advisor

For people with type 1 diabetes, adjustment of short-acting insulin is required to target recommended blood glucose levels. To achieve this, the insulin dose is adjusted based on carbohydrate intake and current glucose intake, taking into account insulin sensitivity. However, the required insulin dose is frequently miscalculated (Ahola et al. 2010). Some newer meters contain algorithms that can either be programmed with the required insulin sensitivity ratios. These meters appear to be safe and acceptable to patients in proof-of-concept studies (Schmidt et al. 2012).

Closed-Loop Systems

The language of engineering, noted at the beginning of this chapter, is reflected in the considerable advances that have been made in the management of diabetes where continuous glucose monitoring and continuous insulin infusion devices have been linked. Technologies evaluated include systems that suspend delivery of insulin when levels reach or are predicted to reach a preset lower limit and closed-loop systems that provide autonomous graduated modulation of insulin above and below preset insulin amounts in a glucose responsive manner (Hovorka et al. 2014).

Many people with type 2 diabetes have welcomed the development of such systems and the way they can provide "time off..." from the demands of diabetes (Hovorka et al. 2014; Barnard et al. 2015).

The control algorithms used in such closed-loop systems include a wide range of predictive approaches based on mathematical models that account for delays in absorption of food and delays in absorption of insulin. Strategies that have been used to overcome the inaccuracies of predictions include the use of hybrid systems that allow use of manual bolus of short-acting insulin.

The major limitations of these systems include the slow absorption of insulin and the difficulties of predicting insulin requirements around exercise and the postprandial state. In addition, there is a lag in blood glucose levels changing in the interstitial fluid compared to plasma. All of these factors limit the performance of closed-loop systems during the daytime, with clinical evaluations to date largely focusing on the overnight phase.

CGM systems are now being evaluated as a sensor within a closed-loop system in which insulin delivery through a pump device is regulated by the use of a control algorithm that automatically reduces and increases subcutaneous insulin delivery according to sensor glucose levels. Recent short-term studies in young adults with diabetes in a home setting indicate that glucose control is improved during the day and night with fewer episodes of hypoglycemia (Hovorka et al. 2014).

Summary

Monitoring of blood glucose levels is appropriate where the purpose of doing so is clear, the technology robust, and it can be done by individuals. Technology for blood glucose monitoring is increasingly simple to use and, although not as accurate as laboratory measurement, offers information on which to adjust insulin therapy. However, the extent to which routine monitoring offers advantages over HbA_{1c} monitoring for adjustment of medication in type 2 diabetes is unclear. Further advances in the technologies linked to SMBG are currently being tested. The rapidly evolving nature of technology and the increasingly ubiquitous presence of devices with significant computing ability represent significant opportunities to support patients with blood glucose control and improve outcomes for people with type 2 diabetes.

Although there is evidence of promise for many solutions across the technology spectrum, there are important barriers posed by the rapid pace of change and significant fragmentation in an environment with different devices, software ecosystems, and stakeholders with different needs (patients, healthcare professionals, and administrators). Together with the known difficulties in establishing uptake and engagement of health professionals and users at scale, there are significant challenges that need to be overcome to deliver sustainable, comprehensive, and accessible technology solutions. Health services need to address these issues to ensure that the potential of new technology is fulfilled to help deliver the increasing cost-efficiencies that are urgently needed to deal with the increasing demands facing health services in the twenty-first century.

References

Ahola AJ, et al. Many patients with Type 1 diabetes estimate their prandial insulin need inappropriately. J Diabetes. 2010;2(3):194–202.

Alkhaldi G, et al. The effectiveness of prompts to promote engagement with digital interventions: a systematic review. J Med Internet Res. 2016;18(1):e6.

Arambepola C, et al. The impact of automated brief messages promoting lifestyle changes delivered via mobile devices to people with type 2 diabetes: a systematic literature review and meta-analysis of controlled trials. J Med Internet Res. 2016;18(4):e86–12.

Arnhold M, Quade M, Kirch W. Mobile applications for diabetics: a systematic review and expert-based usability evaluation considering the special requirements of diabetes patients age 50 years or older. J Med Internet Res. 2014;16(4):e104.

Barnard K, et al. Future artificial pancreas technology for type 1 diabetes: what do users want? Diabetes Technol Ther. 2015;17(5):311–5.
Bin Abbas B, et al. Effect of mobile phone short text messages on glycemic control in type 2 diabetes. Int J Endocrinol Metab. 2015;13(1):e18791.
Bobrow K, et al. Mobile phone text messages to support treatment adherence in adults with high blood pressure (StAR): a single-blind, randomized trial. Circulation. 2016.; Available at: http://circ.ahajournals.org/content/133/6/592.full.html?ijkey=9HjK6o57zyKut6w&keytype=ref
Boulos MN, Brewer, AC, Karimkhani C et al. Mobile medical and health apps: state of the art, concerns, regulatory control and certification. J Public Health Inform. 2014;5(3): 229.
Breland JY, Yeh VM, Yu J. Adherence to evidence-based guidelines among diabetes self-management apps. Transl Behav Med. 2013;3(3):277–86.
Brouwer W. Which intervention characteristics are related to more exposure to internet-delivered healthy lifestyle promotion interventions? A systematic review. J Med Internet Res. 2011;13(1):e2.
Capozza K, Woolsey S, Georgsson M. Going mobile with diabetes support: a randomized study of a text message–based personalized behavioral intervention for type 2 diabetes self-care. Diabetes. 2015;28(2):83–91.
Carver C, Scheier M. Control processes and self-organization as complementary principles underlying behavior. Personal Soc Psychol Rev. 2002;6(4):304–15.
Chaudoir SR, Dugan AG, Barr CH. Measuring factors affecting implementation of health innovations: a systematic review of structural, organizational, provider, patient, and innovation level measures. Implement Sci. 2013;8(1):724.
Clar C, et al. Self-monitoring of blood glucose in type 2 diabetes: systematic review. Health Technol Assess (Winch, Eng). 2010;14(12):1–140.
Coonrod BA, Betschart J, Harris MI. Frequency and determinants of diabetes patient education among adults in the US population. Diabetes Care. 1994;
Coster S, et al. Monitoring blood glucose control in diabetes mellitus: a systematic review. Health Technol Assess (Winch, Eng). 2000;4(12):1–93.
Couper MP. Engagement and retention: measuring breadth and depth of participant use of an online intervention. J Med Internet Res. 2010;12(4):e52.
Cramer JA. A systematic review of adherence with medications for diabetes. Diabetes Care. 2004;27(5):1218–24.
Cresswell K, Sheikh A. Organizational issues in the implementation and adoption of health information technology innovations: an interpretative review. Int J Med Inform. 2013;82(5):e73–86.
Davidson M, et al. The effect of self monitoring of blood glucose concentrations on glycated hemoglobin levels in diabetic patients not taking insulin: a blinded, randomized trial. Am J Med. 2005;118(4):422–5.
Del Toro V, Parker SR. Principles of control systems engineering: McGraw Hill; New York;1960.
van Dijk JAGM. Digital divide research, achievements and shortcomings. Poetics. 2006;34(4-5):221–35.
Donkin L. A systematic review of the impact of adherence on the effectiveness of e-therapies. J Med Internet Res. 2011;13(3):e52.
Egede LE, et al. Medication nonadherence in diabetes. Diabetes Care. 2012;35(12):2533–9.
Eng DS, Lee JM. The promise and peril of mobile health applications for diabetes and endocrinology. Pediatr Diabetes. 2013;14(4):231–8.
Farmer A, et al. A systematic review of telemedicine interventions to support blood glucose self-monitoring in diabetes. Diabet Med. 2005;22(10):1372–8.
Farmer A, et al. Impact of self monitoring of blood glucose in the management of patients with non-insulin treated diabetes: open parallel group randomised trial. Br Med J. 2007;335(7611):132.
Farmer AJ, Rodgers LR, Lonergan M, et al. Adherence to oral glucose–lowering therapies and associations with 1-year HbA 1c: a retrospective cohort analysis in a large primary care database. Diabetes Care. 2016; 39(2):258–263.
Franciosi M, et al. The impact of blood glucose self-monitoring on metabolic control and quality of life in type 2 diabetic patients: an urgent need for better educational strategies. Diabetes Care. 2001;24(11):1870–7.

Franciosi M, et al. ROSES: role of self-monitoring of blood glucose and intensive education in patients with Type 2 diabetes not receiving insulin. A pilot randomized clinical trial. Diabet Med. 2011;28(7):789–96.
Free AH, et al. Simple specific test for urine glucose. Clin Chem. 1957;3(3):163–8.
Free C, et al. The effectiveness of mobile-health technology-based health behaviour change or disease management interventions for health care consumers: a systematic review T. Cornford, ed. PLoS Med. 2013; 10(1):e1001362.
French DP, et al. Self-monitoring of blood glucose changed non-insulin-treated Type 2 diabetes patients' beliefs about diabetes and self-monitoring in a randomized trial. Diabet Med. 2008;25 (10):1218–28.
Garabedian LF, Ross-Degnan D, Wharam JF. Mobile phone and smartphone technologies for diabetes care and self-management. Curr Diab Rep. 2015;15(12):109.
Greenhalgh T, et al. Storylines of research in diffusion of innovation: a meta-narrative approach to systematic review. Soc Sci Med. 2005;61(2):417–30.
Grol R. Personal paper. Beliefs and evidence in changing clinical practice. Br Med J. 1997;315 (7105):418–21.
Guariguata L, et al. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103(2):137–49.
Health and Social Care Information Centre. National diabetes audit 2014–2015 report 1: care processes and treatment targets; 2016 HSCIC Leeds UK.
Hertz RP, Unger AN, Lustik MB. Adherence with pharmacotherapy for type 2 diabetes: a retrospective cohort study of adults with employer-sponsored health insurance. Clin Ther. 2005;27(7):1064–73.
Hex N, et al. Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. Diabet Med. 2012;29 (7):855–62.
Holman R, et al. Addition of biphasic, prandial, or basal insulin to oral therapy in type 2 diabetes. N Engl J Med. 2007;357:1716–30.
Holman R, et al. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359:1577–89.
Hovorka R, et al. Overnight closed-loop insulin delivery in young people with type 1 diabetes: a free-living, randomized clinical trial. Diabetes Care. 2014;37(5):1204–11.
Hunt DL, et al. Effects of computer-based clinical decision support systems on physician performance and patient outcomes: a systematic review. JAMA. 1998;280(15):1339–46.
International Diabetes Federation. Diabetes: facts and figures. n.d.. Available at: http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures.
Juvenile Diabetes Resarch Foundation. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 2008;359:1464–1476
Karter AJ, et al. New prescription medication gaps: a comprehensive measure of adherence to new prescriptions. Health Serv Res. 2009;44(5p1):1640–61.
Kawamoto K, et al. Improving clinical practice using clinical decision support systems: a systematic review of trials to identify features critical to success. Br Med J. 2005;330(7494):765–0.
Klonoff DC. The current status of mhealth for diabetes: will it be the next big thing? J Diabetes Sci Technol. 2013;7(3):749–58.
Law GR, et al. Analysis of continuous glucose monitoring in pregnant women with diabetes: distinct temporal patterns of glucose associated with large-for-gestational-age infants. Diabetes Care. 2015;38(7):1319–25.
Leon N, et al. Improving treatment adherence for blood pressure lowering via mobile phone SMS-messages in South Africa: a qualitative evaluation of the SMS-text Adherence SuppoRt (StAR) trial. BMC Fam Pract. 2015;16:80.
Lester RT, et al. Effects of a mobile phone short message service on antiretroviral treatment adherence in Kenya (WelTel Kenya1): a randomised trial. Lancet. 2010;376(9755):1838–45.

Liang X, et al. Effect of mobile phone intervention for diabetes on glycaemic control: a meta-analysis. Diabet Med. 2011;28(4):455–63.
Malanda UL, Welschen LM, Riphagen II, Dekker JM, Nijpels G, Bot SD. Self-monitoring of blood glucose in patients with type 2 diabetes mellitus who are not using insulin. Cochrane Database Syst Rev. 2012;1:CD005060.
Misono AS, et al. Healthcare information technology interventions to improve cardiovascular and diabetes medication adherence. Am J Manag Care. 2010;16(12 Suppl HIT):SP82–92.
Murray E, et al. Normalisation process theory: a framework for developing, evaluating and implementing complex interventions. BMC Med. 2010;8:63.
National Institute for Health and Clinical Excellence. Type 1 diabetes in adults: diagnosis and management. NICE London; 2015.
Norris SL, et al. Self-management education for adults with type 2 diabetes: a meta-analysis of the effect on glycemic control. Diabetes Care 2002;25(7):1159–71.
O'Connor PJ, et al. Outpatient diabetes clinical decision support: current status and future directions. Diabet Med. 2016;33(6):734–41.
O'Kane MJ, et al. Efficacy of self monitoring of blood glucose in patients with newly diagnosed type 2 diabetes (ESMON study): randomised controlled trial. 2008;336(7654):1174–7.
Office for National Statistics. Internet users. Office for National Statistics; London; 2015.
Pal K, et al. Computer-based diabetes self-management interventions for adults with type 2 diabetes mellitus. Cochrane Database Syst Rev. 2013;3:CD008776.
Pal K, et al. Computer-based interventions to improve self-management in adults with type 2 diabetes: a systematic review and meta-analysis. Diabetes Care. 2014;37(6):1759–66.
Payne HE, et al. Behavioral functionality of mobile apps in health interventions: a systematic review of the literature. JMIR mHealth uHealth. 2015;3(1):e20.
Pereira K, et al. Internet delivered diabetes self-management education: a review. Diabetes Technol Ther. 2015;17(1):55–63.
Pickup JC, Ford Holloway M, Samsi K. Real-time continuous glucose monitoring in type 1 diabetes: a qualitative framework analysis of patient narratives. Diabetes Care. 2015;38(4):544–50.
Pladevall M, et al. Clinical outcomes and adherence to medications measured by claims data in patients with diabetes. Diabetes Care. 2004;27(12):2800–5.
Polonsky WH, Fisher L. Self-monitoring of blood glucose in noninsulin-using type 2 diabetic patients: right answer, but wrong question: self-monitoring of blood glucose can be clinically valuable for noninsulin users. Diabetes Care. 2013;36(1):179–82.
Powers MA, Bardsley J, Cypress M, et al. Diabetes Self-Management Education and Support in Type 2 Diabetes: A Joint Position Statement of the American Diabetes Association, the American Association of Diabetes Educators, and the Academy of Nutrition and Dietetics. J Acad Nutr Diet. 2015;115(8):1323–34.
Quinn CC, et al. Cluster-randomized trial of a mobile phone personalized behavioral intervention for blood glucose control. Diabetes Care. 2011;34(9):1934–42.
Riazi H, et al. Managing diabetes mellitus using information technology: a systematic review. J Diabetes Metab Disord. 2015;14(1):35.
Roberts LG. Beyond Moore's law: internet growth trends. Computer. 2000;33(1):117–9.
Roshanov PS, et al. Features of effective computerised clinical decision support systems: meta-regression of 162 randomised trials. Br Med J. 2013;346:f657.
Samy GN, Ahmad R, Ismail Z. Security threats categories in healthcare information systems. Health Informatics J. 2010;16(3):201–9.
Sarkar U, Lyles CR, Parker MM,et al. Use of the refill function through an online patient portal is associated with improved adherence to statins in an integrated health system. Med Care. 2014;52(3):194–201.
Schaller RR. Moore's law: past, present and future. IEEE Spectr. 1997;34(6):52–57.
Schmidt S, et al. Use of an automated bolus calculator in MDI-treated type 1 diabetes. Diabetes Care. 2012;35(5):984–90.

Schwedes U, et al. Meal-related structured self-monitoring of blood glucose: effect on diabetes control in non-insulin-treated type 2 diabetic patients. Diabetes Care. 2002;25(11):1928–32.
Secher AL, Ringholm L, Andersen HU, et al. The effect of real-time continuous glucose monitoring in pregnant women with diabetes: a randomized controlled trial. Diabetes Care. 2013;36 (7):1877–83.
Seuring T, Archangelidi O, Suhrcke M. The economic costs of type 2 diabetes: a global systematic review. Pharmacoeconomics. 2015;33(8):811–31.
Sherifali D, et al. Evaluating the effect of a diabetes health coach in individuals with type 2 diabetes. Can J Diabetes. 2016;40(1):84–94.
Simon J, et al. Cost effectiveness of self monitoring of blood glucose in patients with non-insulin treated type 2 diabetes: economic evaluation of data from the DiGEM trial. Br Med J. 2008;336 (7654):1177–80.
Tabak RG, Khoong EC, Chambers DA. et al. Bridging research and practice. Am J Prev Med 2012; 43:337–350.
Tattersall RB. Home blood glucose monitoring. Diabetologia. 1979;16(2):71–4.
The Diabetes Control and Complications Trial Epidemiology of Diabetes Interventions and Complications DCCT/EDIC Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353(25):2643–53.
Thom DH, et al. Impact of peer health coaching on glycemic control in low-income patients with diabetes: a randomized controlled trial. Ann Fam Med. 2013;11(2):137–44.
U.S. Department of Commerce, National Telecommunications and Information Administration (NTIA). Falling through the net: a survey of the have nots in rural and urban America. NTIA, Washington, DC; 1995.
van der Sijs H, et al. Overriding of drug safety alerts in computerized physician order entry. J Am Med Inform Assoc. 2006;13(2):138–47.
van der Wulp I, et al. Effectiveness of peer-led self-management coaching for patients recently diagnosed with Type 2 diabetes mellitus in primary care: a randomized controlled trial. Diabet Med. 2012;29(10):e390–7.
van Rooij T, Marsh S. eHealth: past and future perspectives. Personalized Medicine 2016;13(1):15–40
van Vugt M, et al. Uptake and effects of the e-vita personal health record with self-management support and coaching, for type 2 diabetes patients treated in primary care. J Diabetes Res. 2016;2016(2):1–9.
Walford S, et al. Self-monitoring of blood-glucose – improvement of diabetic control. Lancet. 1978;1(8067):732–5.
Wayne N, Ritvo P. Smartphone-enabled health coach intervention for people with diabetes from a modest socioeconomic strata community: single-arm longitudinal feasibility study. J Med Internet Res. 2006;16(6):e149.
Wing R, et al. Does self-monitoring of blood glucose levels improve dietary compliance for obese patients with type II diabetes? Am J Med. 1986;81:830–6.
Winkley K, et al. Patient explanations for non-attendance at structured diabetes education sessions for newly diagnosed Type 2 diabetes: a qualitative study. Diabet Med. 2015;32(1):120–8.
Woolf SH. The meaning of translational research and why it matters. JAMA. 2008;299(2):211–3.
Yu L, Mishra A. An empirical study of Lehman's law on software quality evolution. Int J Software Informatics. 2013;7(3):469–481.

Glycemic Targets and Prevention of Chronic Complications

13

Simona Cernea, Avivit Cahn, and Itamar Raz

Contents

S. Cernea
Department M3/Internal Medicine IV, University of Medicine and Pharmacy, Tîrgu Mureș, Romania

Diabetes, Nutrition and Metabolic Diseases Outpatient Unit, Emergency County Clinical Hospital, Tîrgu Mureș, Romania

A. Cahn
Diabetes Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel

Endocrinology and Metabolism Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel

I. Raz (✉)
Diabetes Unit, Department of Internal Medicine, Hadassah Hebrew University Hospital, Jerusalem, Israel
e-mail: ntv502@netvision.net.il

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_14

Abstract

Hyperglycemia is the main cause and critical initiating factor of chronic microvascular complications of diabetes and also a major contributor to the macrovascular complications. In diabetes care it is therefore essential to set appropriate glycemic targets (or target ranges) that guide the management of the disease in order to reduce the risk of long-term complications, while avoiding unnecessary burden or adverse events. In this chapter we discuss the role of hyperglycemia in inducing diabetes chronic complications and evidence from clinical trials proving the benefit of glycemic control in preventing or ameliorating the progression of micro- and macrovascular complications. We also review the recommendations of current clinical guidelines, including individualization of glycemic targets when treating patients with diabetes.

Keywords

Glycemic targets · Microvascular complications · Macrovascular complications · Glycemic control · Clinical trials · Guidelines

Introduction

The major burden of both type 1 diabetes (T1D) and type 2 diabetes (T2D) stem from the increased risk of microvascular and macrovascular complications. Hyperglycemia, the hallmark of the disease, is the main cause and critical initiating factor of microvascular complications of diabetes (retinopathy, nephropathy, and neuropathy) and also an important contributor (along with other factors, such as insulin resistance, hypertension, and dyslipidemia) to the occurrence of macrovascular complications (cardiovascular disease (CVD)) (Paneni et al. 2013).

Therefore, one of the most important steps in diabetes care is setting glycemic targets (or target ranges) that guide the management of the disease in order to reduce the risk of long-term complications. The challenge resides though from the fact that controlling hyperglycemia to levels as close to normal as possible should be accomplished without unwanted adverse events or encumbering unnecessarily patients' everyday lives and with reasonable costs.

Here we discuss the role of hyperglycemia in inducing diabetic chronic complications as well as evidence from clinical trials proving the benefit of glycemic control in preventing or ameliorating the progression of these complications. Finally, we review the recommendations of current clinical guidelines, including individualization of glycemic targets when treating a given patient.

Pathophysiological Considerations and Glucose Thresholds for Development of Chronic Complications

While in diabetic state all cells in the body are exposed to increased glucose levels, only certain ones (e.g., retinal capillary endothelial cells, glomerular mesangial cells, neuronal and Schwann cells in peripheral nerves) are particularly vulnerable to

hyperglycemia, as they are not capable of downregulating the glucose transport into the cells, resulting in intracellular hyperglycemia (Brownlee 2005).

On the basis of genetic determinants of individual susceptibility, hyperglycemia causes tissue damage through several mechanisms induced by mitochondrial overproduction of reactive oxygen species (ROS): increased fluxes through the polyol and hexosamine pathways, increased formation of advanced glycation end products (AGEs) and expression of AGEs receptor, activation of protein-kinase C (PKC), and NF-kB-mediated inflammation (Paneni et al. 2013; Giacco and Brownlee 2010). An additional trigger of endothelial dysfunction is reduced bioavailability of nitric oxide (NO), caused by hyperglycemia-, insulin resistance-, and free fatty acid (FFA)-induced production of superoxide anion (O_2^-), which further facilitates the pro-inflammatory pathways with increased cytokines production, thrombosis, and vasoconstriction (Creager et al. 2003).

In addition to these well-known biochemical mechanisms, a large body of literature points toward an important role of epigenetic modifications caused by hyperglycemia and AGEs in the pathogenesis of diabetic complications (Reddy et al. 2015; Berezin 2016). These epigenetic mechanisms, such as DNA methylation, posttranslational histone alterations in chromatin, and non-coding RNA (including microRNA), modify in turn gene expression in target tissues (e.g., oxidant/antioxidant, inflammation, fibrosis, cell differentiation, survival), without modifying the DNA sequence, and thus further sustain the alterations in affected cells (Paneni et al. 2013; Reddy et al. 2015; Prattichizzo et al. 2015).

The minimal glucose levels at which detrimental effects begin to be exerted seem to be lower than those commonly recognized as threshold values for diagnosis of diabetes, and in fact there is a "continuum" of glucose values associated with increased risk of vascular complications, beginning at levels beyond normoglycemia (Paneni et al. 2013; Inzucchi and Majumdar 2015). Analysis of data from three population studies suggested that apparently the risk did not respect a clearly defined threshold, but rather there was a continuous relationship between fasting blood glucose and retinopathy (beginning at levels $\leq$4.6 mmol/l (82.8 mg/dl)), while the threshold of 7.0 mmol/l (126 mg/dl) did not accurately identify patients with or without retinopathy (Wong et al. 2008). A Finish study indicated that a fasting glucose level of 6.1 mml/l (109.8 mg/dl) discriminated between a higher and lower prevalence of retinopathy (10.2% (95% CI: 4.8–18.5) in subjects with blood glucose above this value vs. 2.6% (95% CI: 1.5–4.0)) in those with lower levels, but the overall number of subjects with retinopathy in the study was small (Rajala et al. 1998). Moreover, the Hoorn study reported a retinopathy prevalence of 9% in individuals with normal glucose tolerance, 11% in those with impaired glucose metabolism, 13% in subjects with newly diagnosed diabetes, and 34% in those with known diabetes, and interestingly, additional factors like hypertension, increased body mass index (BMI), and hyperlipidemia were also associated with retinopathy (van Leiden et al. 2002). Other epidemiological data support the correlations between blood glucose control and prevalence and progression to retinopathy, microalbuminuria, and neuropathy (Klein et al. 1996). In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, the overall 25-year rate of

progression of diabetic retinopathy was 83%, and the strongest relationship of both progression and regression of retinopathy was with glycated hemoglobin (HbA1c) (Klein et al. 2008).

The continuous association of microvascular complications with glucose levels has been demonstrated for nephropathy and neuropathy as well. Data from the PREVEND study indicated a prevalence of microalbuminuria of 6.6% in non-diabetic population and 16.4% in subjects with diabetes, while other studies showed increased cumulative prevalence of microalbuminuria with increased duration of both T1D and T2D and with HbA1c values (Hillege et al. 2001; Pasko et al. 2013; Amin et al. 2008). Similarly, the Monitoring Trends and Determinants on Cardiovascular Diseases (MONICA)/Cooperative Research in the Region of Augsburg (KORA) study reported progressively higher prevalence of polyneuropathy according to glucose metabolism status: 7.4% in subjects with normal glucose tolerance, 11.3% in those with impaired fasting glucose (IFG), 13.0% with impaired glucose tolerance (IGT), and 28.0% in diabetic subjects (Ziegler et al. 2008). Significant correlations were also described between the presence of peripheral neuropathy and duration of diabetes and with glycemic control (Tesfaye et al. 1996).

Diabetes is also associated with ~twofold excess risk of CVD, independently of other cardiovascular (CV) risk factors (Emerging Risk Factors Collaboration et al. 2010). Data from a meta-analysis of 102 trials demonstrated that in subjects without history of diabetes, the hazard ratio (HR) for CVD was 2.37 (95% CI: 1.79–3.14) in those with fasting blood glucose $\geq$7.0 mmol/l (126 mg/dl) versus those with $<$7.0 mmol/l (Emerging Risk Factors Collaboration et al. 2010). Fasting and post-load glycemia were modestly associated with CVD risk, while HbA1c showed a somewhat stronger association (Emerging Risk Factors Collaboration et al. 2010). Another meta-analysis in subjects without known diabetes confirmed that fasting glycemia was modestly and nonlinearly associated with CVD starting at glucose levels of 5.6 mmol/l (100.8 mg/dl) and statistically higher risk was observed in those with fasting blood glucose $\geq$7.0 mmol/l (Sarwar et al. 2010). A Swedish observational study in patients with T2D followed up for 6 years reported that the risks of coronary heart disease (CHD) and CVD progressively raised with increasing HbA1c levels (HR: 1.11–1.13 and 1.10–1.11, respectively, per 1% unit increase in mean HbA1c, after adjustment for several risk factors) (Eeg-Olofsson et al. 2010). Another prospective study indicated in subjects with diabetes a 14% higher risk of CHD with each 1% point increase in HbA1c (RR: 1.14 (95% CI: 1.07–1.21)) (Selvin et al. 2005). Moreover, in the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients With Diabetes Mellitus (SAVOR)-TIMI53 trial, the baseline HbA1c $\geq$7% was associated with increased risk of CV death, myocardial infarction (MI), or stroke (HR: 1.35, 95% CI: 1.17–1.58) (Cavender et al. 2016). However, the independent impact of glucose per se on the progression of macrovascular complications is more difficult to interpret than in the case of microvascular complications, as the interaction with the additional CV risk factors (i.e., dyslipidemia, hypertension, inflammation, insulin resistance, etc.) is more pronounced (Inzucchi and Majumdar 2015).

It has been discussed that even transient spikes of hyperglycemia are sufficient for initiating the pathogenic mechanisms that cause diabetic chronic complications

(Thomas 2014). It appears that even a brief exposure to high blood glucose concentrations induces the epigenetic modifications that lead to development of long-term effects in target tissues (El-Osta et al. 2008). Moreover, there seems to be increasing evidence that glycemic variability (with hyperglycemic spikes and hypoglycemic troughs), independent of HbA1c, also plays a role in the occurrence of chronic complications, although there is data that refute these observations, so further evaluation is warranted (Suh and Kim 2015; Brownlee and Hirsch 2006; Bergenstal 2015; Ceriello 2012). Oscillating glucose concentrations seem to impair the flow-mediated dilation, a marker of endothelial function, increase oxidative stress, and induce cellular apoptosis (Ceriello et al. 2008; Quagliaro et al. 2003). Some data indicate that the negative effect on oxidative stress is triggered more specifically by the glycemic variations rather than chronic sustained hyperglycemia (Monnier et al. 2006).

More evidence, however, support the fact that the risk of complications is driven by the buildup of the metabolic (hyperglycemic) memory (Berezin 2016; Ceriello 2012). This implies that prior exposure to high blood glucose levels creates an imprint on the development of long-lasting detrimental effects, even after the normalization of blood glucose (Paneni et al. 2013). It may depend on the duration and degree of hyperglycemia (Ceriello 2012). Persistence of the alterations induced by the hyperglycemic stress is explained by multiple intracellular biochemical processes (activation of NF-kB; glycation of mitochondrial proteins, lipids, and nucleic acids, with lasting oxidative stress and release of ROS) and epigenetic modifications that are not reverted by returning to normoglycemia (Berezin 2016; Ceriello 2012; Paneni et al. 2012).

There is sufficient evidence clearly establishing the role of hyperglycemia in the pathogenesis of diabetes complications. The reverse phenomenon also seems to occur, i.e., protection against chronic complications is afforded by an intensive blood glucose control, mainly if implemented early in the course of the disease (legacy effect) (Pozzilli et al. 2014). This was actually demonstrated by the post-trial monitoring of the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) and United Kingdom Prospective Diabetes Study (UKPDS) cohorts. The long-term follow-up of the DCCT cohort in the EDIC study showed that patients randomized to intensive treatment presented lower rates of complications over more than a decade after DCCT trial completion, although the differences in the HbA1c between the two treatment arms evened out soon after trial ended (DCCT/EDIC Research Group 2014; Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015, 2016). During 18 years of follow-up in EDIC, a 45% risk reduction (95% CI: 26–59) for microalbuminuria and 61% (95% CI: 41–74) for macroalbuminuria were noted for the intensively treated group in the DCCT (DCCT/EDIC Research Group 2014). However, the risk reduction of incident macroalbuminuria for intensive versus conventional therapy decreased in time: 79% (95% CI: 58–89; $p < 0.001$) during EDIC 1–8 years and 31% (−20 to 61; p: 0.19) during years 9–16. A similar effect was noted for retinopathy, with decreasing odds of further progression of diabetic retinopathy from the DCCT closeout level

74% (95% CI: 65–81; $p < 0.0001$) at year 4 in EDIC, 59% (95% CI: 47–68; $p < 0.0001$) at year 10, and 43% (95% CI: 27–55; $p < 0.0001$) at years 15–18 (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). During 30 years of DCCT/EDIC follow-up, the incidence of any CVD was reduced by 30% (95% CI: 7–48; p: 0.016) (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group 2016). The long-term beneficial effect of early intensive glycemic control on chronic complications was obvious also for patients with T2D enrolled in the UKPDS, who presented continued reduction in microvascular risk (24%, p: 0.001 in the sulfonylurea–insulin group) and myocardial infarction (15%, p: 0.01 in the sulfonylurea–insulin group and 33%, p: 0.005 in the metformin group) during 10-year post-trial follow-up, despite early loss of differences in blood glucose control after trial completion (Holman et al. 2008). Still, the reversion of hyperglycemia at a later time in the natural history of the disease is less likely to ameliorate the progression of complications to a similar degree.

Given the effects of hyperglycemia on vascular events, several randomized clinical trials (RCTs) were performed in order to establish the relationship between treatment targets and micro- and macrovascular endpoints (Table 1).

Effect of Glycemic Control on the Development and Progression of Microvascular Complications

The main data based on which glycemic targets were initially set in both T1D and T2D came from the two landmark trials (DCCT and UKPDS, respectively) that aimed to establish the benefit of stringent glycemic control on the development and progression of diabetic chronic complications (UK Prospective Diabetes Study (UKPDS) Group 1998a; The Diabetes Control and Complications Trial Research Group 1993).

The DCCT set out to ascertain if maintaining near-normal blood glucose levels by means of intensive insulin therapy regimen (three or more daily insulin injections or insulin pump therapy) versus standard treatment (one to two daily insulin injections) would be associated with prevention of new onset (primary prevention) or of progression of vascular complications (secondary prevention) (The DCCT Research Group 1986). Retinopathy was chosen as the primary outcome (The Diabetes Control and Complications Trial Research Group 1993). The trial included 1,441 patients with T1D, with an average age of 27 years, who were followed-up for a mean of 6.5 years (The Diabetes Control and Complications Trial Research Group 1993). The primary prevention cohort had had diabetes for 1–5 years and no retinopathy, while the secondary prevention cohort had been diagnosed with diabetes for 1–15 years and had very-mild-to-moderate nonproliferative retinopathy and urinary albumin excretion <200 mg/day (The Diabetes Control and Complications Trial Research Group 1993). Both groups were randomized to either intensive or standard therapy. The glycemic targets for the intensive arm were premeal blood

Table 1 Selected characteristics and micro- and macrovascular benefits in major diabetes trials (Holman et al. 2008; UK Prospective Diabetes Study (UKPDS) Group 1998a, b; The Diabetes Control and Complications Trial Research Group 1993; Lachin et al. 2008; Albers et al. 2010; Stratton et al. 2000, 2001; Ismail-Beigi et al. 2010; Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Eye Study Group and the Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Study Group 2016; ADVANCE Collaborative Group et al. 2008; Zoungas et al. 2014; Wong et al. 2016; Duckworth et al. 2009; Agrawal et al. 2011; ACCORD Study Group Writing Committee 2016; Reaven et al. 2009; Hayward et al. 2015; Writing Group for the DCCT/EDIC Research Group et al. 2015)

	DCCT	**UKPDS SU-I**	**UKPDS Met**	**ACCORD**	**ADVANCE**	**VADT**
No. of patients	1441	3867	753	10,251	11,140	1791
Mean age (years)	27	53	53	62	66	60.4
Mean duration of diabetes (years)	2.6[a]/8.6–8.9[b]	0	0	10.8	8	11.5
Mean duration of f/u (years)	6.5	10.0	10.7	3.7	5	5.6
Baseline HbA1c (%)	8.8[a]/8.9–9.0[b]	7.1	7.1–7.3	8.3	7.5	9.4
End HbA1c (int vs. std) (%)	7.2 vs. 9.1	7.0 vs. 7.9	7.4 vs. 8.0	6.4 vs. 7.5	6.5 vs. 7.3	6.9 vs. 8.4
All-cause death (HR (95% CI))	NR	0.94[c] (0.80–1.10)	0.64[c] (0.45–0.91)	1.20 (1.04–1.39)	0.93 (0.83–1.06)	1.07 (0.81–1.42)
Macrovascular benefit	No	No	Yes: nonfatal MI	Yes: nonfatal MI	No	No
Microvascular benefit	Yes: retinopathy	Yes	No	NR	Yes: nephropathy	Yes: nephropathy
	nephropathy					
	neuropathy					
Follow-up studies	**EDIC**	**UKPDS f/up**	**UKPDS f/up**	**ACCORDION**	**ADVANCE-ON**	**VADT f/up**
		SU-I	**Met**			
No. of patients	1375	2998	588	8601	8494	1391
Mean duration of f/u (years)	18	8.5	8.8	7.7[d]	5.4	9.8
End HbA1c (int vs. std) (%)	8.0	7.8 vs. 7.8	8.1 vs. 8.1	7.8 vs. 8.0	7.2 vs. 7.4	8.1 vs. 8.3
All-cause death (HR (95% CI))	0.67 (0.46–0.99)	0.87[c] (0.79–0.96)	0.73[c] (0.59–0.89)	1.01 (0.92–1.10)	1.00 (0.92–1.08)	1.05 (0.89–1.25)
Macrovascular benefit	Yes	Yes: nonfatal MI	Yes: nonfatal MI	No	No	Yes
Microvascular benefit	Yes: retinopathy	Yes	No	Yes: retinopathy	No	NR
	nephropathy					
	neuropathy					

f/up follow-up, *HR* hazard ratio, *NR* not reported
[a]Primary prevention cohort
[b]Secondary prevention cohort
[c]Relative risk
[d]From randomization

glucose 70–120 mg/dl, post-meal glycemia <180 mg/dl, and HbA1c <6.05%, while the aim for the conventional arm was to maintain normal growth and development and avoid symptomatic hyperglycemia and hypoglycemia (The Diabetes Control and Complications Trial Research Group 1993). Over the duration of the study, the subjects allocated to the conventional group had significantly higher mean Hb1c levels, and this was 9.1% at study closeout (vs. 7.2% in the intensive group) (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015; The Diabetes Control and Complications Trial Research Group 1993). In the primary prevention cohort the intensive therapy reduced the adjusted mean risk of retinopathy by 76% (95% CI: 62–85; $p \leq 0.002$), while the risk reduction in the secondary prevention cohort was 54% (95% CI: 39–66; $p \leq 0.002$) (The Diabetes Control and Complications Trial Research Group 1993). Subsequent analysis of the DCCT data showed the cumulative incidence of retinopathy progression was dependent on HbA1c levels (low in the 6.5–7.49% group and higher in 7.5–8.49% and 8.5–9.49%, respectively), regardless of randomization arm (Lachin et al. 2008). Intensive therapy reduced the mean adjusted risk of microalbuminuria (urinary albumin excretion ≥40 mg/24 h) by 34% (95% CI: 2–56; $p < 0.04$) and by 43% (95% CI: 21–58; $p \leq 0.002$) in the primary and secondary intervention cohorts, respectively (The Diabetes Control and Complications Trial Research Group 1993). The risk of albuminuria (urinary albumin excretion ≥300 mg/24 h) was significantly reduced by 56% (95% CI: 18–76; $p < 0.04$) only in the secondary prevention group (The Diabetes Control and Complications Trial Research Group 1993). Reductions in clinical neuropathy at 5 years by intensive therapy were 69% (95% CI: 24–87; $p < 0.04$) and 57% (95% CI: 29–73; $p < 0.04$) in the primary and secondary intervention cohort, respectively (The Diabetes Control and Complications Trial Research Group 1993). Thus the DCCT demonstrated that obtaining near-normal glycemic control in young adults with T1D is associated with prevention of onset and progression of microvascular complications. It also established intensive insulin therapy as standard of care for these patients.

At the end of the trial, the subjects in the conventional group were instructed to take intensive insulin regimens, and all participants returned to their primary diabetes care providers (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). They were invited to take part in the follow-up observational study, EDIC, for which data of over 18 years are now available (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). The differences in the mean HbA1c levels between the former intensive- and standard-therapy cohorts attenuated in time, becoming nonsignificant in the seventh year post DCCT closeout, so that during years 15–18 post-randomization, mean HbA1c values were ~8% in both groups (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). In spite of the loss in the initial difference in the glucose control, the adjusted risk reduction for further progression of retinopathy was 46% (95% CI: 36–54;

$p < 0.0001$) with former intensive treatment (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). The risk reduction was somewhat greater in patients with microaneurysms or mild nonproliferative retinopathy at DCCT end (~55%, $p < 0.0001$) than in patients without retinopathy (30%, p: 0.021), virtually all from the initial primary intervention cohort (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group et al. 2015). The risk reduction for microalbuminuria (albumin excretion rate >30 mg/24 h) was 45% (95% CI: 26–59; $p < 0.0001$) during EDIC, and for macroalbuminuria (albumin excretion rate >300 mg/24 h) it was 61% (95% CI: 41–74; $p < 0.0001$) with initial intensive therapy (DCCT/EDIC Research Group 2014). Similarly, the incidence of clinical neuropathy remained lower in the initial intensive group versus conventional group (22% vs. 28%, p: 0.0125) in EDIC years 13–14 (Albers et al. 2010). Thus the results of EDIC follow-up study indicated persistent long-term microvascular benefits with short-term intensive-therapy and tight glycemic control, despite subsequent loss of stringent target.

UKPDS evaluated the effect of tight glycemic control on chronic complications and mortality in 5,102 patients with newly diagnosed T2D (UK Prospective Diabetes Study (UKPDS) Group 1991). The study started recruitment in 1977 and ended in 1997. At study entry, the median age of patients was 53 years, and their median fasting plasma glucose was 11.3 mmol/l (203.4 mg/dl) (UK Prospective Diabetes Study (UKPDS) Group 1991). The UKPDS aimed to establish, first, whether the risk of complications can be reduced with intensive blood glucose control and, second, which therapy (metformin, sulfonylurea (SU), or insulin) affords particular benefit. Four thousand two hundred and nine T2D patients were randomly assigned to either intensive therapy (with SU or insulin) targeting for fasting glucose levels <108 mg/dl or conventional therapy (diet) aiming for the lowest achievable fasting plasma glucose with diet alone. A subset of patients (overweight, >120% ideal body weight) were randomized to intensive therapy with metformin (UK Prospective Diabetes Study (UKPDS) Group 1998b). In the effort to maintain tight glucose control, insulin or metformin was later added to SU, while in the conventional arm metformin, SU or insulin was added if fasting glucose >270 mg/dl or patients became symptomatic (Wright et al. 2002). Over 10 years of follow-up, HbA1c was 7.0% and 7.9% in the intensive and conventional group, respectively, and this difference was accompanied by a 12% (95% CI: 1–21; p: 0.029) lower risk for any diabetes-related endpoint, most of the risk reduction being due to a 25% (95% CI: 7–40; p: 0.009) reduction in microvascular complications (UK Prospective Diabetes Study (UKPDS) Group 1998a). Similarly, median HbA1c was 7.4% in the metformin group versus 8.0% in the conventional arm (UK Prospective Diabetes Study (UKPDS) Group 1998b). Patients allocated to metformin had risk reductions of 32% (95% CI: 13–47; p: 0.002) for any diabetes-related endpoint and 36% for all-cause mortality (95% CI: 9–55; p: 0.011) compared with the conventional group, but no significant reductions in microvascular endpoints were noted (UK Prospective Diabetes Study (UKPDS) Group 1998b). However, a later combined analysis indicated that fewer patients treated with metformin had diabetes-related endpoints

(risk reduction 19%; 95% CI: 2–33; p: 0.033) (UK Prospective Diabetes Study (UKPDS) Group 1998b). The UKPDS data was subsequently analyzed according to HbA1c strata (<6.2%, 6.2–7.4%, ≥7.5%) and found that the risk of incident or progressive retinopathy increased correspondingly: for patients with no retinopathy at baseline, the relative risk (RR) was 1.4 (95% CI: 1.1–1.8) and 2.5 (95% CI: 2.0–3.2) for the second and third tertiles, respectively, and for patients with pre-existing retinopathy, it was 4.1 (95% CI: 3.1–5.6) and 8.1 (95% CI: 6.3–10.5) for second and third tertiles versus the lowest tertile, respectively (Stratton et al. 2001). Moreover, the UKPDS data revealed each 1% reduction in updated mean HbA1c was associated with 37% (95% CI: 33–41; $p < 0.0001$) reduction in risk for microvascular complications (as well as other endpoints, such as death – or any endpoint related to diabetes) (Stratton et al. 2000). Thus, similar to DCCT, the UKPDS demonstrated that tight glycemic control reduced the risk of microvascular complications in patients with T2D.

After trial completion, the UKPDS cohort was followed-up in an observational study for 10 more years. In the first year post-trial monitoring, the between-group differences in HbA1c levels were lost, and the mean HbA1c by the end of follow-up period was about 8% (Holman et al. 2008). In spite of converging glycemic control, at 10 years there was still a 24% reduction in the risk of microvascular complications in the SU–insulin group versus conventional group (risk ratio: 0.76 (95% CI: 0.64–0.89); p: 0.001), while in the metformin group no significant risk reduction for microvascular complications was seen (risk ratio: 0.84 (95% CI: 0.60–1.17); p: 0.31), although there was a 21% reduction for any diabetes-related endpoint (p: 0.01) (Holman et al. 2008). In both intensive groups, there were significant reductions in all-cause mortality and diabetes-related death (Holman et al. 2008). Therefore, the post-UKPDS long-term follow-up indicated that in newly diagnosed patients with T2D, initial stringent glucose control had long-lasting benefits in terms of microvascular complications of diabetes, despite later deterioration in glycemic control.

The Kumamoto study was an additional early study done in 110 Japanese T2D patients aimed at investigating whether tight glycemic control by intensive insulin treatment (three or more injections per day) could prevent incident (primary prevention) or progression of (secondary prevention) of microvascular complications (Ohkubo et al. 1995). Patients had had diabetes for about 6.5 years (primary prevention cohort) and for about 10.3 years (secondary intervention cohort) with an average age of about 49 years (Ohkubo et al. 1995). In the intensive-therapy arms (primary and secondary cohorts), the aim was to obtain target fasting glycemia levels <140 mg/dl, 2-h postprandial glucose <200 mg/dl, and HbA1c <7%, while in the conventional arms, the aim was to achieve fasting glucose <140 mg/dl and no symptoms of hyper- or hypoglycemia (Ohkubo et al. 1995). The HbA1c levels were 7.1% and 9.4% in the intensive- and standard-therapy groups, respectively (Ohkubo et al. 1995). After 6 years, in the primary intervention cohort the cumulative percentages of retinopathy were 7.7% and 32.0% (p: 0.039) and of nephropathy 7.7% and 28.0% (p: 0.032), in the intensive-therapy and conventional-therapy groups, respectively (Ohkubo et al. 1995). In the secondary prevention cohort, the cumulative percentages of retinopathy were 19.2% and 44.0% (p: 0.049) and of

nephropathy were 11.5% and 32.0% (p: 0.044), in the intensive-therapy and conventional-therapy groups, respectively (Ohkubo et al. 1995). Furthermore, data from the two intervention cohorts were combined and analyzed according to the degree of glycemic control showing that the prevention of onset and progression of microvascular complications occurred at the following glycemic thresholds: HbA1c <6.5%, fasting glycemia <110 mg/dl, and 2-h postprandial blood glucose <180 mg/dl.

Overall, the Kumamoto study in accordance with the two major trials, the DCCT and the UKPDS, advocated the idea that tight glycemic control, to levels close to normal, can prevent the microvascular complications of diabetes. As a result, in adult subjects with T1D and T2D, achieving near-normal glycemic values – if possible – has become a standard of care.

Later studies in patients with diabetes were designed to investigate if achieving near-normal glucose control can decrease macrovascular complications. These trials are discussed in more detail in the next section, but the results regarding the correlation between glycemic control and microvascular complications (which was also studied in these trials) are briefly presented here.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) randomized 10,251 T2D patients and high CV risk, with a disease duration of 10 years and median age of 62 years, to receive intensive therapy (targeting a HbA1c <6%) or standard therapy (targeting a HbA1c of 7.0–7.9%) (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). One-year post-randomization, there was a significant difference between the two arms with regards to median HbA1c (6.4% in the intensive-therapy group and 7.5% in the standard-therapy group), and this was maintained throughout the follow-up period (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). After a median duration of 3.5 years, the intensive-therapy arm was discontinued due to increased mortality rates, and subjects were transitioned to standard treatment (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008; Ismail-Beigi et al. 2010). Intensive therapy did not reduce the risk of advanced measures of microvascular outcomes (dialysis or renal transplantation, high serum creatinine, or retinal photocoagulation or vitrectomy) (HR: 1.00 (95% CI: 0.88–1.14); p: 1.00), but the development of microalbuminuria (HR: 0.81 (95% CI: 0.70–0.94); p: 0.005) or macroalbuminuria (HR: 0.68 (95% CI: 0.54–0.86); p: 0.001) were reduced, as well as several diabetic ocular outcomes and diabetic neuropathy (Ismail-Beigi et al. 2010). Four years after the trial closeout, a subset of the participants (who did not have laser photocoagulation or vitrectomy) ($n = 2856$) were reexamined, and it resulted that retinopathy progressed in 5.8% with intensive treatment versus 12.7% with standard therapy (OR: 0.42 (95% CI: 0.28–0.63); $p < 0.0001$) (Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Eye Study Group and the Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Study Group 2016).

The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified-release Controlled Evaluation (ADVANCE) trial randomized 11,140 patients with T2D (mean age: 66 years, disease duration of approximately 8 years) to achieve target HbA1c <6.5% by means of intensive therapy with gliclazide-modified release (plus additional medication as needed) or to standard care (ADVANCE

Collaborative Group et al. 2008). After a median follow-up of 5 years, the mean HbA1c was 6.5% in the intensive-control group and 7.3% in the standard-control group (ADVANCE Collaborative Group et al. 2008). This difference in glucose control was accompanied by a reduction in the incidence of combined major macro- and microvascular events (HR: 0.90 (95% CI: 0.82–0.98); *p*: 0.01) with intensive therapy, as well as the incidence of microvascular complications (HR: 0.86 (95% CI: 0.77–0.9); *p*: 0.01). These were primarily due to a decrease in the incidence of new or worsening nephropathy (HR: 0.79 (95% CI: 0.66–0.93); *p*: 0.006) and new-onset microalbuminuria (HR: 0.91 (95% CI: 0.85–0.98); *p*: 0.02) (ADVANCE Collaborative Group et al. 2008). There were no significant effects on retinopathy, although only more advanced forms (proliferative retinopathy or blindness) were considered as endpoints in this trial (*p*: 0.50) (ADVANCE Collaborative Group et al. 2008). The post-trial follow-up (ADVANCE-ON) which lasted for a median 5.4 years reported the loss of the between-group difference in HbA1c in the first post-trial visit and that there were no cumulative benefits for major microvascular outcomes (HR: 0.92 (95% CI: 0.80–1.05); *p*: 0.23), except for a reduction in end-stage renal disease (ESRD) (HR: 0.54 (95% CI: 0.34–0.85); *p*: 0.007) (Zoungas et al. 2014). The ESRD risk reduction persisted even after 9.9 years of overall follow-up (HR: 0.54; $p < 0.01$), the effects being greater in patients with earlier stages of kidney disease (*p*: 0.04) (Wong et al. 2016).

The Veteran Affairs Diabetes Trial (VADT) randomized 1,791 patients with T2D (mean age: 60.4 years, mean duration of diabetes: 11.5 years) to receive intensive or standard glucose control (aiming at an absolute reduction in HbA1c of 1.5% between groups) and followed them for 5.6 years (Duckworth et al. 2009). After 6 months the median HbA1c stabilized at 8.4% in the standard-therapy group and 6.9% in the intensive-therapy group (Duckworth et al. 2009). There was no significant difference in new onset or progression of retinopathy (*p*: 0.27) or in progression to clinically important macular edema (*p*: 0.31) between the two treatment arms (Duckworth et al. 2009). However, there was a benefit of intensive glycemic control with regards to any worsening of albumin excretion (*p*: 0.01) and progression to macroalbuminuria (*p*: 0.04), and there was less worsening of renal function (eGFR) in patients with higher baseline urine albumin-to-creatinine ratio (odds ratio (OR): 0.61 (95% CI: 0.37–1.00); *p*: 0.04) (Duckworth et al. 2009; Agrawal et al. 2011).

These three trials together demonstrated a benefit of intensive blood glucose control (to levels close to normal range) on diabetic nephropathy, but not clearly on retinopathy. This may be due to shorter duration of follow-up compared to earlier trials, or it may be possible that the standard care groups in these later trials had lower HbA1c levels, which could have mitigated hyperglycemic effects and attenuated the differences between groups (Inzucchi and Majumdar 2015).

The Steno-2 study randomized 160 patients with T2D and albuminuria (mean age: 55.1 years, mean duration of diabetes: 6 years) to receive either intensive therapy (by a multifactorial risk reduction approach, which included glycemic control (aiming at HbA1c <6.5%), lipid, and blood pressure control) or standard of care (Gaede and Pedersen 2004; Gaede et al. 2003, 2008). The intervention lasted for a mean 7.8 years, but the patients were subsequently followed-up for additional

5.5 years (Gaede et al. 2008). By the end of intervention period, the patients receiving intensive therapy attained an HbA1c of 7.9% (vs. 9.0% for those allocated to conventional therapy) (Gaede et al. 2008). At the end of intervention, there was a significant reduction in microvascular complications with intensive therapy: nephropathy (RR: 0.39 (95% CI: 0.17–0.87); *p*: 0.003), retinopathy (RR: 0.42 (95% CI: 0.21–0.86); *p*: 0.02), and autonomic neuropathy (RR: 0.37 (95% CI: 0.18–0.79); *p*: 0.002), without significant effect on peripheral neuropathy (*p*: 0.66) (Gaede et al. 2003). Reductions in the progression of microvascular complications were maintained during the entire 13.3-years observation period (RR: 0.44 (95% CI: 0.25–0.77); *p*: 0.004 for diabetic nephropathy and RR: 0.57 (95% CI: 0.37–0.88); *p*: 0.01 for retinopathy) (Gaede et al. 2008).

Finally, the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial randomized 12,537 subjects (mean age: 63.5 years) with CV risk factors and T2D, IFG, or IGT to receive insulin glargine (aiming to reach fasting blood glucose $\leq$95 mg/dl) or standard care, and they were followed-up for a median duration of 6.2 years (ORIGIN Trial Investigators et al. 2012). There was no significant difference in microvascular events between the two treatment groups in the overall trial population (HR: 0.97 (95% CI: 0.90–1.05); *p*: 0.43). However, subjects allocated to intensive therapy who had baseline HbA1c levels $\geq$6.4% presented reduction in incidence of primary microvascular composite outcome of kidney and eye disease (HR: 0.90 (95% CI: 0.81–0.99)) in contrast with those with lower HbA1c at baseline (HR: 1.07 (95% CI: 0.95–1.20); *p* for interaction: 0.031) (ORIGIN Trial Investigators et al. 2012, 2014). After randomization, subjects with baseline HbA1c $\geq$6.4% allocated to intensive-therapy arm had a median change in HbA1c of −0.65% (vs. −0.33% in the standard care arm, median difference 0.33%; $p < 0.0001$) (ORIGIN Trial Investigators et al. 2014). No microvascular benefits were seen in the group with baseline HbA1c <6.4%, which reached a median HbA1c difference of 0.22% ($p < 0.0001$), which suggests that it is difficult to prove microvascular protection with glycose lowering strategies if baseline glycemic levels are already close to normal and/or larger glycemic separation between the intervention groups is needed (ORIGIN Trial Investigators et al. 2014).

Taken together, the data seem to suggest that a significant decrease of initially high glycemic levels (HbA1c of at least 6.5%), mainly early in the disease course, is associated with a corresponding reduction in the risk of development of microvascular complications, which persists in time, although even later on during the natural history of the disease the improvement in the level of glycemic control also brings renal benefits.

Effect of Glycemic Control on the Development and Progression of Macrovascular Complications

The hypothesis that improved glycemic control could also result in significant reduction of CVD (the most prevalent complication and the leading cause of mortality in patients with diabetes), as well as of all-cause mortality has been

explored in several studies conducted during the last decades (Conway et al. 2015; Geiss et al. 1995).

The DCCT reported fewer CV events in patients with T1D receiving intensive therapy, but the number of events was too small to clearly determine superiority (Nathan et al. 2005). However, a 17-year follow-up (including post-trial monitoring) has shown that intensive treatment reduced the risk of any CVD event by 42% (95% CI: 9–63; *p*: 0.02), highlighting the importance of tight glycemic control in patients with T1D (Nathan et al. 2005). Moreover, recent data from DCCT/EDIC suggest sustained CV benefit with intensive diabetes therapy up to 30 years of follow-up, with reduced incidence of any CVD by 30% (95% CI: 7–48; *p*: 0.016) and a statistically nonsignificant decreased incidence of major CV events (MACE) (nonfatal MI, stroke, or CV death) by 32% (95% CI: −3 to 56; *p*: 0.07) (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group 2016). The lower HbA1c values obtained during the DCCT/EDIC may have accounted for the long-term effect on CVD risk, and it was estimated that 10% lower updated HbA1c values during DCCT were associated with a 20% risk reduction for a CV event (95% CI: 9–30; $p < 0.001$) (Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group 2016; Nathan et al. 2005).

In the SU–insulin arm of the UKPDS in patients with newly diagnosed diabetes, after a median follow-up of 10 years there was a borderline nonsignificant risk reduction of 16% (*p*: 0.052) in MI events with intensive therapy, while patients allocated to metformin arm had a 39% (*p*: 0.01) risk reduction in MI with intensive glucose control (UK Prospective Diabetes Study (UKPDS) Group 1998a, b). The 10-year post-trial follow-up of the UKPDS indicated sustained macrovascular benefits, despite early loss of between-group difference in HbA1c: the RR reduction in MI in the intensive SU–insulin versus conventional group was similar to that reported at UKPDS closeout (15%), but reached statistical significance (*p*: 0.01), indicating that perhaps a longer duration of observation is needed in order to observe a CV benefit (Holman et al. 2008). For patients allocated to metformin, there was a significant risk reduction of 33% in MI (HR: 0.67 (95% CI: 0.51–0.89); *p*: 0.005) and 27% in all-cause mortality (HR: 0.73 (95% CI: 0.59–0.89), *p*: 0.002) with the intensive approach, after a median follow-up of 17.7 years (Holman et al. 2008). This may suggest a glucose-independent beneficial effect of metformin on CVD (Inzucchi and Majumdar 2015).

The initial UKPDS trial and additional smaller studies did not fully resolved the dilemma of whether intensive glucose control can reduce macrovascular complications in patients with longer disease duration. It was hypothesized that possibly even more stringent glucose control, aiming for normoglycemia, could further improve the macrovascular outcomes. Thus, before the post-UKPDS follow-up results were published, the ACCORD, ADVANCE, and VADT studies were designed to test this assumption in patients with long-standing T2D and high CV risk (35% of patients enrolled in ACCORD, 32% in ADVANCE, and 40% in VADT, respectively, had had established CVD) (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008; Zoungas et al. 2014; Duckworth et al. 2009).

The ACCORD trial reported no significant difference with respect to the primary outcome (MACE) between intensive-therapy and standard-therapy group (HR: 0.90 (95% CI: 0.78–1.04); *p*: 0.16), although nonfatal MI was significantly decreased (HR: 0.76 (95% CI: 0.62–0.92); *p*: 0.004) (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). Further analysis confirmed a beneficial effect of intensive glycemic control on CHD, with lower rates of MI and combined MI, coronary revascularization, and unstable angina during the active-treatment phase and continued mean 1.2 years follow-up (Gerstein et al. 2014). However, there was an unexpected significant increase in death of any cause (HR: 1.22 (95% CI: 1.01–1.46); *p*: 0.04) as well as in CV death (HR: 1.35 (95% CI: 1.04–1.76); *p*: 0.02) (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). It was hypothesized that higher rates of hypoglycemia experienced by patients allocated to the intensive arm ($p < 0.001$) may have been related to increased mortality, but an irrefutable connection between hypoglycemia and differential mortality between treatments has not been proven, although severe hypoglycemic events were associated with higher mortality in both treatment groups (Skyler et al. 2009). A retrospective epidemiological analysis of the ACCORD study pointed out that among patients experiencing at least one episode of severe hypoglycemia requiring any assistance, a nonsignificant lower risk of death was observed in the intensive- versus standard-treatment group (HR: 0.74 (95% CI: 0.46–1.23)) (Bonds et al. 2010). A post-hoc analysis of the ACCORD data identified a subgroup of intensively treated patients with higher average on-treatment HbA1c (that failed to improve glycemic control, despite a more intensive approach and sustained efforts) as having a higher mortality risk (Riddle et al. 2010). This may indicate that in patients refractory to improvement of average HbA1c, attempts at importunate treatment intensification may be hazardous. Moreover, patients with baseline HbA1c $\leq$8% and no prior CVD who were randomized to the intensive-therapy arm benefited the most from treatment intensification (Action to Control Cardiovascular Risk in Diabetes Study Group et al. 2008). Follow-up of the ACCORD patients (ACCORDION) reported a neutral long-term effect of the initial mean 3.7-year intensive glucose-lowering approach on MACE (HR: 0.95, 95% CI: 0.87–1.04; *p*: 0.27) or all-cause mortality (HR: 1.01, 95% CI: 0.92–1.10; *p*: 0.91), while the risk of CV death in the intensive group decreased at follow-up, but remained significant (HR: 1.20 (95% CI: 1.03–1.39); *p*: 0.02), possibly suggesting a carry-on effect (ACCORD Study Group Writing Committee 2016).

Intensive glycemic control in the ADVANCE trial had no significant effect on major macrovascular events (HR: 0.94 (95% CI: 0.84–1.06); *p*: 0.32) or on CV mortality (HR: 0.88 (95% CI: 0.74–1.04); *p*: 0.12) (ADVANCE Collaborative Group et al. 2008). Furthermore, the ADVANCE-ON follow-up did not show any significant difference in the risk of any-cause mortality or major macrovascular events between the intensive- and standard-control groups (HR: 1.00 (95% CI: 0.92–1.08) and 1.00 (95% CI: 0.92–1.08), respectively), possibly due to lower baseline HbA1c (Zoungas et al. 2014).

As with the two aforementioned studies, VADT failed to demonstrate a macrovascular benefit with intensive glucose-lowering therapy, as there was no significant

difference in the primary outcome (time to the first occurrence of a composite of MI, stroke, death from CV causes, congestive heart failure (HF), surgery for vascular disease, inoperable coronary disease, and amputation for ischemic gangrene) with intensive treatment (HR: 0.88 (95% CI: 0.74–1.05); *p*: 0.14) or in the rate of all-cause mortality (HR: 1.07 (95% CI: 0.81–1.42); *p*: 0.62) (Duckworth et al. 2009). Subsequent analysis showed that intensive therapy reduced the risk of CV events in patients with less baseline coronary atherosclerosis (Reaven et al. 2009). In the VADT post-trial extension, which had a median duration of 9.8 years, the between-group difference in HbA1c declined to 0.2–0.3% by the third year and onward (Hayward et al. 2015). The intensive-therapy group had a significantly lower risk of the primary outcome (HR: 0.83 (95% CI: 0.70–0.99); *p*: 0.04), but no significant decrease in CV or overall mortality compared to standard group (Hayward et al. 2015).

Overall, these three trials failed to prove a CV benefit with an intensive glucose-lowering approach (although there was evidence for reduced nonfatal MI) in patients with long-standing T2D and high CV risk and additionally, the higher mortality observed in the ACCORD trial, raised concerns regarding the appropriate glucose targets in these patients and the ways to achieve them (Skyler et al. 2009). A meta-analysis of five RCTs (including UKPDS, ACCORD, ADVANCE, VADT, and PROspective pioglitAzone Clinical Trial In macroVascular Events (PROACTIVE) study) pointed out possible macrovascular benefits of tight glycemic control (Ray et al. 2009). With mean 0.9% lower HbA1c levels in the intensive versus the control group, a significantly lower incidence of nonfatal MI (OR: 0.83 (95% CI: 0.75–0.93)) and CHD (OR: 0.85 (95% CI: 0.77–0.93)), but not of stroke or all-cause mortality, was observed (Ray et al. 2009). Another meta-analysis that included only the first 4 trials, with a total of 27,049 T2D patients, reported that more-intensive glycemic control was associated with a 9% risk reduction for macrovascular events (HR: 0.91 (95% CI: 0.84–0.99)), mainly due to a 5% reduction in MI (HR: 0.85 (95% CI: 0.76–0.94)) (Control Group et al. 2009). The rates of major hypoglycemia were 2.48 higher with intensive treatment; however the all-cause and CV mortality were nonsignificantly increased by 4% and 10%, respectively (HR: 1.04 (95% CI: 0.90–1.20) and 1.10 (95% CI: 0.84–1.42), respectively) (Control Group et al. 2009). Subgroup analyses revealed that only patients without prior CVD exhibited a reduction in major CV events (HR: 0.84 (95% CI: 0.74–0.94)) (Control Group et al. 2009).

On the other hand, a retrospective cohort study that analyzed survival as a function of HbA1c in patients with T2D who had treatment intensification to dual therapy (metformin and SU; mean diabetes duration/deciles: 5.2–5.6 years) or insulin therapy (mean diabetes duration/deciles: 6.8–8.2 years) indicated that the relationship of all-cause mortality and HbA1c is U-shaped and the adjusted HR for all-cause mortality in the lowest HbA1c decile (6.4%; 6.1–6.6) was 1.52 (95% CI: 1.32–1.76) compared to lowest hazard decile (7.5%) (Currie et al. 2010). Additionally, patients on insulin-based regimen had higher HR for all-cause mortality (HR: 1.49 (95% CI: 1.39–1.59)), and similar findings were noted for the risk of first cardiac event (HR: 1.54 (95% CI: 1.28–1.84)) (Currie et al. 2010). This might indicate the need of establishing a lower limit for target HbA1c in certain patients with diabetes (possibly those with higher CV risk and longer duration of diabetes).

Overall these data seem to suggest that the intensive glycemic control strategies might be beneficial on long-term in patients with T1D and in patients with T2D if started early in the course of disease, while it might have deleterious effects if initiated in subjects with long-standing T2D and history of CVD. Several possible mechanisms have been proposed to explain the detrimental CV effects observed with tight glycemic control including hypoglycemia, weight gain (which usually is accompanied with more severe insulin resistance) or other metabolic changes, failure to decrease Hb1Ac despite intensive therapy, or even drug-related adverse events. In patients with established CVD, the complexity of atherosclerotic lesions with extended vascular hyperglycemic memory may yield difficulty of any glucose-lowering strategy to improve CV outcomes, as the damage inflicted on the vasculature does not easily (if at all) revert with improved glycemic control (Paneni et al. 2013; Miyazawa et al. 2012). Therefore, it might be plausible that maintenance of tight blood glucose control over a more extended period of time and a longer observation period is needed to clearly perceive a CV benefit. An interesting hypothesis was also raised to explain the unexpected adverse outcomes seen in these trials (Nolan et al. 2015). It was postulated that the subgroups of obese/overweight T2D subjects with significant insulin resistance and refractory hyperglycemia that fail to reverse the positive energy balance are the ones who are exposed to higher CV risk with an aggressive glucose-lowering approach, since in their case insulin resistance might be an adaptive defense mechanism against the metabolic stress in several insulin-sensitive tissues, including the myocardium (Nolan et al. 2015).

The ORIGIN trial was performed in subjects very early in the disease course of T2D, and in this way it also tested the assumption that early intervention by stringent glycemic control might provide advantages on macrovascular endpoints. The primary outcome of MACE, however, was not significantly different between the two intervention groups (HR: 1.02 (95% CI: 0.94–1.11); *p*: 0.63), suggesting that factors other than timing of intervention might be more relevant for prevention of macrovascular complications (ORIGIN Trial Investigators et al. 2012). The post-trial follow-up (the ORIGIN and Legacy Effects (ORIGINALE) study) for additional 2.7 years showed no differences in CV effects between the two interventions groups (HR: 1.01 (95% CI: 0.94–1.10); *p*: 0.72) (ORIGIN Trial Investigators 2016).

It might be the case that in order to show CV benefit in patients with diabetes, it is not sufficient to target hyperglycemia, but the other CV risk factors should be concomitantly controlled (Stark Casagrande et al. 2013). The STENO-2 trial demonstrated that intensive management of all CV risk factors brings CV benefit (Zoungas et al. 2014). After mean 7.8 years of follow-up, a 53% reduction of CV events was shown in the intensively treated arm (HR: 0.47 (95% CI: 0.24–0.73); *p*: 0.008) (Zoungas et al. 2014). Further on, after another 5.5 years of observation, a reduction in all-cause mortality (HR: 0.54 (95% CI: 0.32–0.89); *p*: 0.02) and in CV mortality (HR: 0.43 (95% CI: 0.19–0.94); *p*: 0.04) were noted (Zoungas et al. 2014; Wong et al. 2016). Notably, the use of statins was negligible at baseline, increasing to 85% in the intensive-therapy arm and to only 22% in the conventional arm at the end of study intervention, so it is not clear which of the interventions contributed most to the CV risk reduction.

Weight gain is a known untoward event of certain therapies (e.g., sulfonylurea, insulin, or TZDs), and mitigating weight gain is an additional target to be pursued parallel to attainment of glycemic targets. Recent clinical trials have therefore viewed the benefit of the new drugs or regimens by their ability to attain the combined triple target of HbA1c (<7.0% or <6.5%) *and* no hypoglycemia *and* no weight gain, and this was also explored in post hoc analysis and meta-analysis of trials data (Lingvay et al. 2016; Rosenstock et al. 2012, 2016; Bron et al. 2014; Zinman et al. 2012).

Many studies evaluating the CV safety of glucose-lowering agents have been performed or are currently underway, but these are not validating glycemic targets (but rather striving for similar glucose control in all study participants) and therefore will not be discussed here. We do briefly mention though two recent trials that proved significant CV benefits with similar magnitude of decrease in blood glucose levels as the other trials that failed to show the same. The EMPA-REG Outcome trial demonstrated a clear benefit of a sodium–glucose cotransporter 2 (SGLT-2) inhibitor (empagliflozin) versus placebo with regard to the primary composite endpoint (MACE) in patients with T2D (mean age: 63 years, mean duration of diabetes: 10 years and mean HbA1c: 8.1%) and established CVD (HR: 0.86 (95% CI: 0.74–0.99); *p*: 0.04 for superiority) after a median duration of follow-up of 3.1 years (Zinman et al. 2015). In addition, a 32% reduction in the risk of all-cause mortality and a 35% reduction in the risk of hospitalization for HF were shown (Zinman et al. 2015). The HbA1c was consistently lower in the active-treatment arms throughout the trial, but the differences in the mean HbA1c at trial end between active-drug arms and placebo were relatively small (0.24% and 0.36%, respectively), and most probably the effect on blood glucose alone is not the only mechanism explaining the proven CV benefit with such short onset. Rather it is proposed that the drug modulates other factors related to the CV risk in this patient population, such as reduction in sympathetic nervous system activity, blood pressure and arterial stiffness, oxidative stress, uric acid, body weight/visceral adiposity, albuminuria, low risk of hypoglycemia, diuretic (and natriuretic) effect, or perhaps a combined effect on these factors, mainly because the reduced CV risk was due to a reduction in death from HF, while the risk of MI remained the same (Inzucchi et al. 2015). The Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial reported lower rates of CV events and mortality with a glucagon-like peptide-1 (GLP-1) receptor agonist (liraglutide) versus placebo in patients with T2D (mean age: 64 years, mean duration of diabetes: 12.8 years and mean HbA1c: 8.7%), followed-up for 3.8 years (Marso et al. 2016). The primary outcome (MACE) occurred in significantly fewer patients in the liraglutide arm (HR: 0.87 (95% CI: 0.78–0.97); *p*: 0.01 for superiority), as was death of all-cause (HR: 0.85 (95% CI: 0.74–0.97); *p*: 0.02) (Marso et al. 2016). Even if there was an important decrease in the HbA1c (of more than 1%), in the first 3 months after treatment initiation with liraglutide the mean difference in the HbA1c levels in the two therapy arms was consistent but relatively small at 36 months (−0.40% points, 95% CI: −0.45, −0.34) and by the end of the trial (Marso et al. 2016). In LEADER trial the observed CV benefits were possibly due to an improvement in progression

of atherosclerotic disease, but given the relatively short duration of the trial, the positive CV outcomes resulted not only from improvement of blood glucose levels but to other factors as well (e.g., weight loss, lower number of hypoglycemic events).

Exploring the Benefit of Reducing Postprandial Glucose or Glycemic Variability

The above-discussed trials titrated treatment by the fasting glucose and HbA1c levels. Several other studies focused on the potential impact of targeting postprandial hyperglycemia on the risk of diabetes complications.

Postprandial hyperglycemia has been discussed as a possible mediator of diabetes complications as well. The Hyperglycemia and Its Effect After Acute Myocardial Infarction on Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus (HEART2D) trial was designed to compare the effect of controlling the postprandial versus the fasting blood glucose on the risk for CV events in T2D patients post-acute MI (Raz et al. 2009). One thousand one hundred and fifteen patients were randomized to either a prandial strategy (targeting the 2-h postprandial glucose to $<$7.5 mmol/l (135 mg/dl) using premeal insulin) or to a basal strategy (targeting fasting glucose to $<$6.7 mmol/l ($<$120.6 mg/dl) using bedtime basal or intermediate acting insulin) (Raz et al. 2009). The postprandial glucose excursions were significantly lower in the prandial- versus the basal-strategy group (0.1 $\pm$ 0.2 mmol/l vs. 1.3 $\pm$ 0.1 mmol/l; $p < 0.001$), and fasting glucose concentrations were lower in the basal- versus the prandial-strategy group (7.0 $\pm$ 0.2 mmol/l vs. 8.1 $\pm$ 0.2 mmol/l; $p < 0.001$), and there was no significant difference in the mean HbA1c between the two groups (7.7 $\pm$ 0.1% vs. 7.8 $\pm$ 0.1%; p: 0.4) (Raz et al. 2009). The study was stopped early (after mean of 2.7 years) due to futility, as there was no significant difference in the risk of additional CV events between the two intervention arms (HR: 0.98 (95% CI: 0.8–1.21)) (Raz et al. 2009). The lack of CV benefit with prandial strategy in this study could be explained either by the modest effect on postprandial glycemia (smaller than expected difference between arms) or by the advanced state of CVD in study participants. However, a post-hoc analysis of trial data identified a subset of patients (older than 65.7 years) in whom a prandial strategy might potentially bring CV benefit (Raz et al. 2011).

The STOP-Noninsulin-Dependent Diabetes Mellitus (NIDDM) trial randomized 1,429 patients with IGT to receive an α-glucosidase inhibitor (acarbose), which mainly reduces postprandial glucose levels, or placebo (Chiasson et al. 2003). The main outcome measure in this study was defined as number of patients developing a major CV event (CHD (MI, new angina, or revascularization), CV death, congestive HF, cerebrovascular events, and peripheral vascular disease), and subjects were followed up for a mean duration of 3.3 years (Chiasson et al. 2003). Even though the number of CV events was very low, the trial reported a significant reduction in the risk of CVD with acarbose therapy (HR: 0.51 (95% CI: 0.28–0.95); p: 0.03), but this correlation needs to be further validated (Chiasson et al. 2003). Also, it is not clear if the drug per se or lowering the postprandial glycemic values was associated with CV

benefit. Similarly, the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) trial evaluated the effect of a short-acting insulin secretagogue (nateglinide) which also primarily targets postprandial hyperglycemia, on the occurrence of CV events (co-primary outcome) (NAVIGATOR Study Group et al. 2010). The trial included 9,306 patients with IGT and high CV risk, who were followed up for a median of 6.5 years for the vital status and showed no significant effect on the composite CV outcome (HR: 0.94 (95% CI: 0.82–1.09); *p*: 0.43) (NAVIGATOR Study Group et al. 2010).

Therefore, the data overall did not conclusively demonstrate CV benefit by controlling postprandial glucose concentrations in patients with T2D. High glycemic variability (with high and low spikes) has been proposed to increase CV risk, but data on the impact of glycemic variability on CV outcomes is limited (Suh and Kim 2015; Hirsch 2015). A subsequent analysis of data from 578 patients with T2D and acute MI included in the Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI)-2 study, who had blood glucose levels measured hourly during the first 48 h of hospitalization, evaluated measures of glycemic variability (root mean square error, range, slope) in relation to a composite endpoint of mortality, stroke, and reinfarction and to mortality (Mellbin et al. 2013). The 1-year risk for the composite endpoint and for mortality did not relate to markers of glycemic variability (Mellbin et al. 2013). There are presently trials underway exploring the association of glycemic variability and adverse outcomes (CV risk, albuminuria) (FLAT-SUGAR Trial Investigators et al. 2015). A systematic review and meta-analysis of 7 studies in patients with T1D and 13 in patients with T2D reported that higher HbA1c variability was positively associated with some micro- and macrovascular complications, independently of the HbA1c levels in both patient populations, but the studies were retrospective, and the definition of HbA1c variability was inconsistent (Gorst et al. 2015). High glycemic variability in the context of similar HbA1c levels might be indicative of higher rates of hypoglycemia, which has been linked to adverse CV consequences, by inducing arrhythmias, inflammation, and oxidative stress (Connelly et al. 2015; Pistrosch et al. 2015). So probably, besides defining the optimal pre- and postprandial blood glucose levels and the definition of HbA1c targets, low hypoglycemia rates should also be taken into consideration when setting glycemic targets.

In conclusion, there is no clear-cut evidence that lowering blood glucose levels to near-normal concentrations in all patients is associated with CV benefits in the short-term, although some CV outcomes (i.e., nonfatal MI) might be improved. On contrary, there is concern that if this therapeutic approach is initiated in patients with long-standing diabetes and advanced CVD, who are unable to ameliorate glycemic control despite intensive treatment, it may even be harmful. On the other hand, lowering the HbA1c (i.e., to a target $<7\%$) mainly early in the disease course, perhaps in younger individuals without evidence of CVD, may be associated with positive macrovascular outcomes in the longer-term. In addition, this approach may possibly alter the natural course of the disease (Phillips et al. 2014). Aiming for diminished glycemic variability has not yet demonstrated a CV benefit, though as it generally mitigates the risk of hypoglycemia it might be a sensible treatment approach.

Current Clinical Guidelines Recommendation and Individualization of Glycemic Targets

With the advent of several new classes of antihyperglycemic medications and with the increasing complexity of treatments, technologies, and complications, diabetes care is becoming increasingly challenging. Therefore, national and international clinical societies of diabetes and endocrinology have put much effort into formulating clinical guidelines/position statements which reflect the standard of care based upon most recent scientific data in patients with diabetes. These guidelines are of course not to be considered prescriptive for the individual patient and do not profess to replace clinical judgment (American Diabetes Association 2016; Garber et al. 2016; International Diabetes Federation Clinical Guidelines Task Force 2012).

The issue of glycemic targets is the pivotal aspect (although obviously not the only one) of diabetes care and reviewed in all guidelines. Glycemic targets include HbA1c and fasting/pre- and postprandial glucose levels; their currently recommended thresholds essentially resulted from the overall evaluation of clinical trials data (Table 2).

The HbA1c target varies between patients and many different factors need to be considered (see below), yet the guidelines' recommended goal for most adult patients is <7% (ADA/EASD, IDF) or ≤6.5%, if it can safely be accomplished (AACE) (American Diabetes Association 2016; Garber et al. 2016; International Diabetes Federation Clinical Guidelines Task Force 2012). HbA1c should be measured at least twice a year in patients who are meeting their glycemic goals and quarterly in patients who have recently adjusted their therapy or are not meeting their treatment targets (American Diabetes Association 2016). In patients suffering of hemoglobinopathies, hemolysis, blood loss, or any other condition which may affect the accuracy of HbA1c measurement, other measures of glycemic control need to be assessed such as fructosamine and/or self-monitoring of blood glucose (SMBG). The measurement of HbA1c does not reflect glycemic variability or hypoglycemia, and therefore patients taking medication with a propensity of causing hypoglycemia must be educated on the predisposing factors, symptoms, and treatment of hypoglycemia at each visit.

Assessing for the attainment of fasting/pre- and postprandial capillary plasma glucose targets requires SMBG, but HbA1c levels could also be indicators of mean

Table 2 Currently recommended glycemic targets by international clinical societies of diabetes and endocrinology (American Diabetes Association 2016; Garber et al. 2016; International Diabetes Federation Clinical Guidelines Task Force 2012)

	ADA/EASD	AACE	IDF
HbA1c (%)	7.0%	<6.5	<7.0
Fasting plasma glucose (mg/dl)	80–130	110	115
Postprandial plasma glucose (mg/dl)	180		160

AACE American Association of Clinical Endocrinologists, *ADA* American Diabetes Association, *EASD* European Association for the Study of Diabetes, *IDF* International Diabetes Federation
According to all guidelines glycemic targets must be individualized

glucose concentrations. For example, HbA1c values of 6.0%, 7.0%, 8.0%, and 9.0% correlate with mean plasma glucose levels of 126 mg/dl, 154 mg/dl, 183 mg/dl, and 212 mg/dl, respectively (American Diabetes Association 2016). Moreover, correlations between HbA1c intervals and mean fasting, premeal, postprandial, and bedtime glucose levels have been suggested (e.g., HbA1c levels of 7–7.49% are correlated with mean fasting and premeal glucose of 152 mg/dl, mean postprandial glucose of 176 mg/dl and mean bedtime glucose of 177 mg/dl) (American Diabetes Association 2016). In case of significant deviation of SMBG values from the HbA1c measurement, confounders of HbA1c or poor SMBG technique should be sought. Patients receiving multiple daily insulin injections may use SMBG values to modify insulin doses and to monitor for and prevent asymptomatic hypoglycemia and hyperglycemia. However, in patients receiving non-insulin glucose-lowering agents or basal insulin alone, the role of SMBG is less well established. Patients should be taught how to use SMBG data to adjust food intake, exercise, or pharmacological therapy and should be educated with respect to their individual SMBG targets. The recommended frequency of SMBG monitoring should be discussed with the patient and aim to improve their glycemic control.

Subgroup analyses of clinical trials in diabetes have highlighted the often differential response of the individual patients to glucose-lowering modalities or drugs. The presence or lack of established CVD is an important consideration. In the ACCORD trial, primary prevention of CVD by intensive glycemic control has been shown to be of benefit versus possible harm of tight glycemic control in patients with established CVD. Similarly, CV benefit was shown in VADT patients with less coronary atherosclerosis. This trend had been further demonstrated in the Look AHEAD and ADVANCE trials and has thus advocated tighter glycemic control in patients without established CVD, and more lenient targets in patients with CVD.

The medications used to attain the target are of utmost importance with regard to several aspects. First, the particular risks associated with the use of each drug or class should be reviewed in each patient. For example, the use of TZDs is not recommended in patients with advanced HF, and SGLT2 inhibitors may increase the risk of genital infections in those prone to develop them. Second, the risk of hypoglycemia from each drug and the patient's inherent risk from hypoglycemia must be considered as well. In a survey conducted among over 150 expert opinion-leading diabetologists the "risk of hypoglycemia from treatment" was considered to be the most important factor in determining the glycemic target for the individual patient (Raz et al. 2013). In other words, the glycemic target is also influenced by the hypoglycemic risk of drugs needed to attain it (Mosenzon et al. 2016). For example, in a patient who is well controlled on metformin alone, a more stringent target may be proposed since the price of attaining it is minimal (if the drug is well tolerated by the patient). Conversely, a patient who requires a complicated regimen of basal-bolus insulin will experience increased rates of hypoglycemia with treatment intensification; therefore a more lenient target may be considered for him/her, depending of course upon additional aspects (Box 1). On the other hand, additional benefits which may be brought to the patient by a particular drug/drug class should also be considered, as these are gradually becoming realized. Patients with established CVD

(particularly those aged ≥65 years and with HbA1c <8.5%) have demonstrated reduced CV mortality and hospitalization for HF with the use of empagliflozin (Zinman et al. 2015). DPP-4 inhibitors have demonstrated a reduction in microalbuminuria, which is independent of their effect on HbA1c, in several trials, although the mechanism responsible for this observation remains to be explored (Groop et al. 2013; Mosenzon et al. 2015). Several glucose-lowering agents have demonstrated the additional benefit of weight loss and BP reduction, which are particularly valuable in patients suffering from these concomitant morbidities and for improving CV risk.

Box 1 Suggested Factors to be Considered When Glycemic Targets Are Chosen for Individual Patients with Diabetes

1. Age
2. Life expectancy
3. Disease duration
4. Macrovascular complications
5. Advanced microvascular complications
6. History of severe hypoglycemia
7. History of previous glycemic control
8. Important comorbidities
9. Cognitive function
10. Adherence and motivation
11. Quality of life
12. Patients attitude, including preference, values, needs, and expectations
13. Risk of hypoglycemia from treatment
14. Other drug-induced adverse effects
15. Family and social support system
16. Economic resources

Therefore, multiple decision elements of different relative importance need to be considered while setting glycemic targets for the individual patient, such as patient-related factors (e.g., disease duration, age, presence of complications or comorbidities, quality of life), drug-related factors (e.g., potential adverse effects, propensity of hypoglycemia), support system, or economic considerations (Box 1) (Raz et al. 2013; Cahn et al. 2015). Current guidelines generally recommend to individualize the glycemic goals according to these factors and to choose more stringent or more relaxed HbA1c targets, without though specifically indicating methods or algorithms to be used for each given patient, which presents with a particular constellation of characteristics (American Diabetes Association 2016). Therefore, personalized, "patient-tailored" therapy is still difficult to attain.

However, several groups have suggested different algorithms that would guide physicians in individualizing glycemic targets for patients with diabetes and improve its management. It should be mentioned, however, that none of these have been

prospectively validated in clinical trials and are rather intuitive, but based on interpretation of landmark trials.

One such simple algorithm based on three factors (age, complications, duration of disease), adapted after the previously published ABCD algorithm, was proposed as a practical tool for choosing the HbA1c target ranges (Pozzilli et al. 2010; Bianchi and Del Prato 2011). It basically categorizes patients as being young (<40 years old), middle aged, or elderly (over 70 years) and then takes into consideration the presence of complications (yes/no) and duration of diabetes more than 10 years (yes/no) (Bianchi and Del Prato 2011).

Another comprehensive review evaluated the needs and ways to individualize glycemic goals and suggested a more complex framework to assist physicians in choosing HbA1c target ranges and treatment intensities, according to the severity and magnitude of several variables (age, disease duration, hypoglycemia risk, comorbid conditions, established vascular complications, and psycho-socioeconomic factors) (Inzucchi et al. 2012). Later, the ADA/EASD guidelines were adapted following this proposal (Ismail-Beigi et al. 2011).

A more recently published algorithm considers the unique patient characteristics and the relative importance of the decision elements, as were defined by a survey of over 150 expert diabetologists worldwide (Cahn et al. 2015). One may input the level of each parameter on a scale of 1–3 (i.e., long, limited, short life expectancy), and the algorithm proposes a glycemic target. This tool may supplement and aid decision-making; yet, it does not intend to replace clinical judgment, which is the cornerstone of clinical care.

Finally, it is clear that there is no "one-size-fits-all" approach for diabetes management and medicine remains an art in which the physician understands and balances the unique characteristics of the patient with the accumulating data and evidence from clinical trials. Perhaps a personalized approach to identifying the most appropriate glycemic target for each patient, based on a well-structured system with clear directives that takes into account various factors (including the characterization of patient's clinical phenotype, risk of hypoglycemia, his/her capacities, expectations) and that is prospectively validated in large clinical trials, would bring the benefit of optimal glycemic control, while minimizing risks and avoiding excessive burden. In time, the glycemic targets should nevertheless be flexible and adapted continuously according to the particular situations/changes in patient's life and health. Clearly, in all this decision-making process of setting the best glycemic targets and ways to achieve them, the patient should be specifically educated, supported, and actively involved.

Conclusions

In conclusion, there is well-established evidence that tight glycemic control reduces microvascular complications, with no clear threshold effect, and an increasing benefit perceived as glucose levels approach normoglycemia. However, there is no clear-cut evidence that lowering blood glucose levels to near-normal

concentrations is associated with CV benefits in the short-term. On contrary, there is concern that if this therapeutic approach is initiated in patients with long-standing diabetes and advanced CVD, particularly when they do not respond well, it may inflict unwanted harm. On the other hand, lowering the HbA1c (i.e., to a target <7%) mainly early in the disease course may be associated with positive macrovascular outcomes in the longer-term. Targeting additional aspects of glycemic control such as postprandial hyperglycemia or reduction of glycemic variability have not clearly demonstrated benefit, though as minimizing variability generally mitigates the risk of hypoglycemia, it might be a sensible treatment approach.

Finally, while clinical guideline recommendations reflecting the standards of care should help in setting glycemic targets, an improved management of diabetes requires their individualization, according to many particular factors and characteristics of each patient.

References

ACCORD Study Group Writing Committee. Nine-year effects of 3.7 years of intensive glycemic control on cardiovascular outcomes. Diabetes Care. 2016;39(5):701–8.

Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Eye Study Group and the Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Study Group. Persistent effects of intensive glycemic control on retinopathy in type 2 diabetes in the action to control cardiovascular risk in diabetes (ACCORD) follow-on study. Diabetes Care. 2016;39(7):1089–100.

Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med. 2008;358 (24):2545–59.

ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med. 2008;358 (24):2560–72.

Agrawal L, Azad N, Emanuele NV, Veterans Affairs Diabetes Trial (VADT) Study Group, et al. Observation on renal outcomes in the Veterans Affairs Diabetes Trial. Diabetes Care. 2011;34 (9):2090–4.

Albers JW, Herman WH, Pop-Busui R, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, et al. Effect of prior intensive insulin treatment during the Diabetes Control and Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) study. Diabetes Care. 2010;33(5):1090–6.

American Diabetes Association. Glycemic targets. Diabetes Care. 2016;39(Suppl 1):S39–46.

Amin R, Widmer B, Prevost AT, et al. Risk of microalbuminuria and progression to macroalbuminuria in a cohort with childhood onset type 1 diabetes: prospective observational study. BMJ. 2008;336(7646):697–701.

Berezin A. Metabolic memory phenomenon in diabetes mellitus: achieving and perspectives. Diabetes Metab Syndr. 2016. https://doi.org/10.1016/j.dsx.2016.03.016.

Bergenstal RM. Glycemic variability and diabetes complications: does it matter? Simply put, there are better glycemic markers! Diabetes Care. 2015;38(8):1615–21.

Bianchi C, Del Prato S. Metabolic memory and individual treatment aims in type 2 diabetes – outcome-lessons learned from large clinical trials. Rev Diabet Stud. 2011;8(3):432–40.

Bonds DE, Miller ME, Bergenstal RM, et al. The association between symptomatic, severe hypoglycaemia and mortality in type 2 diabetes: retrospective epidemiological analysis of the ACCORD study. BMJ. 2010;340:b4909.

Bron M, Wilson C, Fleck P. A post hoc analysis of HbA1c, hypoglycemia, and weight change outcomes with alogliptin vs. glipizide in older patients with type 2 diabetes. Diabetes Ther. 2014;5(2):521–34.

Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54(6):1615–25.

Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. JAMA. 2006;295(14):1707–8.

Cahn A, Raz I, Kleinman Y, et al. Clinical assessment of individualized glycemic goals in patients with type 2 diabetes: formulation of an algorithm based on a survey among leading worldwide diabetologists. Diabetes Care. 2015;38(12):2293–300.

Cavender MA, Scirica BM, Raz I, et al. Cardiovascular outcomes of patients in SAVOR-TIMI 53 by baseline hemoglobin A1c. Am J Med. 2016;129(3):340.e1–8.

Ceriello A. The emerging challenge in diabetes: the "metabolic memory". Vasc Pharmacol. 2012;57 (5–6):133–8.

Ceriello A, Esposito K, Piconi L, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes. 2008;57(5):1349–54.

Chiasson JL, Josse RG, Gomis R, STOP-NIDDM Trial Research Group, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA. 2003;290(4):486–94.

Connelly KA, Yan AT, Leiter LA, et al. Cardiovascular implications of hypoglycemia in diabetes mellitus. Circulation. 2015;132(24):2345–50.

Control Group, Turnbull FM, Abraira C, Anderson RJ, et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. Diabetologia. 2009;52(11):2288–98.

Conway BN, May ME, Fischl A, Frisbee J, et al. Cause-specific mortality by race in low-income Black and White people with type 2 diabetes. Diabet Med. 2015;32(1):33–41.

Creager MA, Lüscher TF, Cosentino F, et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Circulation. 2003;108(12):1527–32.

Currie CJ, Peters JR, Tynan A, et al. Survival as a function of HbA1c in people with type 2 diabetes: a retrospective cohort study. Lancet. 2010;375(9713):481–9.

DCCT/EDIC Research Group. Effect of intensive diabetes treatment on albuminuria in type 1 diabetes: long-term follow-up of the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications study. Lancet Diabetes Endocrinol. 2014;2(10):793–800.

Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: the DCCT/EDIC study 30-year follow-up. Diabetes Care. 2016;39 (5):686–93.

Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group, Lachin JM, White NH, Hainsworth DP, et al. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. Diabetes. 2015;64(2):631–42.

Duckworth W, Abraira C, Moritz T, VADT Investigators, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med. 2009;360(2):129–39.

Eeg-Olofsson K, Cederholm J, Nilsson PM, et al. New aspects of HbA1c as a risk factor for cardiovascular diseases in type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). J Intern Med. 2010;268(5):471–82.

El-Osta A, Brasacchio D, Yao D, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med. 2008;205 (10):2409–17.

Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SR, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet. 2010;375(9733):2215–22.

FLAT-SUGAR Trial Investigators, Probstfield JL, Hirsch I, O'Brien K, et al. Design of FLAT-SUGAR: randomized trial of prandial insulin versus prandial GLP-1 receptor agonist together with basal insulin and metformin for high-risk type 2 diabetes. Diabetes Care. 2015;38(8):1558–66.
Gaede P, Pedersen O. Intensive integrated therapy of type 2 diabetes: implications for long-term prognosis. Diabetes. 2004;53(Suppl 3):S39–47.
Gaede P, Vedel P, Larsen N, et al. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med. 2003;348(5):383–93.
Gaede P, Lund-Andersen H, Parving HH, et al. Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med. 2008;358(6):580–91.
Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the comprehensive type 2 diabetes management algorithm – 2016 executive summary. Endocr Pract. 2016;22(1):84–113.
Geiss LS, Herman WH, Smith PJ. Mortality among persons with non-insulin dependent diabetes. In: Harris MI, Cowie CC, Stern MP, Boyko EJ, Reiber GE, Bennett PH, editors. Diabetes in America. 2nd ed. Bethesda: National Institutes of Health; 1995. p. 233–58.
Gerstein HC, Miller ME, Ismail-Beigi F, ACCORD Study Group, et al. Effects of intensive glycaemic control on ischaemic heart disease: analysis of data from the randomised, controlled ACCORD trial. Lancet. 2014;384(9958):1936–41.
Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058–70.
Gorst C, Kwok CS, Aslam S, et al. Long-term glycemic variability and risk of adverse outcomes: a systematic review and meta-analysis. Diabetes Care. 2015;38(12):2354–69.
Groop PH, Cooper ME, Perkovic V, et al. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. Diabetes Care. 2013;36(11):3460–8.
Hayward RA, Reaven PD, Wiitala WL, VADT Investigators, et al. Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2015;372(23):2197–206.
Hillege HL, Janssen WM, Bak AA, PREVEND Study Group, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Intern Med. 2001;249(6):519–26.
Hirsch IB. Glycemic variability and diabetes complications: does it matter? Of course it does! Diabetes Care. 2015;38(8):1610–4.
Holman RR, Paul SK, Bethel MA, et al. 10-Year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359(15):1577–89.
International Diabetes Federation Clinical Guidelines Task Force. Global guideline for type 2 diabetes. 2012. http://www.idf.org/sites/default/files/IDF-Guideline-for-Type-2-Diabetes.pdf.
Inzucchi S, Majumdar S. Glycemic targets: what is the evidence? Med Clin North Am. 2015;99(1):47–67.
Inzucchi SE, Bergenstal RM, Buse JB, American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), et al. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2012;35(6):1364–79.
Inzucchi SE, Zinman B, Wanner C, et al. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. Diab Vasc Dis Res. 2015;12(2):90–100.
Ismail-Beigi F, Craven T, Banerji MA, ACCORD Trial Group, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet. 2010;376(9739):419–30.
Ismail-Beigi F, Moghissi E, Tiktin M, et al. Individualizing glycemic targets in type 2 diabetes mellitus: implications of recent clinical trials. Ann Intern Med. 2011;154(8):554–9.
Klein R, Klein BE, Moss SE. Relation of glycemic control to diabetic microvascular complications in diabetes mellitus. Ann Intern Med. 1996;124(1 Pt 2):90–6.
Klein R, Knudtson MD, Lee KE, et al. The Wisconsin epidemiologic study of diabetic retinopathy: XXII the twenty-five-year progression of retinopathy in persons with type 1 diabetes. Ophthalmology. 2008;115(11):1859–68.

Lachin JM, Genuth S, Nathan DM, DCCT/EDIC Research Group, et al. Effect of glycemic exposure on the risk of microvascular complications in the diabetes control and complications trial – revisited. Diabetes. 2008;57(4):995–1001.

Lingvay I, Manghi FP, García-Hernández P, DUAL V Investigators, et al. Effect of insulin glargine up-titration vs. insulin degludec/liraglutide on glycated hemoglobin levels in patients with uncontrolled type 2 diabetes: The DUAL V randomized clinical trial. JAMA. 2016;315 (9):898–907.

Marso SP, Daniels GH, Brown-Frandsen K, LEADER Steering Committee, LEADER Trial Investigators, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016;375(4):311–22.

Mellbin LG, Malmberg K, Rydén L, et al. The relationship between glycaemic variability and cardiovascular complications in patients with acute myocardial infarction and type 2 diabetes: a report from the DIGAMI 2 trial. Eur Heart J. 2013;34(5):374–9.

Miyazawa T, Nakagawa K, Shimasaki S, et al. Lipid glycation and protein glycation in diabetes and atherosclerosis. Amino Acids. 2012;42(4):1163–70.

Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA. 2006;295(14):1681–7.

Mosenzon O, Cahn A, Hirshberg B, et al. Cardiovascular outcomes by albumin creatinine ratio categories in the SAVOR trial. Diabetes. 2015;64(Suppl 1). https://ada.scientificposters.com/epsAbstractADA.cfm?id=2. Accessed 3 July 2016.

Mosenzon O, Pollack R, Raz I. Treatment of type 2 diabetes: from "guidelines" to "position statements" and back, recommendations of the Israeli National Diabetes Council. Diabetes Care. 2016;39(Suppl 2):S146–53.

Nathan DM, Cleary PA, Backlund JY, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353(25):2643–53.

NAVIGATOR Study Group, Holman RR, Haffner SM, JJ MM, et al. Effect of nateglinide on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362(16):1463–76.

Nolan CJ, Ruderman NB, Kahn SE, et al. Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. Diabetes. 2015;64(3):673–86.

Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res Clin Pract. 1995;28 (2):103–17.

ORIGIN Trial Investigators. Cardiovascular and other outcomes post-intervention with insulin glargine and omega-3 fatty acids (ORIGINALE). Diabetes Care. 2016;39(5):709–16.

ORIGIN Trial Investigators, Gerstein HC, Bosch J, Dagenais GR, et al. Basal insulin and cardiovascular and other outcomes in dysglycemia. N Engl J Med. 2012;367(4):319–28.

ORIGIN Trial Investigators, Gilbert RE, Mann JF, Hanefeld M, et al. Basal insulin glargine and microvascular outcomes in dysglycaemic individuals: results of the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial. Diabetologia. 2014;57(7):1325–31.

Paneni F, Mocharla P, Akhmedov A, et al. Gene silencing of the mitochondrial adaptor p66(Shc) suppresses vascular hyperglycemic memory in diabetes. Circ Res. 2012;111(3):278–89.

Paneni F, Beckman JA, Creager MA, et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur Heart J. 2013;34(31):2436–43.

Pasko N, Toti F, Strakosha A, et al. Prevalence of microalbuminuria and risk factor analysis in type 2 diabetes patients in Albania: the need for accurate and early diagnosis of diabetic nephropathy. Hippokratia. 2013;17(4):337–41.

Phillips LS, Ratner RE, Buse JB, et al. We can change the natural history of type 2 diabetes. Diabetes Care. 2014;37(10):2668–76.

Pistrosch F, Ganz X, Bornstein SR, et al. Risk of and risk factors for hypoglycemia and associated arrhythmias in patients with type 2 diabetes and cardiovascular disease: a cohort study under real-world conditions. Acta Diabetol. 2015;52(5):889–95.

Pozzilli P, Leslie RD, Chan J, et al. The A1C and ABCD of glycaemia management in type 2 diabetes: a physician's personalized approach. Diabetes Metab Res Rev. 2010;26(4):239–44.

Pozzilli P, Strollo R, Bonora E. One size does not fit all glycemic targets for type 2 diabetes. J Diabetes Investig. 2014;5(2):134–41.

Prattichizzo F, Giuliani A, Ceka A, et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics. 2015;7(1):56.

Quagliaro L, Piconi L, Assaloni R, et al. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes. 2003;52(11):2795–804.

Rajala U, Laakso M, Qiao Q, et al. Prevalence of retinopathy in people with diabetes, impaired glucose tolerance, and normal glucose tolerance. Diabetes Care. 1998;21(10):1664–9.

Ray KK, Seshasai SR, Wijesuriya S, et al. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet. 2009;373(9677):1765–72.

Raz I, Wilson PW, Strojek K, et al. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. Diabetes Care. 2009;32(3):381–6.

Raz I, Ceriello A, Wilson PW, et al. Post hoc subgroup analysis of the HEART2D trial demonstrates lower cardiovascular risk in older patients targeting postprandial versus fasting/premeal glycemia. Diabetes Care. 2011;34(7):1511–3.

Raz I, Riddle MC, Rosenstock J, et al. Personalized management of hyperglycemia in type 2 diabetes: reflections from a Diabetes Care editors' expert forum. Diabetes Care. 2013;36:1779–88.

Reaven PD, Moritz TE, Schwenke DC, Veterans Affairs Diabetes Trial, et al. Intensive glucose-lowering therapy reduces cardiovascular disease events in Veterans Affairs Diabetes Trial participants with lower calcified coronary atherosclerosis. Diabetes. 2009;58(11):2642–8.

Reddy MA, Zhang E, Natarajan R. Epigenetic mechanisms in diabetic complications and metabolic memory. Diabetologia. 2015;58(3):443–55.

Riddle MC, Ambrosius WT, Brillon DJ, Action to Control Cardiovascular Risk in Diabetes Investigators, et al. Epidemiologic relationships between A1C and all-cause mortality during a median 3.4-year follow-up of glycemic treatment in the ACCORD trial. Diabetes Care. 2010;33(5):983–90.

Rosenstock J, Vico M, Wei L, et al. Effects of dapagliflozin, an SGLT2 inhibitor, on HbA(1c), body weight, and hypoglycemia risk in patients with type 2 diabetes inadequately controlled on pioglitazone monotherapy. Diabetes Care. 2012;35(7):1473–8.

Rosenstock J, Guerci B, Hanefeld M, GetGoal Duo-2 Trial Investigators, et al. Prandial options to advance basal insulin glargine therapy: testing lixisenatide plus basal insulin versus insulin glulisine either as basal-plus or basal-bolus in type 2 diabetes: the GetGoal Duo-2 trial. Diabetes Care. 2016;39(8):1318–28.

Sarwar N, Aspelund T, Eiriksdottir G, et al. Markers of dysglycaemia and risk of coronary heart disease in people without diabetes: Reykjavik prospective study and systematic review. PLoS Med. 2010;7(5):e1000278.

Selvin E, Coresh J, Golden SH, et al. Glycemic control and coronary heart disease risk in persons with and without diabetes: the atherosclerosis risk in communities study. Arch Intern Med. 2005;165(16):1910–6.

Skyler JS, Bergenstal R, Bonow RO, American Diabetes Association, American College of Cardiology Foundation, American Heart Association, et al. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. Diabetes Care. 2009;32(1):187–92.

Stark Casagrande S, Fradkin JE, Saydah SH, et al. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988–2010. Diabetes Care. 2013;36(8):2271–9.
Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321(7258):405–12.
Stratton IM, Kohner EM, Aldington SJ, et al. UKPDS 50: risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis. Diabetologia. 2001;44(2):156–63.
Suh S, Kim JH. Glycemic variability: how do we measure it and why is it important? Diabetes Metab J. 2015;39(4):273–82.
Tesfaye S, Stevens LK, Stephenson JM, et al. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. Diabetologia. 1996;39(11):1377–84.
The DCCT Research Group. The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. Diabetes. 1986;35(5):530–45.
The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.
Thomas MC. Glycemic exposure, glycemic control, and metabolic karma in diabetic complications. Adv Chronic Kidney Dis. 2014;21(3):311–7.
UK Prospective Diabetes Study (UKPDS) Group. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia. 1991;34(12):877–90.
UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998a;352(9131):837–53.
UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998b;352(9131):854–65.
van Leiden HA, Dekker JM, Moll AC, et al. Blood pressure, lipids, and obesity are associated with retinopathy: the Hoorn study. Diabetes Care. 2002;25(8):1320–5.
Wong TY, Liew G, Tapp RJ, et al. Relation between fasting glucose and retinopathy for diagnosis of diabetes: three population-based cross-sectional studies. Lancet. 2008;371(9614):736–43.
Wong MG, Perkovic V, Chalmers J, ADVANCE-ON Collaborative Group, et al. Long-term benefits of intensive glucose control for preventing end-stage kidney disease: ADVANCE-ON. Diabetes Care. 2016;39(5):694–700.
Wright A, Burden AC, Paisey RB, U.K. Prospective Diabetes Study Group, et al. Sulfonylurea inadequacy: efficacy of addition of insulin over 6 years in patients with type 2 diabetes in the U. K. Prospective Diabetes Study (UKPDS 57). Diabetes Care. 2002;25(2):330–6.
Writing Group for the DCCT/EDIC Research Group, Orchard TJ, Nathan DM, Zinman B, et al. Association between 7 years of intensive treatment of type 1 diabetes and long-term mortality. JAMA. 2015;313(1):45–53.
Ziegler D, Rathmann W, Dickhaus T, KORA Study Group, et al. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macroangiopathy: the MONICA/KORA Augsburg Surveys S2 and S3. Diabetes Care. 2008;31(3):464–9.
Zinman B, Schmidt WE, Moses A, et al. Achieving a clinically relevant composite outcome of an HbA1c of <7% without weight gain or hypoglycaemia in type 2 diabetes: a meta-analysis of the liraglutide clinical trial programme. Diabetes Obes Metab. 2012;14(1):77–82.
Zinman B, Wanner C, Lachin JM, EMPA-REG OUTCOME Investigators, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373 (22):2117–28.
Zoungas S, Chalmers J, Neal B, ADVANCE-ON Collaborative Group, et al. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N Engl J Med. 2014;371 (15):1392–406.

Prevention of Type 1 Diabetes

14

Jay S. Skyler

Contents

Abstract

There is a genetic predisposition to type 1 diabetes (T1D), particularly conferred by alleles present within the major histocompatibility complex. In susceptible individuals, an environmental trigger initiates an immune response. The immune infiltration into pancreatic islets results in beta cell damage, impairment of beta cell function, and potential destruction of beta cells. Consequently, there have been a number of studies using immune intervention in an attempt to alter the natural history of the disease. These studies have been conducted both before clinical manifestations of T1D, in an attempt to prevent the evolution of the disease, and after the clinical onset of T1D, in an attempt to slow progressive loss of beta cell function. This chapter summarizes the most important clinical trials that have been conducted to date.

Keywords

Type 1 diabetes · Immunotherapy · Prevention

J. S. Skyler (✉)
Diabetes Research Institute, University of Miami, Miami, FL, USA
e-mail: jskyler@miami.edu

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_15

Type 1 diabetes (T1D) results from immune-mediated beta cell dysfunction, in genetically susceptible individuals in whom an environmental trigger initiates the immune response. Consequently, there have been a number of studies that have sought to interrupt the immune response in attempts to either prevent the development of clinical T1D or slow the progressive loss of beta cell function that continues after the clinical onset of T1D. This chapter will summarize those studies.

During the evolution of T1D, one can identify individuals with genetic predisposition by screening at birth for high risk HLA haplotypes (Ziegler and Nepom 2010). This has been done in both cohorts of newborn babies who have a first degree relative (FDR) – either parent or sibling – with T1D and in the general population (GP). Once identified, the risk of progression to T1D is not different in those with (FDR) or without (GP) a family history (Ziegler et al. 2013). These individuals are then followed at routine intervals for the development of diabetes-related autoantibodies. Once there are two or more autoantibodies, over the next two decades 85–90% of such individuals will progress to clinical T1D (Ziegler et al. 2013). Studies of interventions that are initiated in those who have genetic risk but no autoantibodies are "primary prevention" studies. Those initiated after the appearance of autoantibodies are "secondary prevention" studies (Skyler 2013a).

Alternatively, rather than screening newborns and following them longitudinally over time, potential risk of T1D can be sought be cross-sectional screening for autoantibodies among relatives of patients with T1D (Orban et al. 2009). Studies of interventions in these individuals also are "secondary prevention" studies.

Autoantibody-positive individuals progress to clinical T1D over time. Prior to meeting traditional diagnostic criteria for T1D, they will have progressive impairment of beta cell function, often manifested by dysglycemia – either impaired fasting glucose, impaired glucose tolerance (at the two-hour time point of a glucose tolerance test), or indeterminate glucose tolerance (values above 11.1 mmol/L at 30, 60, or 90 min during a glucose tolerance test). Other evidence of beta cell dysfunction also can be seen (Sosenko et al. 2012; Ferrannini et al. 2010). Studies of interventions in these individuals also are considered "secondary prevention" studies. Indeed, in 2015, there was issued a scientific statement by the JDRF, the Endocrine Society, and the American Diabetes Association, on the staging of presymptomatic type 1 diabetes (Insel et al. 2015). In this, those with genetic predisposition only are considered to have "Pre-Stage 1" T1D; those with autoantibodies are considered to have "Stage 1" T1D; those with dysglycemia are considered to have "Stage 2" T1D; and those meeting traditional diagnostic criteria or having symptoms are considered to have "Stage 3" T1D. Thus, stages 1 and 2 together represent "presymptomatic T1D" and intervention studies in these stages are "secondary prevention" studies. In this scheme, trials in individuals with Stage 3 T1D may test disease modifying therapies to slow loss of beta cell function, are considered "tertiary prevention" studies, or are considered by some as "intervention" studies rather than "prevention" studies. Studies in Stage 3 T1D may be in "recent-onset" T1D – generally defined as being initiated within the first 3 months after diagnosis, or in "established" T1D – generally meant to be after 4 months or more from diagnosis provided there is evidence of beta cell function above a predefined threshold.

Primary Prevention Studies

Primary prevention studies have used generally safe interventions, such as dietary manipulation or some form of antigen-based therapy. The ones conducted to date have usually involved a cohort identified at birth, but also have involved studies in autoantibody-negative, genetically at-risk children. One strategy tested was the removal of cow milk from infant formula, based on an epidemiologic analysis that suggests that earlier exposure to cow milk may serve as an environmental trigger (Gerstein 1994). The ambitious Trial to Reduce Incidence of Diabetes in Genetically at Risk (TRIGR) study, a multinational, randomized prospective trial, involved 77 centers in 15 countries and registered over 5000 newborns and randomized 2159 newborns over a 4.7-year period (TRIGR Study Group et al. 2011). A Finnish TRIGR Pilot Study enrolled 230 babies randomized to either a conventional cow-milk-based formula or a casein hydrolysate formula, to be initiated whenever the mother ceased breast feeding (Knip et al. 2010). It reported that babies fed the experimental formula were nearly 50% less likely to develop autoantibodies. The full-scale TRIGR study, using the same strategy, did not find a difference in appearance of autoantibodies by the time the participants had reached age 7 (Knip et al. 2014). Moreover, the development of T1D by age 10, the primary outcome of TRIGR, also did not find a difference in T1D between the two cohorts (Writing Group for the TRIGR Study Group et al. 2018).

Another Finnish study using a similar strategy used a third group that included an insulin-free whey-based formula, the concept being that bovine insulin in infant formula may serve as a trigger for T1D (Vaarala et al. 2012). That study randomized 1104 babies to the 3 formulae and found that there was 60% reduction in appearance of autoantibodies with the insulin-free formula.

The BABYDIET study, involving 150 infants, tested whether a gluten-free diet could reduce the appearance of autoantibodies, but it did not (Hummel et al. 2011). A small pilot study, named NIP, randomized 98 infants to either have supplemental docosahexaenoic acid or placebo (Chase et al. 2015). It had hoped to demonstrate a difference in cytokine profile, but failed to do so.

The pilot PRE-POINT Study tested the safety of high dose oral insulin in a group of 25 autoantibody-negative, genetically at-risk children ages 2–7 (Bonifacio et al. 2015). There were no safety issues and some suggestion of an effect on some immunologic measures. This was followed by another pilot study, the Pre-POINT-Early Study, which tested a refined oral insulin dosing schedule among 44 autoantibody-negative, genetically at-risk children age 6–24 months (Pre-POINT Early Study). That study was completed in December 2017, but results have not yet been reported. Another pilot study, similar to PRE-POINT, the PINIT study, using nasal insulin, involves 38 autoantibody-negative, genetically at-risk children ages 1–7, is expected to be completed in 2018 (PINIT Study).

Further primary prevention studies are underway or being planned. To facilitate them, the Freder1k-Study is screening 168,000 infants, from birth to 4 months of age, to identify infants with increased T1D risk for enrollment into primary prevention trials (Freder1k-Study). The GPPAD-POInT primary prevention study is

enrolling 1040 genetically at-risk infants age 4–7 months, who will be treated with oral insulin or placebo up until age 3 years, and then followed until age 7.5 years, with the primary outcome – the development of persistent confirmed multiple beta-cell autoantibodies or the diagnosis of diabetes (Ziegler et al. 2016).

Secondary Prevention Studies

Most secondary prevention studies have used either some form of antigen – insulin or GAD – or the vitamin nicotinamide. These studies evaluate time from randomization to clinical diagnosis of T1D. Two nicotinamide studies, DENIS (Lampeter et al. 1998) and ENDIT (2004), both were negative. Two studies using injected insulin, the DPT-1 Parenteral Insulin Trial (2002) and the Belgian Parenteral Insulin Trial (Vandemeulebroucke et al. 2009), were both negative. Two studies using oral insulin, the DPT-1 Oral Insulin Trial (Skyler et al. 2005) and the TrialNet Oral Insulin Trial (Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group et al. 2017), which was based on a post hoc identified subgroup in the DPT-1 trial, also were both negative. An ongoing oral insulin trial, Fr1da, in 220 children, ages 2–12 years, with two or more diabetes-related autoantibodies, is using a higher dose of oral insulin treatment for 1 year with immunologic endpoints after two additional years of follow-up, and then following immune-positive versus immune-negative children for progression to dysglycemia or diabetes (Raab et al. 2016).

The Diabetes Prediction and Prevention (DIPP) study in Finland studied nasal insulin, but this too was negative (Näntö-Salonen et al. 2008). Another nasal insulin study, INIT-II, is in progress in Australia and should report soon (Trial of Intranasal Insulin in Children and Young Adults at Risk of Type 1 Diabetes (INIT II)). The DIAPREV-IT study, in Sweden, used a GAD-based vaccine and also was negative (Elding Larsson et al. 2017). A follow-up study, DIAPREV-IT 2, also in Sweden, is using the combination of a GAD-based vaccine plus vitamin D3 (Prevention Trial).

Two TrialNet studies using immune interventions, one with the anti-CD3 monoclonal antibody teplizumab (Teplizumab for Prevention of Type 1 Diabetes in Relatives), the other with the co-stimulation blocker abatacept (CTLA-4 Ig), have both nearly completed enrollment with subjects continuing to be followed for development of T1D. TrialNet has two additional prevention studies soon to be initiated. One, Methyldopa for Reduction of DQ8 Antigen Presentation in At-Risk Subjects for Type 1 Diabetes, will enroll 36 subjects, age 8 years or older, who have HLA-DQ8 and insulin autoantibodies, in a cross-over design, that will determine whether methyldopa can reduce DQ8 antigen presentation (Methyldopa for Reduction of DQ8 Antigen Presentation in At-Risk Subjects for Type 1 Diabetes). The other study, Hydroxychloroquine in Individuals At-risk for Type 1 Diabetes Mellitus, will randomize 201 individuals, age 3 years or older, with two or more diabetes-related autoantibodies, with the primary endpoint being development of diabetes or abnormal glucose tolerance (Hydroxychloroquine in Individuals At-risk for Type 1 Diabetes Mellitus).

Tertiary Prevention Studies

These are the most common studies using immune intervention. There have generally been conducted in recent-onset Stage 3 T1D. Initially, many were small pilot studies (Skyler 1987; Skyler and Marks 1993). Beginning in the mid-1980s, most studies have been randomized controlled clinical trials with sample sizes large enough to draw valid conclusions. These have been comprehensively reviewed recently (Skyler 2015; Skyler et al. 2017.). Here will be discussed the most important of these studies.

The first large randomized controlled clinical trials were with cyclosporine and with azathioprine. There were two large cyclosporine trials of 1 year duration – The French Study (Feutren et al. 1986) and the Canadian-European Study (1988). In both studies, there were attempts to stop insulin therapy or reduce insulin dose, with "remissions" being the primary outcome measure. In both cases, more remissions were achieved with drug than placebo, but unfortunately the remissions were not sustained despite continuing use of cyclosporine. An additional French study (Bougneres et al. 1988) that was not controlled (because "two controlled studies already showed benefit) confirmed that transient remissions could be achieved but again were lost despite continuing cyclosporine use for up to 3 years (Bougneres et al. 1990). A smaller cyclosporine study carefully assessed beta cell function and found beneficial effects when evaluated by stimulation with a mixed meal tolerance test but not by challenging with intravenous glucose or glucagon (Skyler et al. 1992).

Three randomized studies were conducted with azathioprine. Two Australian studies, one in adults (Harrison et al. 1985) and one in children (Cook et al. 1989), failed to demonstrate benefit. A third study, in which there was a 10-week course of corticosteroids followed by 1 year of treatment with azathioprine, showed improved beta cell function at 1 year (Silverstein et al. 1988).

The most extensively studied approach has been with anti-CD3 monoclonal antibodies, targeting activation of T-lymphocytes. Two different anti-CD3 monoclonal antibodies have been studied in clinical trials – teplizumab and otelixizumab. The first study with teplizumab was a small study, but it demonstrated sustained improvement of beta cell function for 2 years despite only 14 days of treatment at enrollment (Herold et al. 2002, 2005). The first study with olelixizumab was larger (80 randomized subjects) and had a 6-day course if treatment following randomization, with the primary outcome measure at 18 months, in which there was improvement of beta cell function which was carefully assessed with the hyperglycemic clamp technique (Keymeulen et al. 2005). At 48 months follow-up, although clamps were not done, subjects previously treated with drug had much lower insulin doses than placebo subjects, despite equivalent levels of glucose control as measured by A1c (Keymeulen et al. 2010). Two further Phase 3 studies with otelixizumab failed to show an effect, but were complicated by the fact that the dose was lowered to only one-sixteenth of that used in the original study (Aronson et al. 2014; Ambery et al. 2014).

Several additional studies have been conducted with teplizumab. In the Abate Trial, there was again beneficial effect, but teplizumab treated subjects could be

divided retrospectively into two groups – "responders" and "nonresponders" to treatment (Herold et al. 2013a). Responders were those who maintained C-peptide better than the randomized, but untreated, comparison group at 24 months, and the constituted about half of the treated subjects. Another study, the Delay Trial, enrolled subjects beyond the recent-onset period, between 4 and 12 months after diagnosis (Herold et al. 2013b). Those enrolled between 4 and 8 months showed beneficial effect on beta cell function, whereas those enrolled 9 months or longer after diagnosis did not. Two large Phase 3 trials with teplizumab were conducted. Unfortunately, the arbitrary primary outcome at 1 year required the combination of A1c <6.5% and insulin dose less than 0.5 units/kg/day (Sherry et al. 2011). This was not achieved, although improved beta cell function measured by C-peptide was seen both after 1 year and after 2 years (Hagopian et al. 2013).

The failures of achieving the primary outcome measures in the Phase 3 trials of otelixizumab and teplizumab represent major setbacks to the field, as this therapeutic approach remains promising (Skyler 2013b).

Phase 3 trials have also been conducted with a glutamic acid decarboxylase (GAD)-based vaccine, in which GAD was administered subcutaneously along with an aluminum hydroxide adjuvant (GAD-Alum). These were based on a pilot study which appeared to show benefit in a small subgroup (Ludvigsson et al. 2008). However, the Phase 3 trials failed to show improvement in beta cell function (Ludvigsson et al. 2012; A Phase III Study to Investigate the Impact of Diamyd in Patients Newly Diagnosed With Type 1 Diabetes (USA) – DIAPREVENT), a failure also shown in a large Phase 2 trial (Wherrett et al. 2011).

Another intervention taken to Phase 3 trials was a peptide component of heat-shock protein, a peptide named DiaPep277. The initial pilot study looked promising (Raz et al. 2001). The initial reports of the Phase 3 trial claimed to have benefit, but were retracted for fraud (Raz et al. 2014; Pozzilli et al. 2014).

A number of other immune interventions have shown transient benefit, including rituximab (Pescovitz et al. 2009, 2014), abatacept (Orban et al. 2011, 2014), alefacept (Rigby et al. 2013, 2015), and the combination of thymoglobulin (ATG) and granulocyte colony stimulating factor (Haller et al. 2015, 2016). A small pilot study suggested benefit with etanercept (Mastrandrea et al. 2009). In contrast, several other approaches have failed to show benefit, including mycophenalte mofetil alone or in combination with daclizumab (Gottlieb et al. 2010), interleukin-1-beta blockade with either canakinumab or anakinra (Moran et al. 2013), thymoglobulin alone (Gitelman et al. 2013), and an altered peptide ligand of insulin B-chain (Walter et al. 2009). Another study in recent-onset T1D using nonimmune therapy aimed at improving beta cell function combined sitagliptin and lansoprazole, but without effect (Griffin et al. 2014).

Several small pilot studies have evaluated safety of alpha-1-antitrypsin (Gottlieb et al. 2014), plasmid-encoded proinsulin (Roep et al. 2013), proinsulin peptide (Thrower et al. 2009; Alhadj Ali et al. 2017), low-dose interleukin 2 (Hartemann et al. 2013), insulin B-chain (Orban et al. 2010), dendritic cells (Giannoukakis et al. 2011), and regulatory T-cells (Bluestone et al. 2015). All of these were safe, but there was inadequate data to determine efficacy.

A controversial approach has been the use of autologous hematopoietic stem cell therapy (AHSCT). A Brazilian group conducted an uncontrolled study in young people within 6 weeks of diagnosis and reported that many could cease insulin therapy and had improved beta cell function (Voltarelli et al. 2007; Couri et al. 2009; Voltarelli et al. 2009). Another report summarized additional subjects treated in Poland and China, with similar results, but some morbidity and mortality (D'Addio et al. 2014). These studies all used high dose immunosuppression as well, making it unclear whether any beneficial results were due to the immunosuppression or the stem cells. A subsequent report found that in the Brazilian study, in those with prolonged remission baseline islet-specific T-cell autoreactivity persisted after transplantation, but regulatory T cell counts increased (Malmegrim et al. 2017). Clearly, more work is needed in this arena, including the need for randomized controlled trials.

Overall, the effects of immune intervention in recent-onset Stage 3 T1D have been disappointing. Although some studies have demonstrated transient beneficial effects, none has resulted in long standing persistence in improvement in beta cell function. Success may require the use of an approach that uses combined immunomodulation, perhaps together with agents that improve beta cell health.

References

A Phase III Study to Investigate the Impact of Diamyd in Patients Newly Diagnosed With Type 1 Diabetes (USA) – DIAPREVENT. NCT00751842. www.ClinicalTrials.gov

Alhadj Ali M, Liu YF, Arif S, Tatovic D, Shariff H, Gibson VB, Yusuf N, Baptista R, Eichmann M, Petrov N, Heck S, Yang JHM, Tree TIM, Pujol-Autonell I, Yeo L, Baumard LR, Stenson R, Howell A, Clark A, Boult Z, Powrie J, Adams L, Wong FS, Luzio S, Dunseath G, Green K, O'Keefe A, Bayly G, Thorogood N, Andrews R, Leech N, Joseph F, Nair S, Seal S, Cheung H, Beam C, Hills R, Peakman M, Dayan CM. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. Sci Transl Med. 2017;9(402):pii: eaaf7779, 9 pp.

Ambery P1, Donner TW, Biswas N, Donaldson J, Parkin J, Dayan CM. Efficacy and safety of low-dose otelixizumab anti-CD3 monoclonal antibody in preserving C-peptide secretion in adolescent type 1 diabetes: DEFEND-2, a randomized, placebo-controlled, double-blind, multi-centre study. Diabet Med. 2014;31:399–402.

Aronson R, Gottlieb PA, Christiansen JS, Donner TW, Bosi E, Bode BW, Pozzilli P, for the DEFEND Investigator Group. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. Diabetes Care. 2014;37:2746–54.

Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, Herold KC, Lares A, Lee MR, Li K, Liu W, Long SA, Masiello LM, Nguyen V, Putnam AL, Rieck M, Sayre PH, Tang Q. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med. 2015;7(315)

Bonifacio E, Ziegler AG, Klingensmith G, Schober E, Bingley PJ, Rottenkolber M, Theil A, Eugster A, Puff R, Peplow C, Eisenbarth G, Hasford J, Achenbach P. The pre-POINT study group. Immune efficacy of high dose oral insulin for the primary prevention of type 1 diabetes in genetically-at-risk children: the primary oral insulin therapy (pre-POINT) study, a multicenter, double-blind, randomized, placebo-controlled clinical study. JAMA. 2015;313:1541–9.

Bougneres PF, Carel JC, Castano L, Boitard C, Gardin JP, Landais P, Hors J, Mihatsch MJ, Paillard M, Chaussain JL, Bach JF. Factors associated with early remission of type I diabetes in children treated with cyclosporine. N Engl J Med. 1988;318:663–70.

Bougneres PF, Landais P, Boisson C, Carel JC, Frament N, Boitard C, Chaussain JL, Bach JF. Limited duration of remission of insulin dependency in children with recent overt type I diabetes treated with low-dose cyclosporin. Diabetes. 1990;39:1264–72.

Chase HP, Boulware D, Rodriguez H, Donaldson D, Chritton S, Rafkin LE, Krischer JP, Skyler JS, Clare-Salzler M, The Type 1 Diabetes TrialNet Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Study Group. Effect of docosahexaenoic acid (DHA) supplementation on infant inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes. Pediatr Diabetes. 2015;16:271–9.

Cook JJ, Hudson I, Harrison LC, Dean B, Colman PG, Werther GA, Warne GL, Court JM. Double-blind controlled trial of azathioprine in children with newly diagnosed type I diabetes. Diabetes. 1989;38:779–83.

Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, Madeira MI, Malmegrim KC, Foss-Freitas MC, Simões BP, Martinez EZ, Foss MC, Burt RK, Voltarelli JC. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2009;301:1573–9.

CTLA-4 Ig (Abatacept) for Prevention of Abnormal Glucose Tolerance and Diabetes in Relatives At-Risk for Type 1 Diabetes Mellitus. ClinicalTrials.gov Identifier: NCT01773707. www.ClinicalTrials.gov

D'Addio F, Valderrama Vasquez A, Ben Nasr M, Franek E, Zhu D, Li L, Ning G, Snarski E, Fiorina P. Autologous nonmyeloablative hematopoietic stem cell transplantation in new-onset type 1 diabetes: a multicenter analysis. Diabetes. 2014;63:3041–6.

Diabetes Prevention Trial – Type 1 Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med. 2002;346:1685–91.

Elding Larsson H, Lundgren M, Jonsdottir B, Cuthbertson D, Krischer J, DiAPREV-IT Study Group. Safety and efficacy of autoantigen-specific therapy with 2 doses of alum-formulated glutamate decarboxylase in children with multiple islet autoantibodies and risk for type 1 diabetes: a randomized clinical trial. Pediatr Diabetes. 2017. https://doi.org/10.1111/pedi.12611. On-line ahead of print.

European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. European nicotinamide diabetes intervention trial (ENDIT): a randomized controlled trial of intervention before the onset of type 1 diabetes. Lancet. 2004;363:925–31.

Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS, DPT-1 Study Group. Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes. 2010;59:679–85.

Feutren G, Assan R, Karsenty G, Du Rostu H, Sirmai J, Papoz L, Vialettes B, Vexiau P, Rodier M, Lallemand A, Bach JF, For the Cyclosporin/Diabetes French Study Group. Cyclosporin increases the rate and length of remissions in insulin dependent diabetes of recent onset. Results of a multicentre double-blind trial. Lancet. 1986;2(8499):119–24.

Freder1k-Study – Testing Infants for Type 1 Diabetes Risk. ClinicalTrials.gov Identifier: NCT03316261. www.ClinicalTrials.gov

Gerstein HC. Cow's milk exposure and type I diabetes mellitus. A critical overview of the clinical literature. Diabetes Care. 1994;17:13–21.

Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. Diabetes Care. 2011;34:2026–32.

Gitelman SE, Gottlieb PA, Rigby MR, Felner EI, Willi SM, Fisher LK, Moran A, Gottschalk M, Moore WV, Pinckney A, Keyes-Elstein L, Aggarwal S, Phippard D, Sayre PH, Ding L, Bluestone JA, Ehlers MR, the START Study Team. Antithymocyte globulin therapy for patients with recent-onset type 1 diabetes: a randomized double-blind phase 2 trial. Lancet Diabetes Endocrinol. 2013;1:306–16.

Gottlieb PA, Quinlan S, Krause-Steinrauf H, Greenbaum CJ, Wilson DM, Rodriguez H, Schatz DA, Moran AM, Lachin JM, Skyler JS, The Type 1 Diabetes TrialNet MMF/DZB Study Group. Failure to preserve beta-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new onset type 1 diabetes. Diabetes Care. 2010;33:826–32.

Gottlieb PA, Alkanani AK, Michels AW, Lewis EC, Shapiro L, Dinarello CA, Zipris D. Alpha1-antitrypsin therapy downregulates toll like receptor-induced IL-1β responses in monocytes and myeloid dendritic cells and may improve islet function in recently diagnosed patients with type 1 diabetes. J Clin Endocrinol Metab. 2014;99:E1418–26.

Griffin KJ, Thompson PA, Gottschalk M, Kyllo JH, Rabinovitch A. Combination therapy with sitagliptin and lansoprazole in patients with recent-onset type 1 diabetes (REPAIR-T1D): 12-month results of a multicentre, randomised, placebo-controlled, phase 2 trial. Lancet Diabetes Endocrinol. 2014;2:710–8.

Hagopian W, Ferry RJ Jr, Sherry N, Carlin D, Bonvini E, Johnson S, Stein KE, Koenig S, Daifotis AG, Herold KC, Ludvigsson J, for the Protégé Trial Investigators. Teplizumab preserves C-peptide in recent-onset type 1 diabetes: 2-year results from the randomized, placebo-controlled protege trial. Diabetes 2013; 62:3901-3908.

Haller MJ, Gitelman SE, Gottlieb PA, Michels AW, Rosenthal SM, Shuster JJ, Zou B, Brusko TM, Hulme MA, Wasserfall CH, Mathews CE, Atkinson MA, Schatz DA. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. J Clin Investig. 2015;2015(125):448–55.

Haller MJ, Gitelman SE, Gottlieb PA, Michels AW, Perry DJ, Schultz AR, Hulme MA, Shuster JJ, Zou B, Wasserfall CH, Posgai AL, Mathews CE, Brusko TM, Atkinson MA, Schatz DA. Antithymocyte globulin plus G-CSF combination therapy leads to sustained immunomodulatory and metabolic effects in a subset of responders with established type 1 diabetes. Diabetes. 2016;65:3765–75.

Harrison LC, Colman PG, Dean B, Baxter R, Martin FI. Increase in remission rate in newly diagnosed type I diabetic subjects treated with azathioprine. Diabetes. 1985;34:1306–8.

Hartemann A, Bensimon G, Payan CA, Jacqueminet S, Bourron O, Nicolas N, Fonfrede M, Rosenzwajg M, Bernard C, Klatzmann D. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2013;1:295–305.

Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med. 2002;346:1692–8.

Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, Rother K, Diamond B, Harlan DM, Bluestone JA. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes. 2005;54:1763–9.

Herold KC, Gitelman SE, Ehlers MR, Gottlieb PA, Greenbaum CJ, Hagopian W, Boyle KD, Keyes-Elstein L, Aggarwal S, Phippard D, Sayre PH, McNamara J, Bluestone JA, the AbATE Study Team. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. Diabetes. 2013a;62:3766–74.

Herold KC, Gitelman SE, Willi SM, Gottlieb PA, Waldron-Lynch F, Devine L, Sherr J, Rosenthal SM, Adi S, Jalaludin MY, Michels AW, Dziura J, Bluestone JA. Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. Diabetologia. 2013b;56:391–400.

Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care. 2011;34:1301–5.

Hydroxychloroquine in Individuals At-risk for Type 1 Diabetes Mellitus. ClinicalTrials.gov Identifier: NCT03428945. www.ClinicalTrials.gov

Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark Å, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015;38: 1964–74.

Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med. 2005;352:2598–608.

Keymeulen B, Walter M, Mathieu C, Kaufman L, Gorus F, Hilbrands R, Vandemeulebroucke E, Van de Velde U, Crenier L, De Block C, Candon S, Waldmann H, Ziegler AG, Chatenoud L, Pipeleers D. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. Diabetologia. 2010;53:614–23.

Knip M, Virtanen SM, Seppä K, Ilonen J, Savilahti E, Vaarala O, Reunanen A, Teramo K, Hämäläinen AM, Paronen J, Dosch HM, Hakulinen T, Akerblom HK, Finnish TRIGR Study Group. Dietary intervention in infancy and later signs of beta-cell autoimmunity. N Engl J Med. 2010;363:1900–8.

Knip M, Åkerblom HK, Becker D, Dosch HM, Dupre J, Fraser W, Howard N, Ilonen J, Krischer JP, Kordonouri O, Lawson ML, Palmer JP, Savilahti E, Vaarala O, Virtanen SM, TRIGR Study Group. Hydrolyzed infant formula and early β-cell autoimmunity: a randomized clinical trial. JAMA. 2014;311:2279–87.

Lampeter EF, Klinghammer A, Scherbaum WA, Heinze E, Haastert B, Giani G, Kolb H. The deutsche nicotinamide intervention study: an attempt to prevent type 1 diabetes. DENIS group. Diabetes. 1998;47:980–4.

Ludvigsson J, Faresjö M, Hjorth M, Axelsson S, Chéramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Ortqvist E, Zerhouni P, Casas R. GAD treatment and insulin secretion in recent-onset type 1 diabetes. N Engl J Med. 2008;359:1909–20.

Ludvigsson J, Krisky D, Casas R, Battelino T, Castaño L, Greening J, Kordonouri O, Otonkoski T, Pozzilli P, Robert JJ, Veeze HJ, Palmer J. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. N Engl J Med. 2012;366:433–42.

Malmegrim KC, de Azevedo JT, Arruda LC, Abreu JR, Couri CE, de Oliveira GL, Palma PV, Scortegagna GT, Stracieri AB, Moraes DA, Dias JB, Pieroni F, Cunha R, Guilherme L, Santos NM8, Foss MC, Covas DT, Burt RK, Simões BP, Voltarelli JC, Roep BO, Oliveira MC. Immunological Balance Is Associated with clinical outcome after autologous hematopoietic stem cell transplantation in type 1 diabetes. Front Immunol 2017; 8:167.

Mastrandrea L, Yu J, Behrens T, Buchlis J, Albini C, Fourtner S, Quattrin T. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. Diabetes Care. 2009;32:1244–9.

Methyldopa for Reduction of DQ8 Antigen Presentation in At-Risk Subjects for Type 1 Diabetes. ClinicalTrials.gov Identifier: NCT03396484. www.ClinicalTrials.gov

Moran A, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Greenbaum CJ, Herold KC, Marks JB, Raskin P, Sanda S, Schatz D, Wherrett D, Wilson DM, Skyler JS, The Type 1 Diabetes TrialNet Canakinumab Study Group, Pickersgill L, de Koning E, Ziegler A-G, Böehm B, Badenhoop K, Schloot N, Bak JF, Pozzilli P, Mauricio D, Donath MY, Castaño L, Wägner A, Lervang HH, Perrild H, Mandrup-Poulsen T, on behalf of the AIDA Study Group. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicenter, randomized double-masked, placebo-controlled trials. Lancet. 2013;381:1905–15.

Näntö-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkala A, Korhonen S, Erkkola R, Sipilä JI, Haavisto L, Siltala M, Tuominen J, Hakalax J, Hyöty H, Ilonen J, Veijola R, Simell T, Knip M, Simell O. Nasal insulin to prevent type 1 diabetes in children

with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. Lancet. 2008;372:1746–55.

Orban T, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G. Diabetes prevention trial-type 1 study group. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the diabetes prevention trial-type 1. Diabetes Care. 2009;32:2269–74.

Orban T, Farkas K, Jalahej H, Kis J, Treszl A, Falk B, Reijonen H, Wolfsdorf J, Ricker A, Matthews JB, Tchao N, Sayre P, Bianchine P. Autoantigen-specific regulatory T cells induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy. J Autoimmun. 2010;34:408–15.

Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Skyler JS, The Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised double-blind, placebo-controlled trial. Lancet. 2011;378:412–9.

Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Peakman M, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Skyler JS, The Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up one year after cessation of treatment. Diabetes Care. 2014;37:1069–75.

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Lachin JM, Skyler JS, The Type 1 Diabetes TrialNet Anti-CD20 Study Group. Rituximab, B-lymphocyte depletion and preservation of Beta-cell function. N Engl J Med. 2009;361:2143–52.

Pescovitz MD, Greenbaum CJ, Bundy BN, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Skyler JS, The Type 1 Diabetes TrialNet Anti-CD20 Study Group. B-lymphocyte depletion with rituximab and Beta-cell function: two-year results. Diabetes Care. 2014;37:453–9.

PINIT Study: Primary Intranasal Insulin Trial. ClinicalTrials.gov Identifier: NCT03182322. www.ClinicalTrials.gov

Pozzilli P, Raz I, Peled D, Elias D, Avron A, Tamir M, Eren R, Dagan S, Cohen IR. Evaluation of long-term treatment effect in a type 1 diabetes intervention trial: differences after stimulation with glucagon or a mixed-meal. Diabetes Care. 2014;37:1384–91. Statement of Retraction. Diabetes Care 2015; 38:179.

Pre-POINT Early Study. ClinicalTrials.gov Identifier: NCT02547519. www.ClinicalTrials.gov

Prevention Trial: Immune-tolerance With Alum-GAD (Diamyd) and Vitamin D3 to Children With Multiple Islet Autoantibodies (DiAPREV-IT2). ClinicalTrials.gov Identifier: NCT02387164. www.ClinicalTrials.gov

Raab J, Haupt F, Scholz M, Matzke C, Warncke K, Lange K, Assfalg R, Weininger K, Wittich S, Löbner S, Beyerlein A, Nennstiel-Ratzel U, Lang M, Laub O, Dunstheimer D, Bonifacio E, Achenbach P, Winkler C, Ziegler AG, Fr1da Study Group. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. BMJ Open. 2016;6(5):e011144.

Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. ß-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet. 2001;358:1749–53.

Raz I, Ziegler AG, Linn T, Schernthaner G, Bonnici F, Distiller LA, Giordano C, Giorgino F, de Vries L, Mauricio D, ProchaÅLzka V, Wainstein J, Elias D, Avron A, Tamir M, Eren R, Peled D, Dagan S, Cohen IR, Pozzilli P, The DIA-AID 1 writing group. Treatment of recent onset type 1 diabetes patients with DiaPep277: results of a double-blind, placebo-controlled, randomized phase 3 trial. Diabetes Care. 2014;37:1392–400. Statement of Retraction. Diabetes Care 2015; 38:178.

Rigby MR, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, Patel CM, Griffin KJ, Tsalikian E, Gottlieb PA, Greenbaum CJ, Sherry NA, Moore WV, Monzavi R, Willi SM, Raskin P, Moran A, Russell WE, Pinckney A, Keyes-Elstein L, Howell M, Aggarwal S, Lim N, Phippard D, Nepom GT, McNamara J, Ehlers MR, The T1DAL Study Team. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Diabetes Endocrinol. 2013;1:284–94.

Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, Griffin KJ, Tsalikian E, Gottlieb PA, Greenbaum CJ, Sherry NA, Moore WV, Monzavi R, Willi SM, Raskin P, Keyes-Elstein L, Long SA, Kanaparthi S, Lim N, Phippard D, Soppe CL, Fitzgibbon ML, McNamara J, Nepom GT, Ehlers MR. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. J Clin Investig. 2015;125:3285–96.

Roep BO, Solvason N, Gottlieb PA, Abreu JR, Harrison LC, Eisenbarth GS, Yu L, Leviten M, Hagopian WA, Buse JB, von Herrath M, Quan J, King RS, Robinson WH, Utz PJ, Garren H, BHT-3021 Investigators, Steinman L. Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific $CD8^+$ T cells in type 1 diabetes. Sci Transl Med. 2013;5 (191):191ra82.

Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ Jr, Bode B, Aronoff S, Holland C, Carlin D, King KL, Wilder RL, Pillemer S, Bonvini E, Johnson S, Stein KE, Koenig S, Herold KC, Daifotis AG, Protégé Trial Investigators. Teplizumab for treatment of type 1 diabetes (protégé study): 1-year results from a randomised, placebo-controlled trial. Lancet 2011; 378:487-497.

Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. N Engl J Med. 1988;319:599–604.

Skyler JS. Immune intervention studies in insulin-dependent diabetes mellitus. Diabetes Metab Rev. 1987;3:1017–35.

Skyler JS. Primary and secondary prevention of type 1 diabetes. Diabet Med. 2013a;30:161–9.

Skyler JS. The compelling case for anti-CD3 in type 1 diabetes. Diabetes. 2013b;62:3656–7.

Skyler JS. The prevention & reversal of type 1 diabetes – past challenges & future opportunities. Diabetes Care. 2015;38:997–1007.

Skyler JS, Marks JB. Immune intervention in type I diabetes mellitus. Diabetes Rev. 1993;1:15–42.

Skyler JS, Rabinovitch A, The Miami Cyclosporine Diabetes Study Group. Cyclosporine in recent onset type I diabetes mellitus: effects on islet beta cell function. J Diabetes Complicat. 1992;6:77–88.

Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E, Diabetes Prevention Trial – Type 1 Diabetes Study Group. Effects of oral insulin in relatives of patients with type 1 diabetes mellitus. Diabetes Care. 2005;28:1068–76.

Skyler JS, Krischer JP, Becker DJ, Rewers M. Prevention of type 1 diabetes, Chapter 37. In: Cowie CC, Casagrande SS, Menke A, Cissell MA, Eberhardt MS, Meigs JB, Gregg EW, Knowler WC, Barrett-Connor E, Becker DJ, Brancati FL, Boyko EJ, Herman WH, Howard BV, KMV N, Rewers M, Fradkin JE, editors. Diabetes in America. 3rd ed. Bethesda: National Institutes of Health, NIH Pub No. 17-1468; 2017. p. 37.1–21.

Sosenko JM, Skyler JS, Herold KC, Palmer JP, Type 1 Diabetes TrialNet and Diabetes Prevention Trial–Type 1 Study Groups. The metabolic progression to type 1 diabetes as indicated by serial oral glucose tolerance testing in the diabetes prevention trial-type 1. Diabetes. 2012;61:1331–7.

Teplizumab for Prevention of Type 1 Diabetes in Relatives "At-Risk". ClinicalTrials.gov Identifier: NCT01030861. www.ClinicalTrials.gov

The Canadian-European Randomized Control Trial Group. Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. Diabetes. 1988;37:1574–82.

Thrower SL, James L, Hall W, Green KM, Arif S, Allen JS, Van-Krinks C, Lozanoska-Ochser B, Marquesini L, Brown S, Wong FS, Dayan CM, Peakman M. Proinsulin peptide immunotherapy in type 1 diabetes: report of a first-in-man phase I safety study. Clin Exp Immunol. 2009;155:156–65.

Trial of Intranasal Insulin in Children and Young Adults at Risk of Type 1 Diabetes (INIT II). ClinicalTrials.gov Identifier: NCT00336674. www.ClinicalTrials.gov

TRIGR Study Group, Akerblom HK, Krischer J, Virtanen SM, Berseth C, Becker D, Dupré J, Ilonen J, Trucco M, Savilahti E, Koski K, Pajakkala E, Fransiscus M, Lough G, Bradley B, Koski M, Knip M. The trial to reduce IDDM in the genetically at risk (TRIGR) study: recruitment, intervention and follow-up. Diabetologia. 2011;54:627–33.

Vaarala O, Ilonen J, Ruohtula T, Pesola J, Virtanen SM, Härkönen T, Koski M, Kallioinen H, Tossavainen O, Poussa T, Järvenpää AL, Komulainen J, Lounamaa R, Akerblom HK, Knip M. Removal of bovine insulin from cow's milk formula and early initiation of beta-cell autoimmunity in the FINDIA pilot study. Arch Pediatr Adolesc Med. 2012;166:608–14.

Vandemeulebroucke E, Gorus F, Decochez K, Weets I, Keymeulen B, De Block C, Tits J, Pipeleers D, Mathieu C, The Belgian Diabetes Registry. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. Diabetes Metab. 2009;35:319–27.

Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KC, Foss-Freitas MC, Simões BP, Foss MC, Squiers E, Burt RK. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2007;297:1568–76.

Voltarelli JC, Martinez ED, Burt RK. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. Author's reply. JAMA. 2009;302:624–5.

Walter M, Philotheou A, Bonnici F, Ziegler AG, Jimenez R. NBI-6024 study group. No effect of the altered peptide ligand NBI-6024 on beta-cell residual function and insulin needs in new-onset type 1 diabetes. Diabetes Care. 2009;32:2036–40.

Wherrett DK, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Herold KC, Marks JB, Monzavi R, Moran A, Orban T, Raskin P, Rodriguez H, Russell WE, Schatz D, Wilson DM, Skyler JS, The Type 1 Diabetes TrialNet GAD Study Group. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. Lancet. 2011;378:319–27.

Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group, Krischer JP, Schatz DA, Bundy B, Skyler JS, Greenbaum CJ. Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. JAMA. 2017;318:1891–902.

Writing Group for the TRIGR Study Group, Knip M, Åkerblom HK, Al Taji E, Becker D, Bruining J, Castano L, Danne T, de Beaufort C, Dosch HM, Dupre J, Fraser WD, Howard N, Ilonen J, Konrad D, Kordonouri O, Krischer JP, Lawson ML, Ludvigsson J, Madacsy L, Mahon JL, Ormisson A, Palmer JP, Pozzilli P, Savilahti E, Serrano-Rios M, Songini M, Taback S, Vaarala O, White NH, Virtanen SM, Wasikowa R. Effect of hydrolyzed infant formula vs conventional formula on risk of type 1 diabetes: the TRIGR randomized clinical trial. JAMA. 2018;319:38–48.

Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. Immunity. 2010;32:468–78.

Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309:2473–9.

Ziegler AG, Danne T, Dunger DB, Berner R, Puff R, Kiess W, Agiostratidou G, Todd JA, Bonifacio E. Primary prevention of beta-cell autoimmunity and type 1 diabetes – the global platform for the prevention of autoimmune diabetes (GPPAD) perspectives. Mol Metab. 2016;5:255–62.

Prevention of Type 2 Diabetes

15

William C. Knowler

Contents

Abstract

Diabetes is defined by elevated plasma glucose concentrations and characterized by metabolic disturbances and widespread tissue damage. Diagnostic criteria and classification of types of diabetes and the risk factors for T2DM are described in other chapters of this book. This chapter considers only T2DM. Diagnostic cut-points for diabetes have often been chosen to correspond to degrees of hyperglycemia associated with diabetes complications, usually retinopathy or nephropathy. Thus, it is widely believed that preventing increases in hyperglycemia to levels that are diagnostic of diabetes and associated with development of complications will also prevent development of the complications. It is also hypothesized that preventing diabetes complications is more feasible in this way than by postponing interventions until the disease is diagnosed, at which time some tissue damage may have already occurred and hyperglycemia may be more difficult to control. While

W. C. Knowler (✉)
Diabetes Epidemiology and Clinical Research Section, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, AZ, USA
e-mail: knowler@nih.gov

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology, https://doi.org/10.1007/978-3-319-45015-5_16

these are very natural assumptions, evidence supporting them has been difficult to obtain. The assumption that preventing T2DM will also prevent its complications provides the main justification for the concept that it is better to prevent diabetes than to wait until it develops and then treat it.

Keywords
Type 2 diabetes · Prevention

Introduction

Randomized clinical trials in prevention of type 2 diabetes mellitus (T2DM) began as early as the 1960s. This chapter reviews randomized clinical trials in prevention of T2DM since that time. While not comprehensive, this review includes clinical trials with historical interest and a large impact on research in diabetes prevention. I also discuss treatment effects on long-term outcomes beyond diabetes itself. This chapter does not cover nonrandomized prevention activities, bariatric surgery, or randomized clinical trials that aim to prevent or delay type 1 diabetes. This chapter is based, on part, on previous commentaries and reviews I have co-authored with others (Knowler et al. 1995; Crandall et al. 2008; Knowler et al. in press) that include more details on some of the randomized clinical trials described in this chapter.

Why Is Preventing T2DM Important and Feasible?

Diabetes is defined by elevated plasma glucose concentrations and characterized by metabolic disturbances and widespread tissue damage. Diagnostic criteria and classification of types of diabetes and the risk factors for T2DM are described in other chapters of this book. This chapter considers only T2DM. Diagnostic cut-points for diabetes have often been chosen to correspond to degrees of hyperglycemia associated with diabetes complications, usually retinopathy or nephropathy. Thus, it is widely believed that preventing increases in hyperglycemia to levels that are diagnostic of diabetes and associated with development of complications will also prevent development of the complications. It is also hypothesized that preventing diabetes complications is more feasible in this way than by postponing interventions until the disease is diagnosed, at which time some tissue damage may have already occurred and hyperglycemia may be more difficult to control. While these are very natural assumptions, evidence supporting them has been difficult to obtain, as discussed later. The assumption that preventing T2DM will also prevent its complications provides the main justification for the concept that it is better to prevent diabetes than to wait until it develops and then treat it.

There has been disagreement over the years as to precise diagnostic criteria, but all widely accepted criteria have been based on some degree of hyperglycemia. The disagreements in diagnostic criteria derive from the continuity of glucose concentrations, both among people and within one person over time. T2DM has a long stage

of development that varies among people. The development of T2DM in an individual over time also appears to be a continuous process, although defining its nature precisely would require frequent (or continuous) measurement of glycemia over a lifetime in persons who develop T2DM. Most longitudinal studies of serial glucose measurements starting in normoglycemic persons measured glucose in intervals of 2 years or more (Mason et al. 2007; Tabak et al. 2009), thus being unable to define the precise trajectory of glucose concentrations prior to their increasing to diagnostic levels. These studies, however, suggest that glucose concentrations may be stable or gradually increasing for many years, with an increasing slope of glycemia over time in the few years prior to diagnosis. There is large variation among persons, however, in these patterns prior to onset of T2DM.

Such patterns of increasing glycemia are relevant for diabetes prevention, because they suggest the possibility of identifying persons on the way to developing T2DM before the disease is diagnosed. Such persons, whether identified by elevated glucose concentrations or other predictive factors, could be considered at high risk of T2DM, a concept allowing for the "high-risk" approach to T2DM prevention – identification and risk factor modification of persons at high risk. The vast majority of published T2DM prevention research, summarized below, has taken the "high-risk" approach. This approach assumes that the greatest benefit (i.e., most cases prevented) with the least cost and harm comes from treating persons at greatest risk where resources can be concentrated. This is likely true when preventive interventions are delivered to individuals, such as through counseling for lifestyle changes or giving medicines. Some argue, however, that the most benefit can come from "population" approaches in which interventions are designed to decrease risk factors in large numbers of the population without targeting individuals.

Population Approach to Prevention

Population interventions might, for example, aim to decrease body weight or increase physical activity in large numbers of people, thus decreasing their risk of developing T2DM. Examples of such interventions involve changes in the built environment that would encourage walking or cycling rather than vehicular transportation or food taxation and subsidization to encourage shifts in consumption from perceived unhealthy foods (such as high simple carbohydrate) to more healthy foods (such as high-fiber complex carbohydrates). Population approaches are described elsewhere (White 2016; Wareham and Herman 2016; Batis et al. 2016; Stevenson et al. 2016) but are not covered in this chapter.

As promising as these approaches are, research in this area has made limited progress because population interventions are difficult to implement and difficult to evaluate. Changes in the built environment generally require political action and may require large economic investment. They presumably fall outside the realm of medical expertise of most readers of this book. Population approaches are also difficult to evaluate (Ackermann et al. 2013, 2015; Knowler and Ackermann 2013).

Population approaches may have the greatest potential to prevent the largest numbers of cases of T2DM. For example, if obesity and sedentary behaviors could be eliminated through changes in food availability and the man-made environment (such as transportation systems and buildings), the incidence of T2DM should be decreased. It remains unknown, however, to what extent making and evaluating such changes is possible.

Individual Approaches to Prevention

Nearly all prevention trials conducted among individuals followed a "high risk" strategy rather than targeting members of the population at large. This has been necessary for practical reasons in that the power to detect treatment effects in a clinical trial depends in part on the number of events (incident diabetes cases) observed. Most trials identified high-risk persons who had impaired glucose tolerance (IGT) during an oral glucose tolerance test (OGTT), with or without requiring other high-risk characteristics such as obesity or elevated fasting plasma glucose (FPG). I am aware of only one major randomized clinical trial that used FPG as its major eligibility criterion (Saito et al. 2011) and none that used nonglycemic risk factors alone. Therefore, there is little information on the effectiveness of preventive interventions in persons who do not have IGT.

The randomized clinical trials of preventive interventions have tested a variety of lifestyle changes involving some combination of dietary change and increased physical activity, various drugs aimed at preventing increasing glucose concentrations or decreasing weight, or combinations of diet, physical activity, and drugs.

Lifestyle Modification Interventions, With or Without Drug Arms

Several randomized clinical trials formally tested whether modifying recognized risk factors for T2DM, namely, lifestyle modification directed at weight loss and/or increased physical activity or exercise, could prevent or delay T2DM.

Da Qing Randomized Clinical Trial of Lifestyle Modification (1997)

The Da Qing study was a cluster-randomized two-by-two factorial clinical trial evaluating four combinations of diet and exercise interventions given for 6 years (Pan et al. 1997). Participants had IGT by 1985 World Health Organization (WHO) criteria (WHO 1985). Interventions were randomly assigned by clinic (33 clusters). The four intervention arms included a program of dietary modification, exercise, both, or neither (the control group). The dietary intervention included increased consumption of vegetables, reduced alcohol, and simple carbohydrates, and, if BMI $\geq$25 kg/m^2, limited total energy intake. The exercise-only intervention was to increase physical activity by at least 20 min per day of brisk walking or equivalent activity. The 6-year cumulative incidence of diabetes was 48% in the diet-only group, 41% in the exercise-only group, 46% in the diet plus exercise group, and 68% in the control group. The incidence rates in cases/100 person-years were 8.3, 5.1, 6.8, and 13.2 in the same four groups.

The interventions lasted 6 years, after which active treatment and formal follow-up were discontinued. Follow-up data were obtained by examination and record review 23 years after randomization. The four randomized groups were collapsed into a comparison of the control group (8 clusters) with the pooled three groups with diet, exercise, or both interventions (25 clusters). Annual incidence rates decreased during long-term follow-up, probably because of less frequent glucose tolerance testing or earlier development of diabetes in the persons at highest risk. Over the entire 23-year period, diabetes incidence rates in the combined intervention groups (diet, exercise, or both) were 0.55 (95% CI = 0.40–0.76) times the incidence rate in the control group (Li et al. 2014).

The study also reported effects on retinopathy, nephropathy, and death rates. Twenty years after randomization, the pooled intervention groups had a 47% reduction in severe retinopathy (hazard ratio = 0.53, 95% CI = 0.29–0.99) (Gong et al. 2011). The hazard ratio for nephropathy was 1.05, 95% CI = 0.16–7.05, which was inconclusive because of the wide confidence interval. The all-cause mortality rates during 23 years of follow-up were reduced by 54% in women, with no effect in men (Li et al. 2014). Limitations of this study included the cluster randomization and variable schedules of follow-up over time.

The Finnish Diabetes Prevention Study (DPS) (2001)

The Finnish DPS (Tuomilehto et al. 2001) was a randomized clinical trial of 522 overweight or obese, middle-aged adults (mean age 55 years) with IGT according to the 1985 WHO criteria (WHO 1985). Participants were randomly assigned to a lifestyle (diet and exercise) intervention or a control group. The lifestyle intervention participants were instructed to reduce fat intake and increase consumption of fiber, whole grains, vegetables, and low-fat dairy products, with a goal of losing at least 5% of body weight. They were also encouraged to participate in moderate-intensity exercise for at least 30 min per day. End-of-study data were available from 92% of the participants after an average follow-up of 4 years. The intervention and control groups lost an average of 4.2 kg and 0.8 kg in the first year of the study. Diabetes incidence was 58% lower in the lifestyle intervention group (32 cases/1000 person-years) than in the control group (78 cases/1000 person-years).

The lower diabetes incidence in the lifestyle group persisted during 9 additional years of follow-up after the end of the intervention (for 13 years after randomization). During the total follow-up, the adjusted hazard ratio for diabetes (intervention group vs. control group) was 0.61 (95% CI = 0.48–0.79) (Lindström et al. 2013), suggesting that the active intervention had somewhat persistent effects. The corresponding hazard ratio during the postintervention follow-up was 0.67 (95% CI = 0.48–0.95).

Compared with the control group, the lifestyle intervention group had a nonsignificantly lower mortality rate (hazard ratio = 0.57, 95% CI = 0.21–1.58) after 10 years of follow-up, but similar cardiovascular morbidity (hazard ratio = 1.04, 95% CI = 0.72–1.51) (Uusitupa et al. 2009). These results suggested a mortality benefit, but with a sample of only 522 persons and resulting wide confidence intervals, the mortality results were inconclusive.

The US Diabetes Prevention Program (DPP) (2002)

The US Diabetes Prevention Program (DPP) was a large prevention randomized clinical trial testing both a lifestyle and a drug intervention (DPP 2002). The trial enrolled 3234 nondiabetic, overweight or obese, mostly middle-aged adults with IGT and FPG values of 95 mg/dl (5.3 mmol/l) to <126 mg/dl (7.0 mmol/l). There were minor variations in eligibility criteria by clinical center, race, and time. The three randomly assigned interventions were an intensive lifestyle modification program, metformin (850 mg twice a day), and placebo. The metformin and placebo groups received printed material containing standard lifestyle recommendations. The participants were racially/ethnically diverse, with 45% recruited from racial/ethnic and age groups at particularly high risk of diabetes (African Americans, Hispanic Americans, American Indians, and Asian Americans). Mean age at baseline was 51 years and mean BMI was 34 kg/m^2.

The main goal of the intensive lifestyle intervention was 7% loss of body weight over 24 weeks with long-term maintenance. Participants were instructed engage in at least 150 min of moderate-intensity physical activity (such as brisk walking) per week and to eat a low-fat, reduced-calorie diet. The lifestyle-intervention group achieved a mean weight loss of 7% (an average of 7.0 kg) within the first year and had an overall mean weight loss of 5.6% (an average of 5.6 kg) during a mean follow-up of 2.8 years.

The initial phase of the trial was stopped in 2001, before the planned end-date, on the advice of the data and safety monitoring board because of the clear benefits of both interventions on development of diabetes. The lifestyle intervention led to a 58% reduction (95% CI = 48–60%) in diabetes incidence, based on annual OGTTs and mid-year FPG levels, compared with placebo plus standard lifestyle recommendations (DPP 2002). Diabetes risk reduction was related to the amount of weight lost (DPP 2006).

The metformin arm experienced a 31% lower diabetes incidence, compared with placebo, during the mean follow-up of 2.8 years. This was accompanied by a modest weight loss of 1.7 kg, compared with a 0.3 kg gain in the placebo group. An estimated 64% of the beneficial effect of metformin on diabetes risk was attributed to weight loss (DPP 2007). Improved estimated insulin sensitivity was also associated with reduced diabetes risk (DPP 2005b).

In a secondary analysis of history of gestational diabetes, women reporting a history of gestational diabetes were compared with women who had given birth at least once but had no history of gestational diabetes. The women with prior gestational diabetes had an especially high risk of developing diabetes in the DPP. Metformin was more effective in these women (50% reduction in incidence compared with placebo) compared to its insignificant 14% risk reduction in parous women without a history of gestational diabetes. By contrast, the lifestyle intervention had similar benefits in those with a history of gestational diabetes (53% reduction compared with placebo) or without such a history (49% reduction) (DPP 2008).

In addition to the 3234 participants randomly assigned to the placebo, metformin, or lifestyle interventions, 585 were randomly assigned to the thiozolidenedione drug troglitazone. This study arm was terminated early when the potential hepatic toxicity of troglitazone became known (DPP 2005a). During the average of 0.9 years of its

use in DPP, troglitazone reduced the incidence of diabetes by 75% compared with placebo – the largest risk reduction of all the DPP interventions among the subset of participants randomized when troglitazone was being used in the DPP. Whether the reduction in incidence would have persisted, had troglitazone therapy been continued, could not be determined. Other randomized clinical trials of thiazolidinediones, however, have been effective in diabetes prevention (see below).

Following unmasking and publication of the primary DPP results (DPP 2002), all participants, regardless of randomized study group, were offered a group-implemented lifestyle intervention because the lifestyle intervention had been the most effective intervention in the DPP. Placebo was discontinued, and unmasked metformin was continued as a study intervention in the original metformin group during the long-term follow-up study, named the Diabetes Prevention Program Outcomes Study (DPPOS) (DPP 2009). Eighty-eight percent of the surviving DPP cohort enrolled in DPPOS.

During the DPPOS, annual diabetes incidence rates in the former placebo and metformin groups fell to approximately equal those in the former lifestyle group, but the cumulative incidence of diabetes remained lowest in the former lifestyle group. Despite the convergence of annual incidence rates during long-term follow-up, the large difference in rates during the active intervention phase resulted in persistent differences between treatment groups during follow-up. During a mean follow-up of 15 years since DPP randomization, diabetes incidence was reduced by 27% in the lifestyle intervention group (hazard ratio = 0.73, 95% CI = 0.65–0.83; $p < 0.0001$) and by 18% in the metformin group (hazard ratio = 0.82, 0.72–0.93; $p = 0.001$), compared with the placebo group. At year 15, the cumulative incidences of diabetes were 55% in the lifestyle group, 56% in the metformin group, and 62% in the placebo group (DPP 2015a).

Other effects seen in the active intervention phase persisted during the DPPOS. For example, over 10 years since randomization, women with a history of gestational diabetes assigned to placebo had a 48% higher risk of developing diabetes compared with women without a history of gestational diabetes who reported at least one delivery. In women with a history of gestational diabetes, the lifestyle and metformin interventions reduced progression to diabetes compared with placebo by 35% and 40%, respectively. Among the women without a history of gestational diabetes, the lifestyle intervention reduced the progression to diabetes by 30%, and metformin did not significantly reduce the progression to diabetes (DPP 2015b).

Eligibility for DPP enrollment was based on fasting and 2-h postload plasma glucose concentration, in addition to BMI and other factors. HbA_{1c} was measured but not used in determining eligibility or defining the primary outcome of diabetes. All DPP participants were judged to be at high risk of developing diabetes by virtue of elevated fasting and 2-h glucose concentrations and BMI $\geq$24 kg/m^2. Nevertheless, baseline HbA_{1c} was an additional predictor of diabetes. After excluding the few participants with $HbA_{1c} > 6.5\%$ at study entry, treatment effects were evaluated in a post hoc analysis with an alternate diabetes definition of $HbA_{1c} \geq 6.5\%$. Metformin and lifestyle interventions were both effective, compared with placebo, in preventing this outcome, and their effects did not differ significantly from each other (DPP 2015c).

Extended follow-up in the DPPOS examines diabetes incidence and long-term outcomes of diabetes and its complications (DPP 2009, 2015a), although incidence of cardiovascular events and mortality rates have not yet been reported. After an average of 15 years since randomization, DPP participants were evaluated for a composite microvascular/neuropathy outcome defined by the average prevalence of diabetic retinopathy, nephropathy, and neuropathy (DPP 2015a). Retinopathy was assessed by central grading of retinal photographs, nephropathy by albuminuria or estimate glomerular filtration rate, and neuropathy by light touch sensation. There were no significant treatment effects overall, but significant sex by treatment interactions, such that in women only, the composite prevalence of complications was ~22% lower in the lifestyle intervention group than in the placebo or metformin treatment groups. Those who had not developed diabetes had a 28% lower prevalence of complications than those who had developed diabetes.

Additional evidence for long-term benefit comes from the 10-year cost-effective analysis of the DPP interventions. Costs of delivering the interventions and costs of medical care outside of the study were estimated from participant reports of hospitalizations, outpatient visits, and drug costs. The lifestyle intervention was estimated to be cost-effective (costing $10,037 per quality adjusted life year gained over the placebo group), and the metformin intervention was estimated to save costs (DPP 2012). Such an analysis may reflect aspects of health that are not captured by the study's assessments of diabetes and its complications.

Lifestyle Intervention in Japanese Men with IGT (2005)

A lifestyle intervention randomized clinical trial was conducted Japanese men with IGT who were recruited at health screening examinations. The mean BMI was 24 kg/m^2, lower than in European and US trials. They were randomly assigned in an approximately 4:1 ratio to a standard intervention group ($n = 356$) or to an intensive weight loss group ($n = 102$) and followed for 4 years. Diabetes incidence was defined by at least two consecutive FPG concentrations of at least 140 mg/dl (7.8 mmol/l), i.e., not by an OGTT as was done in most other diabetes prevention trials. Diabetes incidence was reduced by 67% by the weight loss intervention (Kosaka et al. 2005). Although these results are consistent with those of other lifestyle intervention trials, this study is difficult to compare with the others because of different inclusion criteria and outcome definition.

The Indian Diabetes Prevention Program (IDDP, 2006)

The IDPP extended the findings of US DPP by (1) enrolling 531 Asian Indians who were younger and had lower BMI, on average, then volunteers in the DPP, and (2) testing a lifestyle intervention and metformin as in the DPP, but including a combined lifestyle and metformin intervention group (Ramachandran et al. 2006). At study entry, participants (420 men and 111 women) had mean age of 46 years and mean BMI was 26 kg/m^2. The metformin dose (250–500 mg twice per day) was substantially lower than the dose of 850 mg twice per day used in the DPP. Study volunteers were followed an average of 30 months, during which time cumulative incidence rates of diabetes were 55.0% (control group), 39.3% (lifestyle

modification group), 40.5% (metformin group), and 39.5% (lifestyle modification plus metformin group). The relative risk reductions were 28.5% (95% CI 20.5–37.3, $p = 0.018$) in the lifestyle modification group, 26.4% (95% CI 19.1–35.1, $p = 0.029$) in the metformin group, and 28.2% (95% CI 20.3–37.0, $p = 0.022$) in the lifestyle modification plus metformin group, compared with the control group. Thus, both the lifestyle modification and metformin interventions reduced diabetes incidence, but their effects were not additive. The risk reductions were lower than in the DPP, perhaps because the interventions were less intense.

Lifestyle Intervention in Japanese Men with Impaired Fasting Glucose (2011)

A Japanese randomized clinical trial enrolled 641 overweight Japanese participants (72% were men) in a lifestyle intervention trial (Saito et al. 2011). This was the only randomized clinical trial discussed in this chapter in which IGT was not an eligibility criterion. Eligibility was based on elevated FPG (100–125 mg/dl or 5.5–6.9 mmol/l, defined as IFG), similar to the FPG eligibility criteria of the DPP, but IGT was not required. OGTTs were performed to exclude diabetes at entry and to define the diabetes outcome. The median age was 49 years and the mean BMI was 27 kg/m^2. Subjects were randomized to lifestyle intervention ($n = 311$) or a control group ($n = 330$). The intensive lifestyle intervention reduced diabetes incidence by 44% compared with standard care (i.e., hazard ratio = 0.56, 95% CI = 0.36–0.87).

The hazard rate reduction was greater among subgroups at higher baseline risk as determined either by IGT, FPG $\geq$ 110 mg/dl (6.1 mmol/l), or $HbA_{1c} \geq 5.6\%$ by the Japan Diabetes Society method (approximately 6.0% by the National Glycohemoglobin Standardization Program, NGSP, method). These high-risk subgroups contained fewer than half the participants but the majority of the outcome events (baseline NGSP-equivalent HbA_{1c} was $\geq$6.0% in 29% of the participants who experienced 57% of the outcomes). In those with NGSP-equivalent $HbA_{1c} \geq 6.0\%$, the hazard rate was reduced by 76%, the greatest relative risk reduction of any subgroup presented. There was no risk reduction among the subjects with isolated IFG (i.e., IFG with normal 2-h glucose and HbA_{1c}), although the effect estimate was very imprecise in this lower-risk group that experienced only 22 outcome events. Therefore, in addition to IFG, other glycemic measures such as elevated HbA_{1c} or IGT were needed to identify persons at high enough risk to show a treatment effect. These results are consistent with suggestions that HbA_{1c} could be used to identify persons for prevention interventions (International Expert Committee 2009) or to further stratify risk among persons selected by other criteria (DPP 2015c). They also confirm that intervention effects are hard to establish or nonexistent in persons without multiple risk factors.

Pharmacologic Interventions

Two of the lifestyle intervention trials described above – the Diabetes Prevention Program and the Indian Diabetes Prevention Program – included metformin

treatment arms. The following clinical trials evaluated only drugs for diabetes prevention. Although many included lifestyle intervention advice for all study participants, lifestyle intervention was not a study variable and was not evaluated in these trials.

Early UK and Swedish Prevention Studies Using Drugs (1979–1982)

The modern history of T2DM prevention began with three randomized clinical trials of drug therapy from the 1960s to 1980s. They began before the current definitions were established for IFG, IGT, and diabetes, so these terms used to describe these trials have slightly different definitions that those used today. These trials examined drugs then in common use to treat T2DM.

In the Whitehall study, 204 men with IGT were randomly assigned either the biguanide phenformin or placebo (Jarrett et al. 1979). The study definition of IGT was complicated, making it difficult to compare with other studies. It required a screening blood glucose 6.1–11.0 mmol/l followed by a 50 g OGTT performed in the afternoon with peak blood glucose >10 mmol/l and at least one of the following: 2-h blood glucose 6.7–11.0 mmol/l, two values >10.0 mmol/l, or mean 2-h glucose from the screening test, and the OGTT >6.7 mmol/l. In the 181 patients who completed 5 years of follow-up, the cumulative incidence of diabetes was 14% in the phenformin-treated patients and 16% in placebo-treated patients, with a cumulative incidence rate ratio (drug versus placebo) of 0.9 (95% confidence interval, CI = 0.4–1.8).

The Bedford study randomly assigned 241 men and women with IGT to the sulfonylurea tolbutamide or placebo and to two dietary groups in a 2 by 2 factorial design (Keen et al. 1974, 1982). IGT was defined by a 50 g OGTT with the 2-h plasma postload capillary glucose of 6.7–11.1 mmol/l. The study drugs were tolbutamide 0.5 g twice daily or matching placebo. One diet group was taught to restrict carbohydrate intake to 120 g/day. The other group received only brief advice to limit table sugar. During 10 years, 15% of subjects worsened to diabetes, but there were no effects of either the drug or diet interventions.

The third major study of this era was conducted in 147 men with IGT in Malmöhus County, Sweden (Sartor et al. 1980; Knowler et al. 1997). Diabetes and IGT were classified by an OGTT with a load of 30 g glucose per square meter of body surface area among men initially identified by having glycosuria. Diabetes was diagnosed if the 1-h postload capillary blood glucose was 11.1 mmol/l or more, the 2-h glucose was 8.6 mmol/l or more, and the 3-h glucose was 5.8 mmol/l or more. If these criteria were not met, but at least one of the following values was found – 1-h glucose 8.9 mmol/l or more, 2-h glucose 6.7 mmol/l or more, or 3-h glucose 4.7 mmol/l – subjects met the glycemic eligibility criteria, which here for simplicity are termed "IGT." All study participants were instructed to limit dietary carbohydrate and lipid and, if overweight, total energy intake. They were also randomly assigned to tolbutamide (0.5 mg three times per day), matching placebo, or neither drug nor placebo. The original report from the trial was interpreted as showing prevention by tolbutamide, based on an analysis of a very small number, 23, of those thought to have continued taking tolbutamide throughout, among whom none developed

diabetes. This conclusion was not based on the currently adopted "intention-to-treat" principle, i.e., analysis by assigned treatment group regardless of adherence. When analyzed later by intention-to-treat, the 10-year cumulative incidence of diabetes was 10% in men assigned tolbutamide treatment and 13% in the two groups assigned placebo or no drug (incidence rate ratio = 0.8, 95% CI = 0.3–2.0) (Knowler et al. 1997).

Long-term mortality rates were ascertained after the end of the trial, which was possible because of the availability of national vital statistics in Sweden. The all-cause mortality rate ratio (drug compared with placebo or no drug) was 0.66 (95% CI = 0.39–1.10) and the ischemic heart disease mortality rate ratio was 0.42 (95% CI = 0.16–1.12) (Knowler et al. 1997). While these effects were not statistically significant in this small randomized clinical trial, they were among the first to suggest that drug treatment of IGT might have health benefits beyond reducing hyperglycemia progression to diabetes.

None of these three early studies established whether diabetes could be prevented; their findings were inconclusive, largely owing to the small sample sizes. Whether pharmacologic prevention of T2DM was possible remained unknown until the 2000s.

Randomized Clinical Trials with Orlistat (2000; 2004)

Diabetes prevention has been tested with weight loss drugs, because overweight and obesity are major risk factors for T2DM. Drugs that affect weight, but do not have a known direct effect on plasma glucose concentration, were hypothesized to prevent diabetes development. Several randomized clinical trials have been performed in obese adults using the weight-loss drug orlistat, an intestinal lipase inhibitor. Three such trials were discussed in a pooled analysis (Heymsfield et al. 2000). Compared with placebo, orlistat was reported to reduce 2-year cumulative diabetes incidence by 61% (7.6% in the placebo group vs. 3.6% in the orlistat group) among those with IGT at randomization. Owing to its gastrointestinal side effects, however, only 69% of the subjects completed the 2-year study. The high drop-out rate, which could be associated with drug effects or side effects, makes it difficult to interpret these results.

A subsequent 4-year randomized clinical trial of orlistat reported a 37% reduction in diabetes incidence (Torgerson et al. 2004). As with earlier orlistat studies, a low percentage of participants completed the trial (52% of the orlistat group and 34% of the placebo group), making it difficult to estimate the effects of the drug. Although orlistat may be beneficial in some persons, the high discontinuation rate owing to side effects limits its widespread use for diabetes prevention.

Troglitazone in Prevention of Diabetes (TRIPOD) Study of Women with Previous Gestational Diabetes (2002)

Troglitazone was compared with placebo in 266 nondiabetic Hispanic women with previous gestational diabetes, about 70% of whom had IGT at entry into the randomized clinical trial called TRIPOD. Troglitazone reduced the development of diabetes by 55% over 2.5 years (Buchanan et al. 2002). As in the DPP, the drug was discontinued before planned study-end because of the potential for liver toxicity. The

preventive effect of troglitazone was attributed to improved insulin sensitivity, with resulting lower demand for insulin secretion, thus protecting the beta cells.

Acarbose in the Study to Prevent Noninsulin-Dependent Diabetes Mellitus (STOP-NIDDM)) (2002)

The α-glucosidase inhibitor acarbose was investigated as a diabetes prevention drug because of its lowering postprandial hyperglycemia, which is characteristic of IGT. The STOP-NIDDM randomized clinical trial tested acarbose in preventing diabetes in high risk adults. (Chiasson et al. 2002). This randomized clinical trial included 1429 subjects with IGT and IFG (FPG $\geq$ 5.6 mmol/l or 100 mg/dl and $<$7.0 mmol/l or 126 mg/dl) who were randomized to acarbose gradually titrated to 100 mg 3 times a day or placebo (Chiasson et al. 2002). Incident diabetes was defined by plasma glucose $\geq$11.1 mmol/l (200 mg/dl) at 2 h in a 75 g OGTT. Over a 3.3-year follow-up period, acarbose led to a 25% reduction in the incidence of diabetes. Weight loss contributed to the decreased risk of diabetes, but the acarbose effect persisted after adjustment for age, sex and BMI. Acarbose was associated with reversion of IGT to normal glucose tolerance [hazard ratio = 1.42 (95% CI: 1.24–1.62). Approximately one-quarter of the cohort (including 31% of the acarbose group) did not complete the study, the drop-out rate in acarbose-treated patients attributed to gastrointestinal side effects (flatulence, diarrhea, and abdominal cramps) that may limit its applicability for diabetes prevention in general practice. The STOP-NIDDM trial also studied treatment effects beyond the development of diabetes.

The acarbose arm had a 49% reduction in cardiovascular events [15 vs. 32 subjects; hazard ratio = 0.51 95% CI: 0.01–0.95); p = 0.03] (Chiasson et al. 2003). This difference from the placebo group was statistically significant, but based on few events. Acarbose also slowed the progression of carotid intimal medial thickness, a measure of subclinical atherosclerosis measured in a subset of the cohort (n = 132) (Hanefeld et al. 2004). Beneficial effects on several CVD risk factors (waist circumference, blood pressure and plasma triglycerides) were also reported (Chiasson et al. 2003). Altogether, these observations suggest that acarbose treatment may reduce the risk of cardiovascular events.

Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM, 2006)

Based on a suggestion that angiotensin-converting-enzyme inhibition might reduce diabetes risk (Yusuf et al. 2001), ramipril, a drug in this class, and the thiazolidinedione rosiglitazone were studied for diabetes prevention in DREAM (DREAM 2006a, b). Rosiglitazone is in the same thiazolidinedione class as troglitazone which, in previous randomized clinical trials, led to substantial reductions in diabetes incidence rates, before the drug was withdrawn from the market because of toxicity (as described above). DREAM tested ramipril and rosiglitazone in a 2 by 2 factorial design in 5269 participants with IFG, IGT, or both. IFG was defined by FPG 110 to $<$126 mg/dl and IGT by 2-h plasma glucose 140 to $<$200 mg/dl in an OGTT. For ramipril, the hazard ratio for developing diabetes was 0.91 (95% CI = 0.80–1.03). The incidence of diabetes was reduced by 62% by rosiglitazone (hazard ratio = 0.38,

95% CI = 0.33–0.44), and 50% of rosiglitazone-treated patients reverted to normoglycemia, compared with 30% of placebo-treated patients. There was no synergistic effect of the drugs in participants who were randomly allocated to both ramipril and rosiglitazone, i.e., the effect of each drug was the same in the presence or absence of the other drug.

Side effects, including weight gain (rosiglitazone-treated patients gained 2.2 kg more than placebo-treated patients) and edema, were observed with rosiglitazone. The frequency of congestive heart failure was also increased in the rosiglitazone group (hazard ratio = 7.03, 95% CI = 1.60–30.9), based on few cases (0.5% in the rosiglitazone group and 0.1% in the rosiglitazone-placebo group) in this generally healthy population (DREAM 2006b).

The Voglibose Randomized Clinical Trial (2009)

Voglibose, another α-glucosidase inhibitor, was studied in a randomized clinical trial in Japanese adults with IGT and at least one other diabetes risk factor (Kawamori et al. 2009). The diabetes outcome was defined by $HbA_{1c} \geq 6.5\%$ and, on two occasions, either FPG $\geq$7.0 mmol/l, 2-h plasma glucose $\geq$11.1 mmol/l, or random plasma glucose $\geq$11.1 mmol/l. The study was terminated before its planned end because of efficacy. After approximately 1-year of follow-up, the diabetes hazard rate ratio (voglibose vs. placebo) was 0.60 (95% CI = 0.43–0.82). Participant acceptance was greater than with acarbose in the STOP-NIDDM trial; 86% of the voglibose group and 83% of the placebo group completed the trial. Voglibose appeared to be moderately well tolerated and reduced the incidence of diabetes, at least for the short term. Because follow-up was terminated after about 1 year, long-term acceptance and efficacy of this medicine for diabetes prevention remain uncertain.

The Nateglinide and Valsartan in Impaired Glucose Tolerance Outcome Research (NAVIGATOR) Trial (2010)

This randomized clinical trial employed a 2 by 2 factorial design using the short-acting insulin secretagogue nateglinide (NAVIGATOR 2010a) and the angiotensin receptor blocker valsartan (NAVIGATOR 2010b) in 9306 participants with IGT, FPG from 95 to <110 mg/dl, and CVD or CVD risk factors. The mean age was 64 years and mean BMI was 30.5 kg/m^2. Nateglinide (60 mg three times daily) did not reduce the cumulative incidence of diabetes during the 5-year follow-up compared with placebo (hazard ratio = 1.07, 95% CI = 1.00–1.15) and was associated with increased frequency of hypoglycemic events (19.6% with nateglinide vs. 11.3% with placebo, $p < 0.001$) and slightly greater weight (+ 0.35 kg, $p = 0.001$) over the course of the study. Valsartan (160 mg once daily) was associated with a small reduction in diabetes incidence compared with placebo (hazard ratio = 0.86, 95% CI = 0.80–0.92). There was no significant interaction between the effects of the two drugs. About 80% of the participants completed the trial.

The NAVIGATOR trial had extended follow-up to evaluate treatment effects on CVD. Neither drug, alone or in combination with the other, affected a composite primary outcome of CVD death, nonfatal MI, or stroke, revascularization or hospitalization for angina or congestive heart failure, nor on a "core" composite that

excluded revascularization and angina (NAVIGATOR 2010a, b), despite lower blood pressure with valsartan than with placebo. The lack of prevention of CVD events does not support the hypothesis of a CVD benefit from reducing post-challenge (or postprandial) hyperglycemia with an insulin secretagogue.

The Canadian Normoglycemia Outcomes Evaluation (CANOE) Trial of the Combination of Rosiglitazone and Metformin (2010)

The CANOE randomized clinical trial tested the efficacy of a combination of submaximal doses of two drugs, metformin (500 mg twice daily) and rosiglitazone (2 mg twice daily) vs. placebo on diabetes incidence in 207 persons with IGT (Zinman et al. 2010). In the placebo group, mean age was 55 years and mean BMI was 32 kg/m^2. In the rosiglitazone plus metformin group, mean age was 50 years and mean BMI was 31 kg/m^2. After a median follow-up of 3.9 years, the 2-drug treatment resulted in a relative risk reduction for diabetes of 66% (95% CI = 41–80) and 80% regressed to normoglycemia, compared with 52% in the placebo group ($p = 0.0002$). The low-dose combination therapy was reportedly well tolerated, without excessive weight gain. The efficacy and tolerability of this low dose combination, compared with larger doses of the individual agents, suggest that low dose combinations may lead to similar benefit with greater tolerability.

The Actos Now for the Prevention of Diabetes (ACT NOW) Trial of Pioglitazone (2011)

Another thiazolidinedione drug, pioglitazone, was tested in the ACT NOW randomized clinical trial for the prevention of diabetes (DeFronzo et al. 2011). Six-hundred-two adults with IGT were enrolled. Mean age was 52 years, and mean BMI was 34 kg/m^2. Participants were randomized to treatment with pioglitazone 30 mg per day or placebo with median follow-up of 2.4 years. The study was completed by only 70% of the pioglitazone group and 76% of the placebo group. Pioglitazone led to a 72% reduction in diabetes incidence compared with placebo (hazard rate ratio = 0.28, 95% CI = 0.16–0.49). This study replicated the large effects of the thiazolidinedione drugs troglitazone and rosiglitazone on reducing diabetes incidence. Pioglitazone was associated with weight gain and edema, as are other drugs of this class.

The SEQUEL Secondary Analysis of a Study of Phentermine-Topiramate for Weight Loss (2012)

As with the previous randomized clinical trials of orlistat, a weight loss drug (see above), it was hypothesized that another weight loss drug would prevent diabetes. CONQUER was a randomized clinical trial of combinations of phentermine and topiramate compared with placebo for weight loss (Garvey et al. 2012). SEQUEL was secondary analysis of a subset of centers and participants in CONQUER with additional follow-up for diabetes incidence. Diabetes was lower in the active treatment groups compared with placebo, and the diabetes risk reduction was associated with the amount of weight loss. SEQUEL was a secondary analysis of a subset of participants in the CONQUER weight loss study, but it is not clear how this subset represents all those randomized in the original randomized clinical trial. Loss to

follow-up was not well described. A strategy of carrying forward the last observation was used to impute a substantial fraction of values, but there was not a clear description of the frequency of missing data or the characteristics of participants with missing outcome data. Loss to follow-up in such studies is not likely to be random but rather due to frustration with lack of weight loss or drug side effects.

A Randomized Clinical Trial of Liraglutide in Weight Management (2017)

Liraglutide, a glucagon-like peptide-1 analogue, was evaluated in a 56-week randomized clinical trial of 3731 nondiabetic adults with BMI $\geq$30 kg/m^2 or $\geq$27 kg/m^2 if they also had dyslipidemia or hypertension (Pi-Sunyer et al. 2015). The study was extended for 2 additional years in the subset of participants with prediabetes by American Diabetes Association criteria (American Diabetes Association 2010). During the 3 years of follow-up since randomization, the diabetes incidence rate was reduced by 79% (hazard ratio = 0.21, 95% confidence interval = 0.13–0.34) in this subgroup, although 50% of the participants were lost to follow-up (Le Roux et al. 2017). In a sensitivity analysis making various assumptions to impute missing data, the diabetes incidence rate was estimated to be reduced by 66% (hazard ratio = 0.34, 95% confidence interval = 0.22–0.53).

Role of Genetics in Diabetes Prevention

The complex field of genetic susceptibility to T2DM is described in another chapter. Most of the discoveries of diabetes susceptibility genes have come from large case-control studies, but several prevention randomized clinical trials have evaluated gene variants as predictors of outcomes within the trials and of potential modifiers of treatment effect. Some results of genetics studies within the DPP are described elsewhere (Florez et al. 2006; Hivert et al. 2011; Jablonski et al. 2010; Hivert et al. 2016). A general conclusion is that preventive interventions that are effective in general are also effective regardless of known genetic susceptibility factors for diabetes. Some exceptions have been described, and more are likely to be discovered in the future, in that gene variants associated with drug actions may modify the effects of those drugs, including on diabetes prevention. For example, variants in the *SLC47A1* gene, that is involved in metformin metabolism, modified the metformin effect in the DPP (Jablonski et al. 2010).

In summary, in prevention of type 2 diabetes, the beneficial effects of lifestyle interventions and of some medicines overcome genetic risk.

Discussion

Population-wide approaches to preventing T2DM (e.g., changes in food availability, transportation, and occupational and leisure physical activity) have the potential of lowering diabetes risk in the largest numbers of people. This conclusion is

speculative, however, because such interventions are difficult to implement and evaluate, generally requiring methods other than the randomized clinical trials that are considered the best methods for evaluating individual-based interventions. By contrast, individual-based interventions in high-risk persons have been well studied with varying degrees of success. Most have shown risk reductions with lifestyle interventions and some drugs, such as metformin, α-glucosidase inhibitors, and thiazolidinediones. These interventions can prevent or delay T2DM over the short term, i.e., several years, but there is less evidence for longer term prevention of diabetes, its complications, or mortality. That is, evidence of benefits of preventive interventions beyond glycemia is limited.

How should high-risk persons be identified for prevention interventions? (Knowler 2011). The high-risk approach is based on enrollment of persons with strong risk factors and the assumption that such risk factors can be affected by the intervention. Obesity, sedentary behavior, insulin resistance, and elevated glycemia (but below diagnostic levels) are easily identifiable with available tests and are potentially modifiable with diet, exercise, drugs, or combinations of each. Other risk factors such as genetic susceptibility or history of gestational diabetes in currently nondiabetic women are not modifiable, but may help in selecting high-risk person with other modifiable risk factors. Risk factors such as drug treatment for other conditions (e.g., statins for dyslipidemia) could be removed by discontinuing such treatment, but the balance between potential risks and benefits of such action is usually not obvious. Most of the published prevention randomized clinical trials have selected persons with IGT, requiring performance of an OGTT. Some trials also required overweight or obesity or elevated FPG for eligibility. All were performed only in adults, so there remains a lack of data on children and adolescents, who are also at risk of T2DM, especially in some US minority groups.

The OGTT required for detection of IGT is inconvenient, time-consuming, and often infeasible in large-scale screening programs. The American Diabetes Association defines “pre-diabetes” by elevated levels (but not meeting diabetes diagnostic criteria) of either FPG, 2-hour post-load glucose (i.e., IGT), or HbA1c, i.e., IGT is not required (American Diabetes Association 2010). There is limited evidence that interventions shown effective in persons with IGT will also benefit persons with other high-risk characteristics (including “prediabetes”) but without IGT. The most informative randomized clinical trials in this regard were the lifestyle intervention in Japanese men with impaired fasting glucose and DREAM, described above. In the Japanese lifestyle trial, prevention was very effective in men without IGT but with elevated FPG and HbA1c (Saito et al. 2011). In DREAM, rosiglitazone was nearly equally effective in persons with isolated IFG (i.e., without IGT), isolated IGT, and both IFG and IGT in combination (DREAM 2006b). Replication of these finding is needed before concluding whether IGT is necessary as an eligibility criterion for preventive interventions for T2DM. Elevated HbA1c may be as good at predicting T2DM and subsequent complications as is IGT (McCance et al. 1994; Vijayakumar et al. 2017; Warren et al. 2017), and it has been suggested as a suitable measure for identifying persons for preventive intervention (International Expert Committee

2009). Effectiveness of interventions for preventing T2DM in high-risk persons identified only by HbA1c, however, has not been evaluated to my knowledge.

In summary, lifestyle interventions and several different drugs can prevent T2DM in high-risk persons in the short term, i.e., for at least several years. There is less evidence that these interventions can prevent diabetes in the long term or reduce risk of diabetes complications, including mortality. Applying results of prevention randomized clinical trials to large numbers of people or on a population level remains a major challenge, but one that should be undertaken.

Acknowledgments This work was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases.

References

Ackermann RT, Holmes AM, Saha C. Designing a natural experiment to evaluate a national health care – community partnership to prevent type 2 diabetes. Prev Chronic Dis. 2013;10:120149.

Ackermann RT, Duru OK, Albu JB, Schmittdiel JA, Soumerai WB, Wharam JF, Ali MK, Mangione CM, Gregg ES, NEXT-D Study Group. Evaluating diabetes health policies using natural experiments: the natural experiments for translation in diabetes study. Am J Prev Med. 2015;48:747–54.

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(Suppl 1):S62–9.

Batis C, Rivera JA, Popkin BM, Taillie LS. First-year evaluation of Mexico's tax on nonessential energy-dense foods: an observational study. PLoS Med. 2016;5:e1002057.

Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women. Diabetes. 2002;51:2796–280.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002;359:2072–7.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance. The STOP-NIDDM Trial. JAMA. 2003;290:486–94.

Crandall JP, Knowler WC, Kahn SE, Marrero D, Florez JC, Bray GA, Haffner SM, Hoskin M, Nathan DM, for the Diabetes Prevention Program Research Group. The prevention of type 2 diabetes. Nat Clin Pract Endocrinol Metab. 2008;4:382–93.

DeFronzo RA, Tripathy D, Schwenke DC, Banerji M, Bray GA, Buchanan TA, Clement SC, Henry RR, Hodis HN, Kitabchi AE, Mack WJ, Mudaliar S, Ratner RE, Williams K, Stentz FB, Musi N, Reaven PD. Pioglitazone for diabetes prevention in impaired glucose tolerance. N Engl J Med. 2011;364:1104–15.

Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.

Diabetes Prevention Program Research Group. Prevention of type 2 diabetes with troglitazone in the Diabetes Prevention Program. Diabetes. 2005a;54:1150–6.

Diabetes Prevention Program Research Group. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. Diabetes. 2005b;54:2404–14.

Diabetes Prevention Program Research Group. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care. 2006;29:2102–7.

Diabetes Prevention Program Research Group. Factors associated with diabetes onset during metformin versus placebo therapy in the Diabetes Prevention Program. Diabetes. 2007;56:1153–9.

Diabetes Prevention Program Research Group. Prevention of diabetes in women with a history of gestational diabetes mellitus: effects of metformin and lifestyle interventions. J Clin Endocrinol Metab. 2008;93:4772–9.

Diabetes Prevention Program Research Group. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet. 2009;374:1677–86.

Diabetes Prevention Program Research Group. The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention. Diabetes Care. 2012;35:723–30.

Diabetes Prevention Program Research Group. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. Lancet Diabetes Endocrinol. 2015a;3:866–75.

Diabetes Prevention Program Research Group. The effect of lifestyle intervention and metformin on preventing or delaying diabetes among women with and without gestational diabetes: the Diabetes Prevention Program Outcomes Study 10-year follow up. J Clin Endocrinol Metab. 2015b;100:1646–53.

Diabetes Prevention Program Research Group. HbA1c as a predictor of diabetes and as an outcome in the Diabetes Prevention Program: a randomized controlled trial. Diabetes Care. 2015c;38:51–8.

DREAM Trial Investigators, Bosch J, Yusuf S, Gerstein HC, Pogue J, Sheridan P, Dagenais G, Diaz R, Avezum A, Lanas F, Probstfield J, Fodor G, Holmann RR. Effect of ramipril on the incidence of diabetes. N Engl J Med. 2006a;355:1551–62.

DREAM Trial Investigators, Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman RR. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. Lancet. 2006b;368:1096–105.

Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D, for the Diabetes Prevention Program Research Group. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med. 2006;355:241–50.

Garvey WT, Ryan DH, Look M, Gadde KM, Allison DB, Peterson CA, Schwiers M, Day WW, Bowden CH. Two-year sustained weight loss and metabolic benefits with controlled-release phentermine/topiramate in obese and overweight adults (SEQUEL): a randomized, placebo-controlled, phase 3 extension study. Am J Clin Nutr. 2012;95:297–308.

Gong Q, Gregg EW, Wang J, An Y, Zhang P, Yang W, Li H, Li H, Jiang Y, Shuai Y, Zhang B, Zhang J, Gerzoff RB, Roglic G, Hu Y, Li G, Bennett PH. Long-term effects of a randomised trial of a 6-year lifestyle intervention in impaired glucose tolerance on diabetes-related microvascular complications: the China Da Qing Diabetes Prevention Outcome Study. Diabetologia. 2011;54:300–7.

Hanefeld M, Chiasson JL, Koehler C, Henkel E, Schaper F, Temelkova-Kurktschiev T. Acarbose slows progression of intima-media thickness of the carotid arteries in subjects with impaired glucose tolerance. Stroke. 2004;35:1073–8.

Heymsfield SB, Segal KR, Hauptman J, Lucas CP, Boldrin MN, Rissanen A, Wilding JP, Sjöström L. Effects of weight loss with orlistat on glucose tolerance and progression to type 2 diabetes in obese adults. Arch Intern Med. 2000;160:1321–6.

Hivert MF, Jablonski KA, Perreault L, Saxena R, McAteer JB, Franks PW, Hamman RF, Kahn SE, Haffner S, the DIAGRAM Consortium, Meigs JB, Altshuler D, Knowler WC, Florez JC, for the Diabetes Prevention Program Research Group. Updated genetic score based on 34 confirmed type 2 diabetes loci is associated with diabetes incidence and regression to normoglycemia in the Diabetes Prevention Program. Diabetes. 2011;60:1340–8.

Hivert M-F, Christophi CA, Franks PW, Jablonski KA, Ehrmann DA, Kahn SE, Horton ES, Pollin TI, Mather KJ, Perreault L, Barrett-Connor E, Knowler WC, Florez JC, for the Diabetes

Prevention Program Research Group. Lifestyle and metformin ameliorate insulin sensitivity independently of the genetic burden of established insulin resistance variants in Diabetes Prevention Program participants. Diabetes. 2016;65:520–6.

International Expert Committee. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes. Diabetes Care. 2009;32:1327–34.

Jablonski KA, McAteer JB, deBakker PIW, Franks PW, Pollin TI, Hanson RL, Saxena R, Fowler S, Shuldiner AR, Knowler WC, Altshuler D, Florez JC, for the Diabetes Prevention Program Research Group. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle interventions in the Diabetes Prevention Program. Diabetes. 2010;59:2672–81.

Jarrett RJ, Keen H, Fuller JH, McCartney M. Worsening to diabetes in men with impaired glucose tolerance ("borderline diabetes"). Diabetologia. 1979;16:25–30.

Kawamori R, Tajima N, Iwamoto Y, Kashiwagi A, Shimamoto K, Kaku K. Voglibose Ph-3 Study Group: Voglibose for prevention of type 2 diabetes mellitus: a randomised, double-blind trial in Japanese individuals with impaired glucose tolerance. Lancet. 2009;373:1607–14.

Keen H, Jarrett RJ, Fuller JH. Toldbutamide and arterial disease in borderline diabetics. In: Malaisse WJ, Pirart J, Vallance-Owen J, editors. Proceedings of the eighth congress of the international diabetes federation. Amsterdam: Excerpta Medica; 1974.

Keen H, Jarrett RJ, McCartney P. The ten-year follow-up of the Bedford survey (1962–1972): glucose tolerance and diabetes. Diabetologia. 1982;22:73–8.

Knowler WC. Prevention of type 2 diabetes: how and in whom? Arch Intern Med. 2011;171:1361–2.

Knowler WC, Ackermann RT. Preventing diabetes in American Indian communities. Diabetes Care. 2013;36:1820–2.

Knowler WC, Narayan KM, Hanson RL, Nelson RG, Bennett PH, Tuomilehto J, Scherstén B, Pettitt DJ. Preventing non-insulin-dependent diabetes. Diabetes. 1995;44:483–8.

Knowler WC, Sartor G, Melander A, Scherstén B. Glucose tolerance and mortality, including a substudy of tolbutamide treatment. Diabetologia. 1997;40:680–6.

Knowler WC, Crandall JP, Chiasson J-L, Nathan DM. Prevention of type 2 diabetes. Chapter 38 in Diabetes in America. In: Cowie CC et al (ed). 3rd ed. Bethesda, MD, National Institute of Health, NIH Pub No. 17–1468. (in press).

Kosaka K, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. Diabetes Res Clin Pract. 2005;67:152–62.

Le Roux CW, Astrup A, Fujioka K, Greenway F, Lau DC, Van Gaal L, Ortiz RV, Wilding JP, Skjøth TV, Manning LS, Pi-Sunyer X, SCALE Obesity Prediabetes NN8022-1839 Study Group. 3 years of liraglutide versus placebo for type 2 diabetes risk reduction and weight management in individuals with prediabetes: a randomised, double-blind trial. Lancet. 2017;389:1399–409.

Li G, Zhang P, Wang J, An Y, Gong Q, Gregg EW, Yang W, Zhang B, Shuai Y, Hong J, Engelgau MM, Li H, Roglic G, Hu Y, Bennett PH. Cardiovascular mortality, all-cause mortality, and diabetes incidence after lifestyle intervention for people with impaired glucose tolerance in the Da Qing Diabetes Prevention Study: a 23-year follow-up study. Lancet Diabetes Endocrinol. 2014;2:474–280.

Lindström J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention Study (DPS). Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). Diabetologia. 2013;56:284–93.

Mason CC, Hanson RL, Knowler WC. Progression to type 2 diabetes characterized by moderate then rapid glucose increases. Diabetes. 2007;56:2054–61.

McCance DR, Hanson RL, Charles MA, Jacobsson LTH, Pettitt DJ, Bennett PH, Knowler WC. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. Br Med J. 1994;308:1323–8.

Navigator Study Group. Effect of nateglinide on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010a;362:1463–76.

Navigator Study Group. Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010b;362:1477–90.

Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care. 1997;20:537–44.

Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, Lau DC, le Roux CW, Violante Ortiz R, Jensen CB, Wilding JP, SCALE Obesity and Prediabetes NN8022-1839 Study Group. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. N Engl J Med. 2015;373:11–22.

Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49:289–97.

Saito T, Watanabe M, Nishida J, Izumi T, Omura M, Takagi T, Fukunaga R, Bandai Y, Tajima N, Nakamura Y, Ito M, Zensharen Study for Prevention of Lifestyle Diseases Group. Lifestyle modification and prevention of type 2 diabetes in overweight Japanese with impaired fasting glucose levels: a randomized controlled trial. Arch Intern Med. 2011;171:1352–60.

Sartor G, Scherstén B, Carlström S, Melander A, Nordén A, Persson G. Ten-year follow-up of subjects with impaired glucose tolerance: prevention of diabetes by tolbutamide and diet regulation. Diabetes. 1980;29:41–9.

Stevenson M, Thompson J, Hérick de Sá T, Ewing R, Mohan D, McClure R, Roberts I, Tiwari G, Giles-Corti B, Sun X, Wallace M, Woodcock J. Urban design, transport, and health 2: land use, transport, and population health: estimating the health benefits of compact cities. Lancet. 2016;388:2925–35.

Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet. 2009;373:2215–21.

Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 2004;27:155–61.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M, Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344:1343–50.

Uusitupa M, Peltonen M, Lindström J, Aunola S, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Valle TT, Eriksson JG, Tuomilehto J, Finnish Diabetes Prevention Study Group. Ten-year mortality and cardiovascular morbidity in the Finnish Diabetes Prevention Study–secondary analysis of the randomized trial. PLoS One. 2009;4:e5656.

Vijayakumar P, Nelson RG, Hanson RL, Knowler WC, Sinha M. HbA1c and the prediction of type 2 diabetes in children and adults. Diabetes Care. 2017;40:16–21.

Wareham NJ, Herman WH. The clinical and public heath challenges of diabetes prevention: a search for sustainable solutions. PLoS Med. 2016;10:e1002097.

Warren B, Pankow JS, Matsushita K, Punjabi NM, Daya NR, Grams M, Woodward M, Selvin E. Comparative prognostic performance of definitions of prediabetes: a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) study. Lancet Diabetes Endocrinol. 2017;5:34–42.

White M. Population approaches to prevention of type 2 diabetes. PLoS Med. 2016;13:e1002080.

World Health Organization. Diabetes mellitus: report of a WHO study group. WHO Tech Rep Ser. 1985;727:7–113.

Yusuf S, Gerstein H, Hoogwerf B, Pogue J, Bosch J, Wolffenbuttel BH, Zinman B, HOPE Study Investigators. Ramipril and the development of diabetes. JAMA. 2001;286:1882–5.

Zinman B, Harris SB, Neuman J, Gerstein H, Retnakaran RR, Raboud J, Qi Y, Hanley AJG. Low-dose combination therapy with rosiglitazone and metformin to prevent type 2 diabetes mellitus (CANOE trial): a double-blind randomized controlled study. Lancet. 2010;376:103–11.

Patient Education and Empowerment

16

Martha M. Funnell, Robert M. Anderson, and Gretchen A. Piatt

Contents

Abstract

The clinical care of both type 1 and type 2 diabetes has changed dramatically in recent years. While new therapies and technological advances improve outcomes in diabetes, these can also increase the burden of daily care for people with diabetes and their family members. In order to use these technologies effectively, patients need the information required for advanced decision-making, the skills to incorporate self-management into their lives, and the self-efficacy to assume this level of responsibility. Diabetes self-management education, on-going support, and patient empowerment are strategies that can be used to facilitate patient engagement and active participation, prevent acute complications, and ultimately to improve long-term outcomes and quality of life among people with diabetes.

M. M. Funnell (✉) · R. M. Anderson · G. A. Piatt
Department of Learning Health Sciences, The University of Michigan Medical School, Ann Arbor, MI, USA
e-mail: mfunnell@umich.edu; boba@umich.edu; piattg@umich.edu

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_17

Keywords
Self-management · Diabetes self-management education · Diabetes self-management support · Patient empowerment · Self-directed goal setting

Introduction

It is widely recognized that diabetes is a largely self-managed disease, with patients assuming more than 99% of their own day-to-day care. Thus, the implementation of therapeutic recommendations, changes in lifestyle, healthy coping, and ultimately outcomes are largely in the hands of the person with diabetes. This responsibility cannot be negotiated, assigned, or diminished (Anderson et al. 2002). It is therefore fundamental to diabetes that medical treatment and patient behaviors must intersect to guide the course of this illness. (Marrero et al. 2013) Within this context, the role of health professionals is to facilitate self-management, informed decision-making, engagement and empowerment through on-going diabetes expertise, education, and psychosocial support.

Diabetes Self-Management

Diabetes self-management is defined as the tasks patients undertake in order to live well with their illness. (Barlow et al. 2002) It includes the patients' ability, knowledge, skills, and confidence to make daily decisions; select and make behavioral changes; and cope with the emotional aspects of their disease within the context of their lives.

Because of the essential nature of self-management in diabetes, patient education has long been viewed as a cornerstone of diabetes care. Unfortunately, early educational efforts to provide a one-time "inoculation of information" designed to get patients to comply or adhere with their physicians' orders for a lifetime were largely ineffective. The concept of patient empowerment was introduced in 1991 (Funnell et al. 1991) as an alternative approach for people with diabetes and patient education. The resulting efforts to design, implement, and evaluate educational and behavioral interventions has led to significant improvements in both our understanding of and ability to provide effective self-management education and on-going behavioral and psychosocial support for people with diabetes.

Diabetes Self-Management Education (DSME) and Diabetes Self-Management Support (DSMS)

The goal of DSME/S is currently defined as "supporting informed decision-making, self-care behaviors, problem solving, and active collaboration with the health care team, and improving clinical outcomes, health status and quality of life" (Haas et al.

2012). It is also recognized that both DSME and on-going support (DSMS) are essential to "enable people with or at risk for diabetes to make informed decisions and to assume responsibility for the day-to-day management of their disease or risk factors" (NDEP 2015).

The Standards of Care from the American Diabetes Association state that "all people with diabetes should participate in DSME to facilitate the knowledge, skills, and ability necessary to carry out diabetes self-care and receive DSMS to assist with implementing and sustaining skills and behaviors needed for on-going self-management, both a diagnosis and as needed thereafter" (ADA 2017). While the need for self-management is well established and the difficulties patients experience implementing provider recommendations is viewed as a major barrier in clinical care and source of frustration, DSME and DSMS are largely underutilized services. The large multinational second Diabetes Attitudes, Wishes, and Needs Study (DAWN2) found that "most people with diabetes are not actively engaged by their healthcare professionals to take control of their condition; education and psychosocial care are often unavailable" (Nicolucci 2013). In the DAWN2 sample of over 8,000 patients with type 1 and type 2 diabetes from 17 different countries, less than half had received formal diabetes education. Of those who had participated in DSME, however, the majority (81.1%) found it helpful.

A review of claims data in the United States revealed that only 6.8% of privately insured, newly diagnosed adults (ages 18–64) participated in DSME during the first year after diagnosis between 2009 and 2012 (Li et al. 2014). Although the reasons for this are largely unknown and likely complex, the misperception that DSME/S is ineffective, costly, and unnecessary is a limiting factor for health professional recommendations and referrals.

Effectiveness of DSME/S

Multiple studies, reviews, and meta-analysis have documented that DSME is effective for improving A1C and other metabolic outcomes and quality of life, and is also cost-effective for reducing hospitalizations and readmissions (Brunisholz et al. 2014; Steinsbekk et al. 2012; Duncan et al. 2009; Heinrich et al. 2010; Pillay et al. 2015a, b). In general, DSME has a positive effect on diabetes-related health and psychosocial outcomes; specifically, glycemic control, blood glucose monitoring, dietary and exercise behaviors, foot care, medication-taking, diabetes-related distress, and healthy coping. (Powers et al. 2015).

DSME/S Content

The International Diabetes Federation (IDF 2009) and many countries have developed Standards for Diabetes Education that include content areas and methods as well as program structure, process, evaluation, and outcomes. In the USA, National Standards for Diabetes Self-management Education and Support (DSME/S) were

Table 1 Recommended diabetes self-management content areas

Diabetes pathophysiology and treatment options
Healthy eating
Physical activity
Medication usage
Monitoring and using patient-generated health data
Preventing, detecting, and treating acute and chronic complications
Healthy coping with psychosocial issues and concerns
Problem solving

From Beck et al. 2017

first published in 1982 and are revised every 5 years based on the current evidence (Beck et al. 2017). Content areas identified by these Standards are outlined in Table 1. Evidence for meeting these Standards through either recognition by the American Diabetes Association (ADA) or certification by the American Association of Diabetes Educators (AADE) is required for reimbursement by Medicare, Medicaid, and most private insurers.

DSME/S Frequency

A joint Position Statement was recently published by the ADA, AADE, and the Academy of Nutrition Sciences to better define the provision of DSME and DSMS for adults with type 2 diabetes (Powers et al. 2015). Critical times to assess and refer for DSME, DSMS, and Medical Nutrition Therapy (MNT) are at diagnosis, during the annual visit, when new or complicating factors affect self-management, and when transitions in care occur (see Fig. 1). As examples, adults with type 2 diabetes who begin insulin therapy, experience depression, are struggling with self-management, or move from home to extended care all need to be assessed to determine if DSME/S is needed. Specific content and action steps for each of these critical times are described in Fig. 2.

DSME/S Methods

Although the evidence supports the efficacy of DSME/S, it is not possible to define an optimal DSME/S program (Norris 2002). However, characteristics that enhance effectiveness have been identified in both clinical and nonclinical settings and are summarized in Table 2. As examples, educational programs that provide more contact than the 10 h typically covered by reimbursement in the USA are more effective than programs that provide 10 contact h or less (Pillay et al. 2015b). In addition, programs that integrate psychosocial and behavioral content and are empowerment-based report better outcomes than traditional, lecture-based educational programs (Norris et al. 2002). There is no difference in the effectiveness of group DSME/S compared with individually provided education.

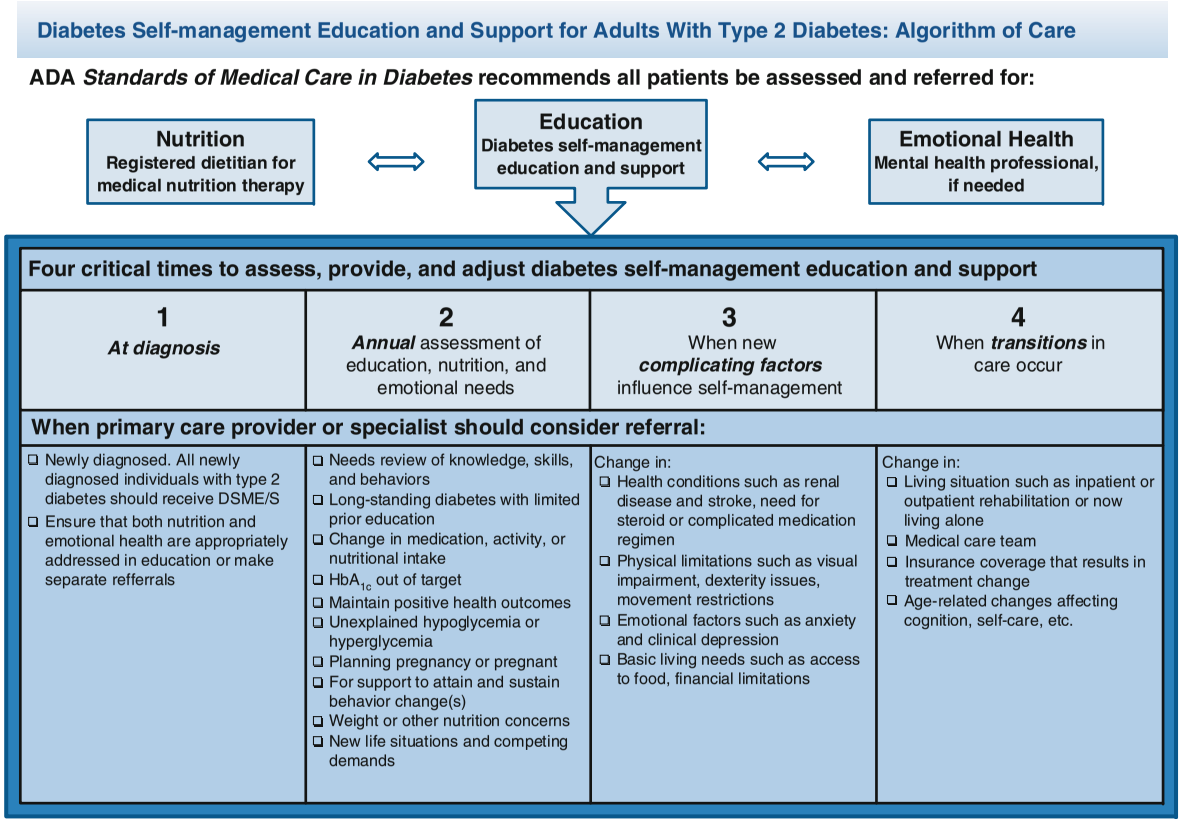


Fig. 1 DSME/S algorithm of care. (Reprinted with permission Powers et al. 2015)

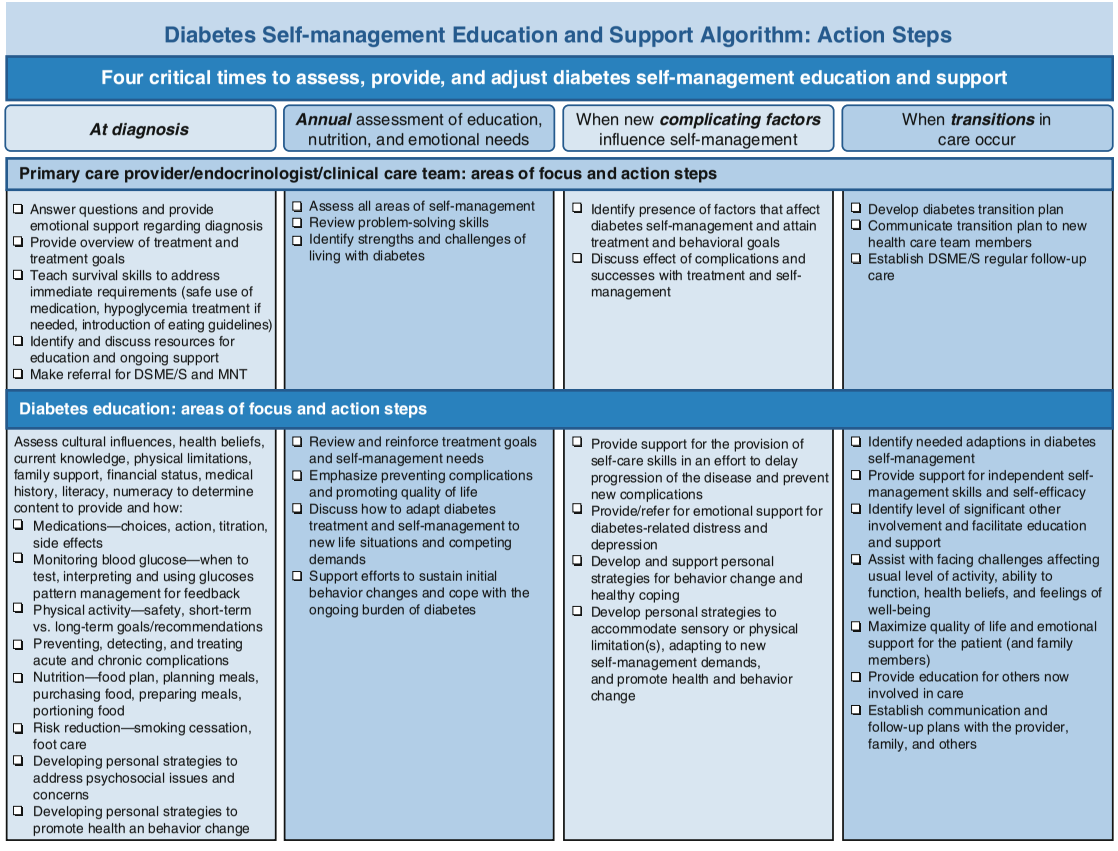


Fig. 2 DSME/S algorithm of care: action steps. (Reprinted with permission Powers et al. 2015)

Table 2 Effectiveness of DSME and DSMS

Characteristics of effective interventions
Regular reinforcement is more effective than one-time or short-term education.
Patient participation and collaboration appear to produce more favorable results than didactic interventions.
Group education is more effective than one-on-one education for lifestyle interventions and appears to be equally effective for improving knowledge and accuracy of self-monitoring of blood glucose (SMBG).
Studies with short-term follow up are more likely to demonstrate positive effects on glycemic control and behavioral outcomes than studies with long-term follow-up.
Programs with less than 10 contact hours and without added support provide limited long-term benefit.
Effectiveness in clinical settings
In the short term (<6 months), DSME improves knowledge levels, SMBG skills, and dietary habits (per self-report).
In the short term (<6 months), glycemic control improves.
Improved glycemic control does not appear to correspond to measured changes in knowledge or SMBG skills.
Weight loss can be achieved with repetitive interventions or with short-term follow-up (<6 months).
Physical activity levels are variably affected by interventions.
Effects on lipids and blood pressure are variable but are more likely to be positive with interactive or individualized repetitive interventions.
Effectiveness in nonclinical settings
Some evidence indicates that DSME is effective when given in community gathering places (e.g., churches and community centers) for adults with type 2 diabetes.
The literature is insufficient to assess the effectiveness of DSME in the home for adults with diabetes.
The literature is insufficient to assess the effectiveness of DSME in the workplace.

Adapted from Norris et al. 2002

DSME/S is designed to match the health literacy of participants and that is culturally relevant to the target population is more effective (AHRQ 2015). Functional health literacy is defined as a measure of a patient's ability to perform basic reading and numerical tasks required to function in the health care environment and is distinct from education level and language ability (AlSayah et al. 2013; Bailey et al. 2014). Patients with low functional health literacy often:

- Have greater difficulty understanding their condition
- Are less likely to engage in self-management
- May have worse glycemic control
- Have poorer communication with providers
- Are less confident managing their diabetes

It is recommended that "universal precautions" (AHRQ 2015) be applied during all patient interactions, which include:

- Use of plain language in speaking and written and spoken materials (no jargon; words less than three syllables)
- Explain medical terms
- Avoid phrases with two interpretations (e.g., positive test results; stable test results)
- Open-ended questions ("What questions do you have?" not "Do you have questions?")
- Highlight key recommendations

The DAWN2 US Study evaluated ethnic differences in psychological outcomes among adult non-Hispanic whites, Mexican Americans, African Americans, and Chinese Americans with diabetes and their adult family members (Peyrot et al. 2014). While there were differences among and between groups and a substantial amount of diabetes distress was found for both people with diabetes and their family members, those in minority groups experienced more diabetes distress than non-Hispanic whites. However, a large social support network was found to positively influence better psychosocial outcomes and health behaviors. Asking patients about cultural or religious influences on their diabetes self-management, use of traditional medicines, inviting family members to participate in care and educational visits, and tailoring education to match ethnic and religious dietary and other preferences are effective strategies for DSME/S. (Funnell et al. 2015).

With the advent of and greater access to various forms of technology, its use has been proposed as an efficient and effective method for providing DSME/S. Although there is a great deal of information available to patients, unfortunately much of it is provided by those who are uninformed, misinformed, or promoting products. The current evidence indicates that the data are mixed in terms of technology-based DSME with some studies reporting modest improvements in glycemic outcomes (Pal et al. 2014). However, technology has been effective for delivering diabetes prevention programs DSMS, including on-going psychosocial support, behavioral and educational reinforcement, tracking behaviors, and patient-provider communication. It is also clear that the use of technology will increase as it becomes more widely available and desired.

Incorporating DSME and DSMS into Clinical Care

DSME and DSMS also need to occur during clinical visits. However, studies have shown that patients typically remember less than 50% of what was said by the provider, and patients with low functional health literacy may remember even less (Schillinger et al. 2002). Use of effective strategies such as the "ask, tell, ask" interactive communication loop can improve the effectiveness of DSME/S during a clinical visit (Schillinger et al. 2003). The visit begins with the provider asking the patient the issue that is most important to address or what is most difficult about their diabetes or current treatment. Information, support, or referrals are then provided based on the patient's issue, specific questions are addressed, and the patient is

Table 3 Key messages

Diabetes is self-managed which means you have an active role to play.
In order to self-manage effectively, you need both education and ongoing support.
Negative emotions (anger, fear, frustration, and guilt) are common.
Learning how to make changes in behavior is essential.
Your treatment will change over time, which does not mean that you have failed but simply that your body needs more help.
Complications are not inevitable.
Diabetes self-management is not easy, but it is worth it.

then asked to repeat or "teach back" the information. This patient-centered communication strategy not only checks for recall but can also provide the opportunity to take advantage of "teachable moments" related to a patient-identified issue, reinforce and tailor DSME/S education, uncover inaccurate beliefs and misunderstandings, and actively engage patients to participate in their treatment and self-care. Table 3 outlines key educational messages to provide during a clinical visit.

On-going care visits also provide an excellent opportunity to provide DSMS in order to sustain improvements and maintain motivation for diabetes self-management. The use of care managers, care navigators, peers, community health workers, and referral to virtual or in person support groups are effective strategies for DSMS.

Patient Empowerment

Self-management requires considerable effort that must be sustained over a lifetime of diabetes. Adults with diabetes are often expected to make significant changes in their lifestyle without taking into account their competing priorities, work and other life goals, family responsibilities, and other demands on their time and energy. In addition, patients are also dealing with the emotional consequences of a serious chronic illness and the potential for complications. It is therefore unsurprising that adults with both type 1 and type 2 diabetes and their family members perceive diabetes to be a significant burden and experience diabetes-related distress (NIcolucci et al. 2013). Empowerment is a patient-centered approach based on the understanding that motivation is most effective when it is internally determined and directed towards behaviors that are personally relevant and meaningful (Funnell et al. 1991; Funnell and Piatt 2017). Patient empowerment involves creating a collaborative (rather than a directive) relationship with patients and actively engaging them in shared-decision-making, incorporating their abilities, goals, needs, barriers, and values.

Effective communication skills are critical to the success of using the empowerment approach. The ALE approach (Ask, Listen, Empathize) is a nondirective communication style using questions to elicit the patient's concerns and active listening and empathy to encourage further discussion, in order to identify personally

Table 4 Five-step goal setting model

Identify the problem
What is the most difficult or frustrating part of caring for your diabetes at this time?
Determine feelings and their influence on behavior
How do you feel about this issue? How are your feelings influencing your behavior?
On a scale of 1–10, how important is it for you to address this problem? On a scale of 1–10, how confident do you feel that you can resolve this issue?
Set a long-term goal
What do you want? What do you need to do? What problems to you expect to encounter? What support do you have to overcome these problems? Are you willing/able to take action to address this problem?
Create an I-SMART plan
What will you do this week to get started working toward your goal?
I-important
S-specific action step
M-measurable
A-attainable
R-relevant to long-term goals
T-time specific
Assess how the experiment worked
How did it work? What did you learn? What might you do differently next time?

Adapted from Funnell and Anderson 2004

meaningful and relevant solutions and set behavioral goals (Anderson et al. 2002). An example of empowerment-based communication when choosing treatments is shared-decision-making which has been shown to improve medication-taking behaviors. (Veroff et al. 2013).

These same communications skills are used when setting behavioral goals and providing empowerment-based DSME and DSMS. Self-directed behavioral goal-setting is an effective intervention to facilitate self-management and behavioral change (ADA 2017; Glasgow et al. 2003). Goal-setting is a process beginning with the patient identifying a problem that is personally meaningful and results in an action plan developed by the patient. Table 4 outlines the empowerment-based five-step process for goal-setting that supports a collaborative approach, addresses both behavioral and psychosocial issues, and includes the development of an I-SMART plan. This action plan is designed as an experiment with the goal of learning about what will and will not work to facilitate goal attainment and improve outcomes. (Funnell and Piatt 2017).

Empowerment-based DSME and DSMS interventions are patient-guided rather than content-driven and designed to provide participants with the knowledge and skills needed to engage with their provider, make informed decisions, solve problems, choose and achieve goals and cope with the demands of diabetes. This approach to DSME and DSMS, which is designed to meet the needs identified by patients, is effective for improving clinical, psychosocial, and behavioral outcomes (Funnell et al. 2014).

Summary

Diabetes self-management education and on-going support strategies improve outcomes and quality of life among people with diabetes. Although access and reimbursement has increased over the last decade, many people with diabetes and their families do not receive referral to or take advantage of these important services. In addition, making the shift to more collaborative, patient-centered models of care has been slow among providers, although the advent of Medical Homes and Accountable Care Organizations has led to renewed interest in empowerment-based approaches to care and education.

Outcomes in diabetes, including long-term morbidity and mortality, are dependent on the ability of people with diabetes to effectively make decisions and care for themselves for a lifetime with this burdensome disease. They therefore have a right to receive effective diabetes self-management education and on-going support, and health care professionals have a responsibility to ensure that they are aware and take advantage of these essential aspects of their treatment.

References

AlSayah F, Majumdar SR, Williams B, Robertson S, Johnson JA. Health literacy and health outcomes in diabetes: a systematic review. J Gen Intern Med. 2013;28:444–52.

American Diabetes Association. Standards in Medical Care in Diabetes-2017. Diabetes Care. 2017;40(Suppl 1):S1–S107.

Anderson RM, Funnell MM, Arnold MS. Using the empowerment approach to help patients change behavior. In: Anderson BJ, Rubin RR, editors. Practical psychology for diabetes clinicians. 2nd ed. Alexandria: American Diabetes Association; 2002. p. 3–12.

Bailey SC, Brega AG, Crutchfield TM, Elasy T, Herr H, et al. Update on health literacy and diabetes. Diabetes Educ. 2014;40:581–604.

Barlow J, Wright C, Sheasby J, Turner A, Hainsworth J. Self-management approaches for people with chronic conditions: a review. Patient Educ Couns. 2002;48:177–87.

Beck J, Greenwood DA, Blanton L, Bollinger ST, Butcher MK. Et on behalf of the 2017 standards revision task force. 2017 national for diabetes self-education and support. Diabetes Care. 2017;40:1409–19.

Brunisholz KD, Briot P, Hamilton S, Joy EA, Lomax M, Barton N, Cunningham R, Savitz LA, Cannon W. Diabetes self-management education improves quality of care and clinical outcomes determined by a diabetes bundle measure. J Multidiscip Healthc. 2014;7:533–42.

Duncan I, Birkmeyer C, Coughlin S, Li Q, Sherr D, Boren S. Assessing the value of diabetes education. Diabetes Educ. 2009;35:752–60.

Funnell MM, Anderson RM. Empowerment and self-management education. Clin Diabetes. 2004;22:123–7.

Funnell MM, Piatt GA. Incorporating diabetes self-management education into your practice: when, what, and how. J Nurs Pract. 2017;13:468–74.

Funnell MM, Anderson RM, Arnold MS, Barr PA, Donnelly MB, Johnson PD, Taylor-Moon D, White NH. Empowerment: an idea whose time has come in diabetes patient education. Diabetes Educ. 1991;17:37–41.

Funnell MM, Anderson RM, Piatt GA. Empowerment, engagement and shared decisions in the real world of clinical practice. Consultant. 2014;53:358–62.

Funnell MM, Bootle S, Stuckey HL. The diabetes attitudes wishes and needs second study. Clinical Diabetes. 2015;33:32–5.

Glasgow RE, Davis CL, Funnell MM, Beck A. Implementing practical interventions to support chronic illness self-management. Jt Comm J Qual Saf. 2003;29:563–74.

Haas L, Maryniuk M, Beck J, Cox CE, Duker P, Edwards L, Fisher E, Hanson L, Kent D, Kolb L, McLaughlin S, Orzeck E, Piette JD, Rhinehart AS, Rothman R, Sklaroff S, Tomky D, Youssef G. National standards for diabetes self-management education and support. Diabetes Care. 2012;35:2393–401.

Health Literacy Universal Precautions Toolkit, 2nd Edition. Content last reviewed February 2015. Agency for Healthcare Research and Quality, Rockville. http://www.ahrq.gov/professionals/quality-patient-safety/quality-resources/tools/literacy-toolkit/healthlittoolkit2.html. Last accessed 10/11/17.

Heinrich E, Schaper NC, de Vries NK. Self-management interventions for type 2 diabetes: a systematic review. Eur Diabetes Nurs. 2010;7:71–6.

International Diabetes Federation. Standards for diabetes self-management education 2009. http://d-net.idf.org/en/library/123-international-standards-for-diabetes-education.html?tag=52-self-management. Last accessed 2/12/16.

Li R, Shrestha SS, Lipman R, Burrows NR, Kolb LE, Rutledge S. Diabetes self-management education and training among privately insured persons with newly diagnosed diabetes – United States 2011–2012. MMWR. 2014;63:1045–9.

Marrero DG, Ard J, Delamater AM, Peragallo-Dittko V, Mayer-Davis EJ, Nwankwo R, Fisher EB. Twenty-first century behavioral medicine: a context for empowering clinicians and patients with diabetes. A consensus report. Diabetes Care. 2013;36:463–70.

National Diabetes Education Program. Guiding principles for diabetes care. 2015. http://ndep.nih.gov/hcp-businesses-and-schools/guiding-principles/. Last accessed 2.4.16.

Nicolucci A, Burns KK, Holt RIG, on behalf of the DAWN2 Study Group, et al. Diabetes Attitudes, Wishes and Needs second study (DAWN2). Cross-national benchmarking of diabetes-related psycho- social outcomes for people with diabetes. Diabet Med. 2013;30:767–77.

Norris SL, Lau J, Schmid CH, Engelgau MM. Self-management education for adults with type 2 diabetes: a meta-analysis of the effect on glycemic control. Diabetes Care. 2002;25:1159–71.

Pal K, Eastwoood SV, Michie S, Farmer A, Barnard ML, et al. Computer-based interventions to improve self-management in adults with type 2 diabetes: a systematic review and meta-analysis. Diabetes Care. 2014;27:1759–67.

Peyrot M, Egede LE, Campos C, Cannon AJ, Funnell MM, Hsu WC, Ruggerio L, Siminerio LM, Stuckey HL. Ethnic differences in psychological outcomes among people with diabetes: USA results from the second Diabetes Attitudes, Wishes and Needs (DAWN2) study. Curr Med Res Opin. 2014;30:2241–54.

Pillay J, Armstrong MJ, Butalia S, Donovan LE, Sigal RJ, et al. Behavioral programs for type 2 diabetes mellitus: a systematic review and network meta-analysis for effect moderation. Ann Intern Med. 2015a;163:848–60.

Pillay J, Armstrong MJ, Butalia S, Donovan LE, Sigal RJ, et al. Behavioral programs for type 1 diabetes mellitus: a systematic review and network meta-analysis for effect moderation. Ann Intern Med. 2015b;163:836–47.

Powers MA, Bardsley J, Cypress M, Duker P, Funnell MM, Fischl AH, Maryniuk MD, Siminerio L, Vivian E. Diabetes self-management education and support in type 2 diabetes: a joint position statement of the American Diabetes Association, the American Association of Diabetes Educators and the Academy of Nutrition and Dietetics. Diabetes Care. 2015;38:1372–82.

Schillinger D, Grumbach K, Piette J, Wang F, Osmond D, Daher C, et al. Association of health literacy with diabetes outcomes. JAMA. 2002;288:475–82.

Schillinger D, Piette J, Grumbach K, Wang F, Wilson C, Daher C, Leong-Grotz K, Castrto C, Bindman AB. Closing the loop. Physician communication with diabetic patients who have low literacy. Arch Intern Med. 2003;163:83–90.

Steinsbekk A, Rygg LO, Lisulo M, Rise MB, Fretheim A. Group based diabetes self-management education compared to routine treatment for people with type 2 diabetes mellitus. A systematic review with meta-analysis. BMC Health Serv Res. 2012;12:213.

Veroff D, Marr A, Wennberg DE. Enhanced support for shared decision-making reduced costs of care for patients with preference-sensitive conditions. Health Aff (Millwood). 2013;32(2):285–93.

Treatment of Diabetes with Lifestyle Changes: Diet

17

Gabriele Riccardi, Marilena Vitale, and Rosalba Giacco

Contents

Abstract

The present chapter critically reviews scientific evidence on the impact of the diet and its components on the metabolic control, cardiovascular risk factors, and morbidity/mortality in diabetic patients.

Three main topics are included in this chapter: (1) the effects of dietary treatment on body weight control in diabetic patients; (2) the optimal dietary composition in order to achieve blood glucose control and reduce other cardiovascular risk factors associated with type 2 diabetes; (3) the effects of lifestyle modifications and dietary changes on the risk to develop type 2 diabetes.

The overall body of evidence seems to confirm the efficacy of current recommendations for diabetes management. However, although dietary strategies based on structured interventions are often successful, particularly in relation to body

G. Riccardi (✉) · M. Vitale
Department of Clinical Medicine and Surgery, Università degli Studi di Napoli "Federico II", Naples, Italy
e-mail: riccardi@unina.it; marilena.vitale@unina.it

R. Giacco
Institute of Food Science of National Research Council, Avellino, Italy
e-mail: rgiacco@isa.cnr.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_18

weight control, they are not easily applicable in clinical practice and, therefore, more feasible strategies should be identified.

In addition, further intervention studies focused on the effects of lifestyle on hard endpoints in diabetic subjects are needed to definitively prove the role of diet in the prevention of both cardiovascular and microvascular complications in these patients over and above their impact on weight reduction.

Keywords

Healthy diet · Diabetes · Body weight control · Dietary fat · Fiber · Glycemic index

List of Abbreviations

BMI	Body Mass Index
CVD	Cardiovascular diseases
DPS	Diabetes Prevention Study
EASD	European Association for the Study of Diabetes
T2D	Type 2 diabetes
HDL-chol	High density lipoprotein-cholesterol
MedD	Mediterranean diet
NHANES	National Health and Nutrition Examination Survey

Dietary Treatment of Type 2 Diabetes

Weight Loss

Weight gain is a major problem for people with type 2 diabetes. The results of the *Third National Health and Nutrition Examination Survey* (NHANES III) have indicated that the 85.2% of diabetic people were overweight or obese, and the 54.8% were obese, during the years 1999–2002 (Flegal et al. 2002); so, most adults with diabetes are overweight or obese. In addition, it is important to underline that body weight increases with age, and widely prescribed oral hypoglycemic drugs facilitate weight gain. Therefore, encouraging patients to achieve and maintain a healthy weight should be a priority for all diabetes care programs.

In overweight and obese patients with type 2 diabetes, modest and sustained weight loss has been shown to improve glycemic control (by reducing insulin resistance) and plasma lipid, and to reduce blood pressure levels, the need for glucose-lowering, blood pressure, and lipids medications, and cardiovascular mortality (UK Prospective Diabetes Study 1990; Goldstein 1992; Pastors et al. 2002; Wing et al. 2013; Look AHEAD Research Group 2014).

In particular for mortality, an observational study conducting in the United States and involving 4970 overweight people with diabetes (body mass index – BMI $\geq$27 kg/m^2) has showed that an intentional weight loss is associated with a reduction in total mortality of 25% and in diabetes-related and cardiovascular mortality of 28% (Williamson et al. 2000). But, in the same study, the authors also report an U-shaped

relationship between mortality and weight loss; more in detail, the authors show that a body weight reduction ≥30% is associated with slightly increased of mortality (Williamson et al. 2000).

In order to improve blood glucose control and reduce body weight and waist circumference, some studies have demonstrated that an intensive dietary intervention, based on the nutritional recommendations for people with diabetes, is more effective than usual care, in particular in diabetic patients not adequately controlled despite an optimized hypoglycemic drug treatment (Coppell et al. 2010). However, it is important to underline that among overweight or obese patients with type 2 diabetes and inadequate glycemic, blood pressure, and lipid control and/or other obesity-related medical conditions, lifestyle changes that include diet, exercise, and daily/weekly contacts with health professionals are the most effective interventions, as demonstrated by the Look AHEAD trial (Look AHEAD Research Group 2010 and 2014). The Look AHEAD trial is the first study that has investigated the effects of a moderate body weight reduction, obtained by an intensive lifestyle intervention combining a moderate energy restriction with a significant increase of the habitual physical activity, on cardiovascular risk factors and the incidence of cardiovascular events and mortality in a large cohort of overweight and obese individuals with type 2 diabetes. In relation to the cardiovascular risk factors, this study has shown that an intensive lifestyle intervention, compared with an usual education program, represents a good strategy to reduce body weight, improve significantly blood pressure and blood glucose control also in long-term (4 years of follow-up) (Look AHEAD Research Group 2010 and 2014). In addition, in a small number of patients, the intervention has been able to induce a partial or total remission of diabetes. Particularly remarkable is the effect on HDL-cholesterol (HDL-chol), with an increase greater at 4 years than at 1 year. More in detail, in the lifestyle group the HDL-chol was approximately 8–9% higher at each year than the baseline levels, whereas in the control group it remained at 3–6% above baseline. Interestingly, although severely obese participants did not reach their ideal body weight, a significant reduction of blood pressure, plasma glucose, HbA1c, and triglycerides was achieved, confirming the benefits of moderate weight loss (7–10% of initial body weight) in the management of diabetes (Look AHEAD Research Group 2010 and 2014).

Although this approach is clinically meaningful, it is not easily applicable in clinical practice for the great investments in terms of economic and professional resources; thus, more feasible strategies should be identified, considering that there is no single intervention or pattern of interventions suitable for all; weight reducing strategies should be tailored to the individual needs.

To date, studies demonstrating the benefits of weight reduction in people with type 2 diabetes are largely of short duration (up to 6 months); moreover, it is known the effort to keep over time the weight loss, particularly in the absence of the intensive support provided in a clinical trial. Usually, successful individuals can lose approximately 10% of baseline body weight with a hypocaloric regimen, though many regain one-third of this in the following year and all the weight loss within 5 years. Experience in the US National Weight Control Registry suggests that most people who successfully lose weight and maintain weight loss have

experienced a triggering event such as an acute medical condition, so it is possible that a new diagnosis of type 2 diabetes could help to motivate an individual to lose weight (Wing and Phelan 2005).

Very low calorie diets, providing only 800 kcal/day, produce rapid weight loss but are not more effective of conventional diets in the long term; they should be reserved for people with severe obesity (BMI $\geq$35 kg/m^2) as part of a supervised weight management program. Nowadays, a 10 kg weight loss in the first 3–6 months, or 1–2 kg per month, has been proposed for people with diabetes. This weight loss can be attained with lifestyle programs that achieve a 500–750 kcal/day energy deficit or provide approximately 1200–1500 kcal/day for women and 1500–1800 kcal/day for men, adjusted for the individual's baseline body weight. In older people with diabetes, since body weight tends to increase with age, weight stabilization may be a more appropriate strategy.

Optimal Diet Composition

Nutrition therapy has an integral role in overall diabetes management and has the following goals:

- To control plasma glucose levels
- To prevent hypoglycemia, if the patient is treated with oral hypoglycemic drugs or with insulin
- To achieve and maintain a normal body weight
- To prevent or delay complications
- To control blood lipid levels and blood pressure
- To improve the quality of life

The current nutritional recommendations for people with diabetes emphasize the healthful eating patterns containing nutrient-dense, high-quality foods and a focus on specific nutrients. In this context, the Mediterranean diet (Estruch et al. 2013), dietary approaches to stop hypertension (DASH) (Cespedes et al. 2016; Ley et al. 2014), and plant-based diets (Rinaldi et al. 2016) are all examples of healthful eating patterns for people with diabetes, and the "Plate model" could be an example of the simple method applying these recommendations in daily life.

Current dietary recommendations for diabetic patients are particularly focused on optimizing the quantities and food sources of fat and carbohydrates within the same recommendations for healthy eating that applies to the general population. The composition of the diet recommended for people with diabetes is listed in Table 1 (American Diabetes Association 2017; Mann et al. 2004). These recommendations should take into account individual nutrition needs based on personal and cultural preferences, health literacy and numeracy, and access to healthful foods.

Fats

Total fat intake should be reduced to provide no more than 35% of energy. The type of fats consumed is more important than total amount of fat when looking at

Table 1 Medical Nutrition Therapy recommendations for people with diabetes from Scientific Associations of Diabetes specialists

Topic	Specific intake[a]	Recommendations[b]
Energy balance	–	• Modest weight loss achievable by the combination of reduction of calorie intake and lifestyle modification benefits overweight or obese adults with type 2 diabetes and those with prediabetes. Intervention programs to facilitate this process are recommended
Dietary carbohydrates **Added sugar** **Fiber**	45–60% of TE <10% of TE >20 g/ 1000 kcal	• Carbohydrate intake from whole grains, vegetables, fruits, legumes, and dairy products, with an emphasis on foods higher in fiber and lower in glycemic load, should be advised over other sources, especially those containing sugars. • People with diabetes and those at risk should avoid sugar-sweetened beverages in order to control weight and reduce their risk for CVD and fatty liver B and should minimize the consumption of foods with added sugar that have the capacity to displace healthier, more nutrient-dense food choices
Dietary fat **SAFA** **MUFA** **PUFA** **Cholesterol**	<35% of TE <10% of TE 10–20% of TE <10% of TE <300 mg/day	• Whereas data on the ideal total dietary fat content for people with diabetes are inconclusive, an eating plan emphasizing elements of a Mediterranean-style diet rich in monounsaturated fats may improve glucose metabolism and lower CVD risk and can be an effective alternative to a diet low in total fat but relatively high in carbohydrates. • Eating foods rich in long-chain v-3 fatty acids, such as fatty fish (EPA and DHA) and nuts and seeds (ALA) is recommended to prevent or treat CVD; however, evidence does not support a beneficial role for v-3 dietary supplements
Protein	10–20% of TE	• In individuals with type 2 diabetes, ingested protein appears to increase insulin response without increasing plasma glucose concentrations. Therefore, carbohydrate sources high in protein should not be used to treat or prevent hypoglycemia
Sodium	2300 mg/day	• As for the general population, people with diabetes should limit sodium consumption to 2300 mg/day, although further restriction may be indicated for those with both diabetes and hypertension

TE total energy, *SAFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

[a]Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. Diabetes and Nutrition Study Group (DNSG), 2004

[b]American Diabetes Association. Standards of Medical Care in Diabetes 2017

metabolic goals and CVD risk (Office of Disease Prevention and Health Promotion 2015–2020; Estruch et al. 2013; Ros 2003; Forouhi et al. 2016; Wang et al. 2016). Therefore, the fat content of the diet should be manipulated to reduce intake of fats which promote alterations in plasma lipid profile. In particular, saturated and trans fatty acids (found in meat and dairy products, and in hard margarines, some salad dressing, and processed foods, respectively) should provide no more than 10% of

total energy and dietary cholesterol should be limited to less than 300 mg/day (and less than 200 mg/day in people with alteration of lipid metabolism).

Recommendations for the dietary content of other fats reflect a balance between their favorable and adverse metabolic effects. Cis-monounsaturated fats (found in olive oil, peanut oil, sunflower oil, almonds, avocado) have a more favorable metabolic impact and may reduce insulin-resistance and plasma LDL-cholesterol concentrations as compared with saturated fat (Vessby et al. 2001). When they replace carbohydrate in a weight-maintaining diet, these fats are also associated with lower postprandial glycaemia and plasma triglyceride levels. They should nevertheless be limited because they are energy-dense and may cause weight gain, and in large amounts, increase insulin resistance.

Polyunsaturated fatty acids of n-3 series (found in fish and soybean oils) have favorable effects on plasma triglyceride levels and antithrombotic activity but may increase plasma LDL-cholesterol concentrations if consumed in large amounts, usually at pharmacological doses. Dietary n-6 polyunsaturated fatty acids are associated with reduced total and LDL-cholesterol compared with a diet high in saturated fat. Their hypocholesterolemic effect is slightly higher than that achieved with similar amounts of monounsaturated fat; in addition, they have also a small hypotriglyceridemic effect; however, they tend to decrease HDL cholesterol (Ooi et al. 2013).

Carbohydrates

Dietary carbohydrates represent the largest contributor to the energy intake in most countries and the main dietary component able to influence blood glucose levels, particularly in the postprandial state. Therefore, their intake is considered extremely important in the regulation of blood glucose levels in people with diabetes.

In the last few years, the debate on the pros and cons of carbohydrate-rich diets has been very hot on the basis of the possible unfavorable effects of dietary carbohydrates on glycemic control and plasma lipid levels, in particular on increase of plasma triglycerides and decrease of plasma HDL-cholesterol. Many of these controversies arise because it is not always recognized that carbohydrates are a heterogeneous class of nutrients with marked differences in their rate of digestion, absorption, and, therefore, on metabolic effects. So, in order to evaluate the variety of the blood glucose response in vivo after a meal containing carbohydrates, it is important to consider other important component, as the fiber content, the chemical composition of carbohydrates, and the physical structure of the foods present in the meal. To account for all these factors, Jenkins et al. (1981) introduced the concept of the glycemic index (GI), which attempts to quantify the potency of carbohydrate foods to raise blood glucose levels in vivo. Most trials that have compared the effects of low-GI and high-GI diets have shown that low-GI foods have more favorable effects on glycemic control (Riccardi et al. 2008). In nondiabetic populations, low-GI diets have been associated with lower plasma levels of insulin and lipids and improved glucose tolerance (Bell et al. 2015; Brand-Miller et al. 2009). In people with type 2 diabetes, a low-GI diet is associated with better glycemic control (as indicated by lower HbA1C or fructosamine levels) (Brand-Miller et al. 2003;

Thomas and Elliott 2010). However, some studies have detected no difference between low- and high-GI foods on plasma lipids in people with diabetes.

A recent meta-analysis comparing the effects of low-GI diets with conventional or high-GI diets on glycemic control in patients with diabetes concluded that the low-GI diets improve the glycemic control reducing HbA1C by about 6% (for example, reducing an HbA1c of 8% to approximately 7.5%) (Wang et al. 2015). This effect is very important in people with diabetes because it is known that HbA1c is continuously related to the risk of diabetes complications, as demonstrated by the results of the United Kingdom Prospective Diabetes Study (UKPDS) in which the reduction of HbA1c by one percentage point was associated with a reduction of 21% in diabetes-related deaths and complications (UK Prospective Diabetes Study Group, 1998). Therefore, any reduction of glycated hemoglobin is welcome.

Based on this context, all nutritional recommendations available for people with diabetes consider the GI of foods the most important parameters for carbohydrates consumption. More in detail, European dietary recommendations state that "*Carbohydrate-containing foods which are high in dietary fibre or have a low glycaemic index are especially recommended*" (The Task Force on diabetes, pre-diabetes, and cardiovascular diseases 2013). The justification for this stance is that low-GI foods may help to improve glycemic control and lipid levels.

Also the American Diabetes Association and the UK recommendations recommend the utilization of the GI in the diet for people with diabetes (American Diabetes Association 2017; Dyson et al. 2011). However, according to ADA, it should always be taken into account that many healthy foods have a higher GI than foods with little nutritional value. For example, oatmeal has a higher GI than chocolate. Therefore, it is important to remember that the GI represents the type of carbohydrate in a food but says nothing about the amount of carbohydrate typically eaten. For this reason, the glycemic load (GL) has been proposed as a marker of the impact of a food on postprandial blood glucose since it takes into account both the amount of carbohydrate present in the portion of food eaten and the glycemic impact of that specific food as compared with a reference food like white bread.

The glycemic load (GL) is calculated by multiplying the GI of a food by the amount of carbohydrate in grams per serving and dividing the total by 100.

There are other important reasons for encouraging consumption of low-GI foods. In general, foods with a low glycemic index tend to be high in fiber and micronutrients – for example, legumes, oats, pasta, and some raw fruits have low GI values (Atkinson et al. 2008).

Dietary Fibers

Intake of dietary fiber is associated with lower all-cause mortality in people with diabetes. There is a large body of evidence that a diet moderately rich in carbohydrates and fibers, and, consequently, with a low glycemic index and mainly based on consumption of legumes, vegetables, fruits, and whole grain cereals improves blood glucose control and reduces plasma cholesterol levels in diabetic patients as compared with a low carbohydrate-low fiber diet. In particular, this type of diet keeps low plasma insulin and triglyceride concentrations despite its higher carbohydrate intake

and induces also a significant reduction in postprandial blood glucose and triglyceride rich lipoprotein levels which play a relevant role in modulating the cardiovascular risk in patients with type 2 diabetes (De Natale et al. 2009). The net LDL-cholesterol reduction due to the doubling of fiber intake can be more than 10%. The beneficial effects of high-fiber diets on LDL-cholesterol have been confirmed by a meta-analysis comparing the effects of low-GI vs. high-GI diets. The significant decrease in LDL-cholesterol observed with the low-GI diets was related to their fiber content and, in fact, it was not any more evident when studies with high-fiber diets were excluded from the analysis (Goff et al. 2013).

Dietary fiber seems able to counteract the rising effect of carbohydrates on fasting triglycerides. In the last years, much attention has been paid to postprandial lipemia as a cardiovascular risk factor and, indeed, large epidemiological studies suggest that postprandial triglycerides are a stronger cardiovascular risk factor than fasting triglyceride levels (Bansal et al. 2007). Different dietary components modulate the postprandial triglyceride response. Recently, much attention has been devoted to the effects of dietary fiber on postprandial triglycerides. In people with type 2 diabetes, a fiber-rich diet reduces the postprandial triglyceride response, mainly due to the reduction of lipoproteins carrying exogenous lipids. On the same line, a diet based on wholegrain cereals, as compared to a diet with refined cereals, reduces postprandial triglyceride levels by 40% in people with the metabolic syndrome (Giacco et al. 2014). In this study, the decrease in postprandial triglycerides was significantly and inversely correlated with the intake of cereal fiber, supporting the role of cereal fiber in the modulation of the postprandial metabolism.

The effects of dietary fiber on HDL-cholesterol are negligible. Together with the reduction of LDL-cholesterol, a small decrease in HDL-cholesterol has been reported with high-fiber diets in some studies; however, on the overall, the magnitude of this effect is much less relevant than that obtained on LDL-cholesterol. Although solubility of fiber was thought to determine physiological effect, more recent studies suggest that other properties of fiber, such as fermentability and viscosity may be more important (Slavin 2013). As a matter of fact, dietary fibers improve glucose and lipid metabolism slowing food digestion and nutrient absorption and producing in the colon short-chain fatty acids that, in turn, modulate liver glucose production and lipid synthesis. Therefore, people with diabetes should not be excluded from the public health campaign that encourages eating five portions of fruit and vegetables a day and promotes wholegrain cereal foods as a substitute for the refined ones. In addition, for diabetic patients it may be helpful, among the high fiber foods, to tilt the balance of consumption in favor of those with a low GI.

Sugar

In the past, people with diabetes were recommended to completely avoid sugar. In fact, it was believed that eating sugar would raise blood glucose. Conversely, available scientific evidence from clinical studies shows that dietary sucrose influences blood glucose levels not more than an equivalent caloric amounts of starch. It is important to underline that the excess of energy intake from nutritive sweeteners or foods and

beverages containing high amounts of nutritive sweeteners should be avoided, since they provide "empty" calories and can lead to weight gain (Evert et al. 2013).

Fructose is a common naturally occurring monosaccharide found in fruits, in some vegetables, and honey and is also widely used as sweetener of drink or added in processed foods in substitution of sucrose. Fructose consumed as "free fructose" (i.e., naturally occurring in foods such as fruit) may result in better glycemic control compared with isocaloric intake of sucrose or starch, and free fructose is not likely to have detrimental effects on triglycerides as long as its intake is kept low (less than 5% energy).

People with diabetes should limit or avoid intake of sugar-sweetened beverages (SSBs) (from any caloric sweetener, including high-fructose corn syrup and sucrose) to reduce the risk of weight gain and worsening the cardiometabolic profile.

A meta-analysis of controlled intervention studies with a duration of less than 12 weeks in people with diabetes compared the impact of fructose with that of other sources of carbohydrate on glycemic control (Cozma et al. 2012). The results showed that an isocaloric exchange of fructose for other carbohydrates did not significantly affect fasting glucose or insulin and reduced glycated blood proteins. However, strong evidence exists that consuming high levels of fructose-containing beverages may have particularly adverse effects on selective deposition of visceral fat, lipid metabolism, blood pressure, and insulin sensitivity (Evert et al. 2013). Thus, recommendations for diabetic people about sugar intake should on the one hand consider the unfeasibility of too stringent limitations of added sugar, particularly for children but, on the other hand, should take into account potential metabolic consequences of excessive consumption of sweetened foods and, even more, beverages, particularly soft drinks, that could lead to further deterioration of insulin resistance and obesity.

Protein

Protein intake in economically developed countries is high and exceeds metabolic needs; in the United States, for example, it is estimated that protein accounts for 10–20% of the energy intake. Current recommended limits are based on a pragmatic interpretation of the available evidence and awareness that attempts to restrict protein intake below 0.6 g/kg/day may precipitate nutritional deficiency.

Protein does not affect the rate at which glucose is absorbed from a meal or postprandial blood glucose levels. In people with type 1 diabetes and incipient nephropathy, high protein intake may increase the progression of renal disease; however, little is known about the effects of high protein consumption in people with type 2 diabetes. Protein increases satiety; diets that are high in protein but low in carbohydrate do achieve weight loss but not more so than other types of calorie restriction diets. Furthermore, such diets tend to be high in fat and this may induce plasma LDL-cholesterol increases. There is no evidence that high protein diets are beneficial in people with type 2 diabetes.

There is no strong evidence to suggest higher benefits from plant protein as compared to animal protein; however, considering that foods as meat, processed meat, milk products, eggs, although rich in essential aminoacids are also rich in saturated fats, it is appropriate to limit animal protein intake (National Kidney Foundation 2012).

Diet and Cardiovascular Morbidity/Mortality

The inverse association between the adherence to a healthy diet (Mediterranean diet, DASH diet, Prudent diet) and cardiovascular disease has been found in many large prospective studies in nondiabetic populations (Sofi et al. 2010; Salehi-Abargouei et al. 2013; Hu et al. 2000) and some data are available also for diabetic patients.

More in detail, a greater adherence to a healthy diet, characterized by high consumption of whole-grains, vegetables, fruit, nuts, and fish compared to meat, poultry, and eggs, is associated with a reduction by 20% of recurrent cardiovascular events in a large cohort of patients with previous CVD and/or diabetes. These data indicate that a healthy diet may be important not only in primary prevention but also in secondary prevention or in high CV-risk individuals, such as diabetic patients. Moreover, the beneficial effects are in addition to those obtained with the pharmacological therapy generally used in secondary prevention (Dehghan et al. 2012).

The association between diet and mortality in type 1 diabetic subjects has been investigated in the EURODIAB study that is the first European prospective study on this issue; the results have shown that, in a cohort of almost 2000 subjects, a 5 g-increase of fiber intake, especially soluble fiber, within the range commonly consumed in patients with type 1 diabetes (11.3–28.3 g/day) is associated with lower CVD mortality (−16%) and all-cause mortality (−28%) (Schoenaker et al. 2012) confirming the importance of dietary fibers in diabetes management also for what concerns type 1 diabetes.

The casual relationship between diet and cardiovascular risk in type 2 diabetic patients has been evaluated in the Look AHEAD trial (The Look AHEAD Research Group 2013). This study has shown that an intensive lifestyle modification program focused on weight reduction is able to improve all cardiovascular risk factors, as reported above, whereas does not reduce the occurrence of cardiovascular events and mortality compared to the usual care group in the long term. Completely different are the results obtained in the PREDIMED study (Estruch et al. 2013) which was not focused on reducing excessive body weight but aimed exclusively at achieving dietary modifications resembling the traditional Mediterranean diet. In fact, in this study a Mediterranean diets supplemented with either extra-virgin olive oil or nuts was able to reduce significantly (almost 30%) the incidence of major CV events compared to the control diet in high-risk individuals, including subjects with T2D ($n = 3614$, almost 50% of the total population).

Although the early termination of the trial may lead to an overestimation of treatment effects (Bassler et al. 2010), the results suggest that changes in diet composition, even small, may be really effective, possibly more than weight reduction, in reducing CVD in type 2 diabetic subjects.

Diet and Type 2 Diabetes Prevention

Diet represents the cornerstone of diabetes treatment since it can induce significant improvements of blood glucose control and other metabolic cardiovascular risk factors (Franz et al. 2010; Lindström et al. 2006) and might potentially reduce the

Table 2 Foods, nutrients and dietary patterns associated with risk of developing type 2 diabetes

	Increased risk	Degree of evidence	Decreased risk	Degree of evidence
Foods	Soft drinks	++	Whole grains	++
	Red meat and processed meat	++	Tea and coffee	++
	Oil and hydrogenated margarines	+	Milk and dairy products low in fat	++
	Eggs	+	Fruits, vegetables, legumes	++
	High alcohol consumption	++	Moderate alcohol consumption	+
			Nuts	+
Nutrients	Saturated fatty acids	+	Fibers	++
	Trans fatty acids	+	Unsaturated fatty acids	++
			Antioxidants	+
			Magnesium	+
Dietary patterns	High glycemic load	++	Mediterranean diet	+++
	Western diet	++		

Degree of evidence from prospective epidemiological studies = +++ High; ++ Moderate; + Reasonable

risk of long-term complications (Laakso 1999). A healthy diet, as part of an appropriate lifestyle, is also able to prevent type 2 diabetes. Studies using lifestyle interventions in people with impaired glucose tolerance have shown a reduction in diabetes incidence (Eriksson and Lindgärde 1991; Pan et al. 1997; Tuomilehto et al. 2001; Knowler et al. 2002; Ramachandran et al. 2006; Kosaka et al. 2005). Lifestyle intervention in these studies lasting for 3–6 years emphasized body weight control (weight reduction >5–10% of initial body weight), physical activity, and dietary modifications such as a fat intake <30% of daily energy intake, saturated fat <10% of daily energy intake, and a fiber intake >15 g/1000 kcal. In particular, both the Finnish Diabetes Prevention Study (Tuomilehto et al. 2001) and the US Diabetes Prevention Program (Knowler et al. 2002) showed a 58% relative risk reduction in the progression from impaired glucose tolerance to type 2 diabetes, during a mean intervention period of about 3 years.

Beside these studies focused on the effects of lifestyle modifications and dietary changes on the risk to develop type 2 diabetes, several observational studies have shown an association between consumption of specific food groups or healthy dietary patterns and the risk of type 2 diabetes (Table 2). The EPIC-Potsdam study, in line with previous studies, has confirmed that higher intakes of whole-grain bread, fruits, raw vegetables, and coffee are inversely associated with type 2 diabetes risk in a large cohort of healthy subjects, during an average follow-up of 8 years (vonRuesten et al. 2013). These foods are good sources of dietary fiber and antioxidant, vitamins and minerals that could contribute to their protective role against type 2 diabetes. In support of their role in diabetes prevention is also the evidence that fiber rich foods have a lower impact on blood glucose levels after a meal. Indeed all pharmacological (Chiasson et al. 2002) and nonpharmacological interventions tested so far in people

with pre-diabetes, able to reduce glycemia after meals with whatever mechanism, have proven effective in the prevention of type 2 diabetes.

Conversely, high intakes of red meat, butter, sauces and fat dairy are associated with an increased risk of type 2 diabetes (vonRuesten et al. 2013). Fish consumption in some studies has been found to be associated with a lower risk of diabetes (Nkondjock and Receveur 2003; Adler et al. 1994). Whether the protective effect of fish is due to its n-3 fatty acid content or to other components, such as protein, is a matter of debate. The mechanisms by which fat consumption could influence the development of diabetes is strictly linked to insulin sensitivity. In fact, dietary fat can influence insulin sensitivity independently of any change in body weight; this influence will obviously affect also the development of the Metabolic Syndrome which is strongly associated with impaired insulin sensitivity. Animal studies have clearly shown that a high-fat diet, particularly if high in saturated fat, decreases insulin sensitivity. Several cross-sectional studies have examined dietary fat in relation to fasting and post-load plasma insulin concentrations, which are both markers of insulin resistance. The consistent finding is a positive association between saturated fat intake and hyperinsulinemia, independently of body fat. These data have been partly confirmed in human intervention studies using more accurate techniques to evaluate insulin resistance. Why dietary fat quality can influence insulin sensitivity is not completely understood; however, the effects of dietary fatty acids on insulin sensitivity are thought to be mediated, at least partially, by the fatty acid composition of cell membranes (Riccardi et al. 2004). A specific fatty acid profile in cell membranes could influence insulin action through several potential mechanisms, including altered insulin receptor binding or affinity, and by influencing ion permeability and cell signaling. Insulin resistant states are associated with a plasma fatty acid pattern characterized by an increased proportion of palmitic acid and a low proportion of linoleic acid, with a distribution of other fatty acids that indicates an increased activity of D9- and D6-desaturases. These changes are possibly related, to a large extent, to the type of fat in the diet and are consistent with a diet where animal (saturated) fat consumption is increased and vegetable (unsaturated) fat consumption is reduced. The deteriorating effect of saturated fat on insulin sensitivity is supported by controlled intervention studies in which the comparison was performed between saturated fat and either monounsaturated or polyunsaturated fat (Vessby et al. 2001).

Looking at the association between dietary pattern and risk of type 2 diabetes, data from epidemiological study have shown that an higher adherence to a Mediterranean dietary pattern is associated with a significant reduction by 12% of type 2 diabetes risk compared with individuals with lower adherence to Mediterranean diet in a large cohort of healthy subjects from Mediterranean and non-Mediterranean countries (InterAct Consortium et al. 2011).

The role of dietary patterns and, in particular, of the Mediterranean diet in reducing type 2 diabetes risk has been clearly reinforced by the results of the PREDIMED study (Salas-Salvadó et al. 2011). After a median follow-up of 4 years, a multivariable adjusted hazard ratio for the incidence of type 2 diabetes was almost 50% lower in the participants assigned to the MedD as compared to the

control diet. In addition, increased adherence to MedD was inversely associated with the development of diabetes. It has to be underlined that in this study the reduction of type 2 diabetes was observed in absence of any significant changes in body weight or physical activity, suggesting that the mechanisms involved in diabetes risk reduction in this study are independent from body weight loss and could be related to an improvement in insulin sensitivity and/or a reduction of oxidative stress and inflammation.

In addition to the effect of diet composition, new data from the Diabetes Prevention Study (DPS) have further outlined the importance of more global lifestyle modifications on the reduction of type 2 diabetes; in fact, the benefits of moderate weight reduction, together with an increase of physical activity and changes in diet composition, are preserved in the long term, even many years after the conclusion of the intervention (Lindström et al. 2013).

Although lifestyle interventions are not easily applicable in real-life, the European Diabetes Prevention Study (EDIPS) has recently shown that the Finnish DPS protocol can be applicable with success in other European countries reducing by 57% the cumulative type 2 diabetes incidence during a mean follow-up of 3.1 years (Penn et al. 2013).

References

Adler AI, Boyko EJ, Schraer CD, et al. Lower prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption among Alaska natives. Diabetes Care. 1994;17:1498–501.

American Diabetes Association. Standards of medical care in diabetes – 2017. Diabetes Care. 2017;40(Suppl 1):S1–S135.

Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care. 2008;31(12):2281–3.

Bansal S, Buring JE, Rifai N, et al. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA. 2007;298(3):309–16.

Bassler D, Briel M, Montori VM, et al. Stopping randomized trials early for benefit and estimation of treatment effects: systematic review and meta-regression analysis. JAMA. 2010;303:1180–7.

Bell KJ, Bao J, Petocz P, Colagiuri S, Brand-Miller JC. Validation of the food insulin index in lean, young, healthy individuals, and type 2 diabetes in the context of mixed meals: an acute randomized crossover trial. Am J Clin Nutr. 2015;102(4):801–6.

Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. Diabetes Care. 2003;26(8):2261–7.

Brand-Miller JC, Stockmann K, Atkinson F, Petocz P, Denyer G. Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects: analysis of a database of more than 1,000 foods. Am J Clin Nutr. 2009;89(1):97–105.

Cespedes EM, Hu FB, Tinker L, et al. Multiple healthful dietary patterns and type 2 diabetes in the women's health initiative. Am J Epidemiol. 2016;183:622–33.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, STOP-NIDDM Trail Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002;359(9323):2072–7.

Coppell KJ, Kataoka M, Williams SM, et al. Nutritional intervention in patients with type 2 diabetes who are hyperglycaemic despite optimised drug treatment–lifestyle over and above drugs in diabetes (LOADD) study: randomised controlled trial. BMJ. 2010;341:c3337.

Cozma AI, Sievenpiper JL, de Souza RJ, et al. Effect of fructose on glycemic control in diabetes: a systematic review and meta-analysis of controlled feeding trials. Diabetes Care. 2012;35:1611–20.

De Natale C, Annuzzi G, Bozzetto L, Mazzarella R, Costabile G, Ciano O, Riccardi G, Rivellese AA. Effects of a plant-based high-carbohydrate/high-fiber diet versus high-monounsaturated fat/low-carbohydrate diet on postprandial lipids in type 2 diabetic patients. Diabetes Care. 2009;32(12):2168–73.

Dehghan M, Mente A, Teo KK, et al. Relationship between healthy diet and risk of cardiovascular disease among patients on drug therapies for secondary prevention: a prospective cohort study of 31 546 high-risk individuals from 40 countries. Circulation. 2012;126:2705–12.

Dyson PA, Kelly T, Deakin T, Duncan A, Frost G, Harrison Z, Khatri D, Kunka D, McArdle P, Mellor D, Oliver L, Worth J, Diabetes UK Nutrition Working Group. Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. Diabet Med. 2011; 28(11):1282–8.

Eriksson KF, Lindgärde F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmö feasibility study. Diabetologia. 1991;34(12):891–8.

Estruch R, Ros E, Salas-Salvadó J, PREDIMED Study Investigators, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368:1279–90.

Evert AB, Boucher JL, et al. Nutrition therapy recommendations for the Management of adults with diabetes: position statement by the ADA. Diabetes Care. 2013;36:3821–42.

Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999–2000. JAMA. 2002;288(14):1723–7.

Forouhi NG, Imamura F, Sharp SJ, et al. Association of plasma phospholipid n-3 and n-6 polyunsaturated fatty acids with type 2 diabetes: the EPIC-InterAct Case-Cohort Study. PLoS Med. 2016;13:e1002094.

Franz MJ, Powers MA, Leontos C, et al. The evidence for medical nutrition therapy for type 1 and type 2 diabetes in adults. J Am Diet Assoc. 2010;110:1852–89.

Giacco R, Costabile G, Della Pepa G, et al. A whole-grain cereal-based diet lowers postprandial plasma insulin and triglyceride levels in individuals with metabolic syndrome. Nutr Metab Cardiovasc Dis. 2014;24(8):837–44.

Goff LM, Cowland DE, Hooper L, Frost GS. Low glycaemic index diets and blood lipids: a systematic review and meta-analysis of randomised controlled trials. Nutr Metab Cardiovasc Dis. 2013;23(1):1–10.

Goldstein DJ. Beneficial health effects of modest weight loss. Int J Obes Relat Metab Disord. 1992;16:397–415.

Hu FB, Rimm EB, Stampfer MJ, et al. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr. 2000;72:912–21.

InterAct Consortium, Romaguera D, Guevara M, et al. Mediterranean diet and type 2 diabetes risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study: the InterAct project. Diabetes Care. 2011;34:1913–8.

Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981;34:362–6.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.

Kosaka K, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. Diabetes Res Clin Pract. 2005;67(2):152–62.

Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes. 1999;48:937–42.

Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet. 2014;383:1999–2007.

Lindström J, Peltonen M, Eriksson JG, et al. High-fibre, low-fat diet predicts long-term weight loss and decreased type 2 diabetes risk: the Finnish Diabetes Prevention Study. Diabetologia. 2006;49:912–20.

Lindström J, Peltonen M, Eriksson JG, et al. Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). Diabetologia. 2013;56:284–93.

Look AHEAD Research Group. Eight-year weight losses with an intensive lifestyle intervention: the Look AHEAD study. Obesity (Silver Spring). 2014;22:5–13.

Look AHEAD Research Group. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med. 2010;170:1566–75.

Mann JI, De Leeuw I, Hermansen K, Diabetes and Nutrition Study Group (DNSG), et al. Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. Nutr Metab Cardiovasc Dis. 2004;14:373–94.

National Kidney Foundation. KDOQI clinical practice guidelines for diabetes and chronic kidney disease. Am J Kidney Dis. 2012;49(Suppl 2):S1–S179.

Nkondjock A, Receveur O. Fish-seafood consumption, obesity, and risk of type 2 diabetes: an ecological study. Diabetes Metab. 2003;29:635–42.

Office of Disease Prevention and Health Promotion, U.S. Department of Health and Human Services. Dietary Guidelines for Americans: 2015–2020. 8th ed. Available from https://health.gov/dietaryguidelines/2015/guidelines/.

Ooi EM, Ng TW, Watts GF, Barrett PH. Dietary fatty acids and lipoprotein metabolism: new insights and updates. Curr Opin Lipidol. 2013;24(3):192–7.

Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care. 1997;20(4):537–44.

Pastors JG, Warshaw H, Daly A, Franz M, Kulkarni K. The evidence for the effectiveness of medical nutrition therapy in diabetes management. Diabetes Care. 2002;25:608–13.

Penn L, White M, Lindström J, et al. Importance of weight loss maintenance and risk prediction in the prevention of type 2 diabetes: analysis of European Diabetes Prevention Study RCT. PLoS One. 2013;8:e57143.

Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V, Indian Diabetes Prevention Programme (IDPP). The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49(2):289–97.

Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr. 2004;23(4):447–56. Review.

Riccardi G, Rivellese AA, Giacco R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. Am J Clin Nutr. 2008;87(1):269S–74S.

Rinaldi S, Campbell EE, Fournier J, O'Connor C, Madill J. A comprehensive review of the literature supporting recommendations from the Canadian Diabetes Association for the use of a plant-based diet for management of type 2 diabetes. Can J Diabetes. 2016;40:471–7.

Ros E. Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. Am J Clin Nutr. 2003;78(Suppl):617S–25S.

Salas-Salvadó J, Bulló M, Babio N, et al. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes Care. 2011;34:14–9.

Salehi-Abargouei A, Maghsoudi Z, Shirani F, Azadbakht L. Effects of dietary approaches to stop hypertension (DASH)-style diet on fatal or nonfatal cardiovascular diseases–incidence: a systematic review and meta-analysis on observational prospective studies. Nutrition. 2013;29:611–8.

Schoenaker DA, Toeller M, Chaturvedi N, et al. Dietary saturated fat and fibre and risk of cardiovascular disease and all-cause mortality among type 1 diabetic patients: the EURODIAB Prospective Complications Study. Diabetologia. 2012;55:2132–41.

Slavin J. Fiber and prebiotics: mechanisms and health benefits. Nutrients. 2013;5(4):1417–35. https://doi.org/10.3390/nu5041417. Review.

Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr. 2010;92:1189–96.

The Look AHEAD Research Group. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. N Engl J Med. 2013;369(2):145–54.

The Task Force on diabetes, pre-diabetes, and cardiovascular diseases. ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2013;34:3035.

Thomas DE, Elliott EJ. The use of low-glycaemic index diets in diabetes control. Br J Nutr. 2010;104(6):797–802.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M, Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344(18):1343–50.

UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854–65.

UK Prospective Diabetes Study 7. UK Prospective Diabetes Study 7: response of fasting plasma glucose to diet therapy in newly presenting type II diabetic patients, UKPDS Group. Metabolism. 1990;39:905–12.

Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nälsén C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH, KANWU Study. Diabetologia. 2001;44(3):312–9.

vonRuesten A, Feller S, Bergmann MM, Boeing H. Diet and risk of chronic diseases: results from the first 8 years of follow-up in the EPIC-Potsdam study. Eur J Clin Nutr. 2013;67:412–9.

Wang Q, Xia W, Zhao Z, Zhang H. Effects comparison between low glycemic index diets and high glycemic index diets on HbA1c and fructosamine for patients with diabetes: a systematic review and meta-analysis. Prim Care Diabetes. 2015;9(5):362–9. https://doi.org/10.1016/j.pcd.2014.10.008. Epub 2014 Dec 16.

Wang DD, Li Y, Chiuve SE, et al. Association of specific dietary fats with total and causespecific mortality. JAMA Intern Med. 2016;176:1134–45.

Williamson DF, Thompson TJ, Thun M, Flanders D, Pamuk E, Byers T. Intentional weight loss and mortality among overweight individuals with diabetes. Diabetes Care. 2000;23(10):1499–504.

Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr. 2005;82(Suppl 1):222S–5S.

Wing RR, Bolin P, Brancati FL, et al. Look AHEAD Research Group. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. N Engl J Med. 2013;369:145–54.

Treatment of Diabetes with Lifestyle Changes: Physical Activity

18

Roberto Codella, Ileana Terruzzi, and Livio Luzi

Contents

Abstract

Lifestyle improvements, like dietary changes and increased physical activity, are typically advocated for the cure, prevention, and reversion of several metabolic diseases, including diabetes mellitus. The non-pharmacological low-cost nature, along with the health-related benefits, increases the therapeutical *appeal* of regular physical activity. In the comprehensive approach of diabetes management, regular physical activity reduces risk of many diseases to which individuals

R. Codella · L. Luzi (✉)
Department of Biomedical Sciences for Health, IRCCS Policlinico San Donato Milanese, Università degli Studi di Milano, Milan, Italy

Metabolism Research Center, IRCCS Policlinico San Donato, San Donato Milanese, Italy
e-mail: roberto.codella@unimi.it; livio.luzi@unimi.it; Livio.Luzi@grupposandonato.it

I. Terruzzi
Diabetes Research Institute, Metabolism, Nutrigenomics and Cellular Differentiation Unit, San Raffaele Scientific Institute, Milan, Italy

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_25

with diabetes, particularly those with type 2 diabetes mellitus, are predisposed: hypertension, coronary heart diseases, and obesity.

The present chapter covers how exercise can facilitate optimal glucose control and lipid levels, assist in weight management, and prevent exacerbation of underlying diabetes-complications, moving medicine forward, far beyond the simplistic *motto* of "exercise more."

Keywords

Diabetes management · Exercise benefits · Physical activity

List of Abbreviations

CGM	Continuous glucose monitoring
CVD	Cardiovascular diseases
FBG	Fasting blood glucose
GLUT4	Glucose transporter 4
HbA1c	Glycosylated hemoglobin
HDL	High density lipoproteins
ICT	Information and communication technologies
IGT	Impaired glucose tolerance
PI3-kinase	Phosphoinositide 3-kinase
SMBG	Self-monitoring of blood glucose

Being Physically Active: An Ancient *Leitmotiv*

We have been repeated for a lifetime that exercise is a cornerstone in the treatment of a myriad of modern-day diseases such as diabetes and cardiovascular diseases. However, epidemiological studies confirm that diabetes, prediabetes, and related metabolic disorders are still on the rise, and their associated burden is undoubtedly growing. This is certainly a side-effect of the achieved longevity and expanded life expectancy. By just looking at the history of our evolution, being physically active for humans seems more natural than remaining "sedentary," so that someone argued, in different contexts, that we were "born to run" (Bramble and Lieberman 2004; Luzi and Pizzini 2004; Livio Luzi 2012).

The *International Diabetes Federation* (IDF) recognizes diabetes as one of the largest global health emergencies of the twenty-first century. According to the IDF, in 2015, 415 million of people were estimated to have diabetes worldwide, and this number was projected to increase up to 642 million by 2040. In addition to those, there are 318 million adults with impaired glucose tolerance (IGT), which puts them at high risk of developing the disease in the future (International Diabetes Federation 2015).

Despite the health-promoting benefits of exercise, like obtaining optimal blood glucose control, hardly more than half of individuals with diabetes currently are committed to accomplish the prescribed treatment goals (Cheung et al. 2009), including exercise recommendations. Nowadays we have compelling evidence that

exercise has a greater therapeutic potency than most drugs for treating type 2 diabetes, and likely several other chronic diseases.

The fascinating challenge will be whether we can capture the intimate mechanisms by which exercise-related healthspan has come to the forefront of biological investigation. The ultimate goal will be not to provide a "polypill," abstaining people from exercising to maintain health, rather to transmit these long-term exercise benefits to subjects with debilitating illnesses. To switch on this exercise revolution, it will be necessary an extensive educational approach, involving not only clinical research and practice but also many health-care providers and fitness professionals, frequently unprepared to the heavy task of the exercise prescription.

Exercise-Related Health Benefits

Human physiology is challenged by exercise, whole-body and in particular at muscular level, depending on type, intensity, duration, physical fitness, and nutritional status. Most of the exercise-related health benefits are measurable over time, such as modifications in musculoskeletal fitness, body composition, cardiovascular fitness, and metabolic control. These health indicators are critical because they allow monitoring patients' overall conditions, the compliance to exercise training, and, ultimately, the success of a certain therapeutic strategy.

In spite of being one of the most efficacious treatments of type 2 diabetes, physical activity has been too often underperforming by diabetic patients. This is possibly due to the incomplete comprehension of the morbidic phenomenon as well as insufficient self-management of the disease. Surprisingly, many health-care providers are still hesitant to prescribe exercise, and fitness professionals might not be fully aware of the precautions to be taken for tackling complications, especially if exercise is an added variable.

On the whole, exercise as a therapeutic modality remains particularly beneficial, given its impact on insulin resistance – the underlying abnormality of this metabolic disorder.

Specifically, on the one hand, exercise increases insulin-mediated muscular glucose uptake, and on the other, it augments insulin sensitivity and insulin action. It is noteworthy that although type 2 diabetic subjects are insulin resistant, they are not resistant to the stimulatory effects of exercise on glucose stimulation (Kennedy et al. 1999). Individuals with type 2 diabetes maintain the capacity to translocate GLUT4 to the sarcolemma in response to exercise. However, glucose transport induced by the simple muscle contraction has to be distinguished from insulin-stimulated glucose transport (Sakamoto and Goodyear 2002; Wojtaszewski et al. 2002). Although GLUT4 translocation to the sarcolemma is been increased by both insulin and exercise (both aerobic and resistance), stimuli for recruiting this carrier originate from different intracellular pools (Coderre et al. 1995; Hayashi et al. 1997). Separated cellular signals for insulin-stimulated and exercise-induced glucose uptake are evidenced by the fact that muscle contraction does not enhance phosphorylation of insulin receptor substrate (IRS-1, IRS-2) nor does it with other

intracellular proteins such as phosphatidylinositol kinase (PI-3 kinase), i.e., the insulin-dependent proteins (Goodyear et al. 1995). Furthermore, wortmannin, a PI-3 kinase inhibitor, interdicts insulin-stimulated glucose uptake but not the one induced by muscle contraction (Lund et al. 1995; Wojtaszewski et al. 1996). Albeit exercise and training markedly increase insulin-stimulatory effect on recruitment of transporters, a prominent part of these effects relies on insulin-independent component of glucose uptake (Manetta et al. 2000). In closing, physical activity has an additive action to insulin, and altogether they exert a synergistic force in insulin-sensitive tissues.

Diverse mechanisms have been postulated to explain how exercise enhances insulin action. For example, hemodynamic adaptations to training promote insulin availability within tissues (increasing in the surface diffusion of the endothelial cells of the capillaries) (Goodyear and Kahn 1998). Furthermore, glycogen concentration pre-exercise is another key contributing factor in the regulation of the glucose transport and of the glycogen-synthase activity (Wojtaszewski et al. 1999). Particularly, the pre-exercise glycogen availability is inversely correlated to the intensity of the insulin response (Richter et al. 2001). Also, restoration of muscle glycogen following exercise occurs in two phases (Garetto et al. 1984). During the first phase, glucose uptake is elevated as well as glycogen-synthase activity; therefore, muscle glycogen is rapidly replenished. This post-exercise (first) phase is insulin-independent. During the second phase, instead, insulin action is boosted. However, the augmented insulin-sensitivity in the exercising muscle persists even when glycogen resynthesis has been completed (Cartee et al. 1989). Yet, exercise can enhance insulin action through indirect effects mediated by insulin-induced suppression of NEFA levels (Suh et al. 2007).

Persons with type 2 diabetes may report these defects in insulin action (glucose transport, phosphorylation), genetically or acquired (abdominal obesity). Chronic hyperglycemia and increased NEFA levels may also worsen insulin resistance (Randle et al. 1963). However, these defects may be reversible (ACSM-ADA 2010). Exercise not only improves insulin sensitivity but also modifies hypertension and lipid abnormalities.

Acute Effects of Physical Activity in Type 2 Diabetic Subjects

The metabolic response to acute exercise is influenced by different factors (diet, age, type of exercise, pre-exercise conditions); however, either in type 2 diabetic or healthy subjects, the extent of the glucose-lowering effect is correlated with the exercise intensity (Ohlson et al. 1985). Improvements in glucose tolerance and insulin sensitivity start to deteriorate after 2–72 h from last session of mild- and moderate-intensity exercise (Boulé et al. 2001; Cartee et al. 1989; Galbo et al. 2007; O'Gorman et al. 2006). Subsequently, exercise emerges as an obvious treatment modality to maintain:

- Low blood glucose
- Augmented insulin sensitivity

Due to insulin resistance, those with early stage type 2 diabetes have a reduced insulin-mediated glucose uptake by 35–40% with respect to healthy individuals (Caro et al. 1989; DeFronzo et al. 1982). In type 2 diabetic subjects, moderate exercise is accompanied by a rise in blood glucose uptake which exceeds hepatic glucose production (Minuk et al. 1981), although exercise-induced hypoglycemia is rare in off-insulin/insulin secretagogues persons, even with prolonged exercise (Koivisto and DeFronzo 1984). In obese, hyperinsulinemic type 2 diabetic subjects, short-term, high-intensity exercise might increase glycemic values because of the counter-regulatory hormone-raising. This hyperglycemic state might persist for about 1 h post-exercise (Marliss and Vranic 2002). Likewise, in IGT patients, exercise might induce hyperglycemia, hypoinsulinemia, and ketonuria (Sigal et al. 2004). Insulin deficiency as well as decreased insulin secretion due to exercise inhibits muscle glucose reuptake, without stopping hepatic glucose production. The latter is enabled by counter-regulatory hormones (adrenaline, noradrenaline, etc.), determining, in turn, an increment in lipolysis with an accelerated conversion from NEFA to ketone bodies. Glucose-raising hormones like epinephrine and norepinephrine are released during exercise in an intensity-dependent manner (Kreisman et al. 2003). Other hormones like glucagon, cortisol, and growth hormone contribute to fuel substrate mobilization during exercise.

A single bout of different-intensity exercise has been shown to enhance splanchnic and peripheral insulin sensitivity in type 2 diabetic subjects for a duration of 12–24 h post-exercise (Burstein et al. 1990; Caro et al. 1989; Devlin et al. 1987). Nevertheless, literature reports divergent results on this. Distinct pre-exercise conditions (baseline blood glucose and insulin, the degree of metabolic control) may variously affect glucose-lowering effects of exercise. All in all, the beneficial effect of acute exercise on insulin action is dissolved in a few days, and at least is short-lived for people with type 2 diabetes (Heath et al. 1983; Schneider et al. 1984).

Chronic Effects of Physical Activity in Type 2 Diabetic Subjects

In those with type 2 diabetes long-term physical activity determines:

- Activation and overexpression of GLUT4
- Changes in intracellular enzymes activity (pyruvate-dehydrogenase, glycogen-synthase, glycogen-phosphorylase)
- Enhanced oxygen extraction
- Increment in mitochondrial enzymes activity
- Lower resting and submaximal heart rate
- Lower resting and exercise blood pressure
- Increased insulin sensitivity
- Reduction of multiple risk factors for cardiovascular disease

As low as 1 week of moderate-to-vigorous aerobic training can ameliorate whole-body insulin sensitivity in type 2 diabetic subjects (Winnick et al. 2008). It is

important to note that improvements in insulin action are "transient," i.e., they last for a period of hours to days (Schneider et al. 1984). That is why physical activity must be performed constantly in order to benefit of its glucose-lowering effects. Also resistance exercise training is effective in sustaining a favorable metabolic control in type 2 diabetes (Black et al. 2010). Resistance training determines an increase in muscle mass which mainly contributes to blood glucose uptake and disposal, being the "metabolically active" mass.

Type 2 diabetic individuals register less commonly than healthy subjects a greater capillary density in response to exercise training (Devlin 1992; Henriksson 1992; Regensteiner et al. 1995). Aerobic power is inversely related to mild and advantageous changes in glycosylated hemoglobin (HbA1c) and/or glucose tolerance (Albright et al. 2000). Augmented aerobic power in people with type 2 diabetes has been associated to a less atherogenic profile: numerous studies acknowledge ameliorations of triglycerides levels, total cholesterol, and high-density lipoprotein (HDL)-cholesterol-to-total cholesterol ratio following chronic exercise stimulation (Kohl et al. 1992). Owing to physical training, lipid metabolism benefits from both accelerated fat oxidation and fatty acids utilization (Albright et al. 2000). In addition, exercise training amplifies sensitivity to catecholamines, especially in adipose tissue, therefore increasing lipolysis and NEFA supply to active muscle mass (Borghouts et al. 2002). Finally, loss of visceral adiposity achieved with exercise training may facilitate weight loss/maintenance optimizing metabolic indexes, lowering CVD risks, and increasing insulin sensitivity. Thus, there are addictive and synergistic effects from the combination of dietary and exercise training interventions for the long-term positive management of body weight (Sigal et al. 2004). This concomitant action results in a virtuous circle in which exercising people with type 2 diabetes (and perhaps overweight/obese) better adhere to nutritional programs and increase their self-esteem and positive mood states. At least five sessions a week of aerobic exercise, lasting 60 min at an intensity of about 50% of VO_{2max}, are necessary to improve body weight and body composition (Bryner et al. 1999). However, exercise seems to optimize insulin action regardless of changes in body composition: exercise and diet (decreased adiposity) allow to gain worthy results more rapidly and consistently (Sigal et al. 2004).

Promoting a Lifestyle Revolution in the Management of Diabetes Mellitus

Exercise prescription per se is not enough to obtain relevant effects on long-term behavior of type 2 diabetic or overweight/obese subjects. A variety of issues make exercise adherence harshly compatible with these kinds of patients' lifestyle. These individuals are frequently deconditioned and unaccustomed to exercise, with a remarkable history of sedentary behaviors. Secondly, many of these patients may result uncompliant to structured exercise programs because of their massive body *habitus*. For these people, exercise recommendations must be tailored on their actual capabilities: the gradual increase of exercise habits (walking, houseworking, baseline daily

Table 1 Exercise recommendations in diabetic subjects with long-term complications, according to ADA/ACSM guidelines

Complication	Exercise recommendation
Vascular disease	Low-to-moderate walking, arm-crank, cycling, lower extremity resistance exercise (treadmill walking, star climbing ability)
Peripheral neuropathy	Without acute ulceration, moderate weight-bearing exercise; moderate walking
Autonomic neuropathy	Tolerance test required before exercise initiation. Intensity is best prescribed using heart reserve (HR) method with direct measurement of maximal HR
Retinopathy	Low-to-moderate intensity exercise training, avoiding activities that greatly increase intraocular pressure and hemorrhage risk
Nephropathy	Aerobic and (especially) resistance training, initiating at low intensity and volume. Supervised (but also home-based), moderate aerobic training during dialysis sessions

activities) might be the first step for promoting a lifestyle revolution. Additionally, if diabetic complications exist (see Table 1), target workloads must be carefully considered and adjusted when a physician or health-care provider is formulating an exercise prescription. Nonetheless, in diabetic people without complications, the risk-to-benefit ratio for low-to-moderate-intensity exercise is clearly diminished. Low-intensity, prolonged aerobic exercise has been the most recommended type of exercise (WHO 2000) and, in fact, the regularity of practicing exercise, regardless of the type, is the key to initiate and maintain a higher activity level, in the long term. A growing body of evidence suggests that modest increments of physical fitness in diabetic subjects decrease by twofold the risk of overall mortality (Church et al. 2004; Myers et al. 2002). Even a single bout of low-intensity exercise has been shown to mainly reduce the prevalence of hyperglycemia in the 24 h post-exercise period in type 2 diabetic individuals (Manders et al. 2010). Once a decent fitness base has been built, one can progress slowly to a more intense and/structured exercise program.

Furthermore, in order to be successful, exercise program might be pleasant and easy-to-be performed, i.e., availability of on-purpose facilities should not represent a critical factor. On a psychological standpoint, it is essential for the patient to receive a positive reinforcement from his/her exercise practice. Those who train regularly can await positive feedback also from their environment and social interaction. Besides, one should dedicate time for exercising in the most energetic fraction of the day.

Two modalities of interventions are possible for those who want to engage in whatever sort of physical activity: structured or unstructured programs. The former is usually more effective than the second one, although in the short term. As previously precised, a long-lasting behavior requires a complex modification of one's lifestyle. A supervised approach, either home-based or in dedicated facilities, needs diversified resources such as equipped clinics, exercise professionals, and time. Unstructured activity is likely to grant health benefits, especially in the management of body weight and blood glucose (Levine et al. 2005). However, the trade-off would be to convert a first-step structured approach in a proper modified healthy lifestyle, highly dynamic, and prone to minimize any daily sedentary time. At this purpose, a multifaceted educational plan is warranted, firstly to provide

patients with a basic knowledge on exercise and its benefits. In a second plane, type 2 diabetic individuals should be encouraged to incorporate more ambitious goals into their daily living, so to move from unstructured activities to durable hyper-dynamic lifestyles.

General and Novel Strategies with Physical Activity

Two major complications are common to both type 1 and type 2 diabetes mellitus, especially in association with exercise: hypoglycemia and hyperglycemia. Several efforts have been made to reduce the risk of both. Management of blood glucose means to maintain quasi-normal balance between hepatic glucose production and peripheral glucose uptake, in combination with effective insulin responses (Wahren and Ekberg 2007). One simple option is represented by the self-monitoring of blood glucose (SMBG), pertaining three to six glucose checks per day by people with type 1 diabetes, and less frequent glucose monitoring by non-insulin users, typically individuals with type 2 diabetes. SMBG benefits glycemic control, independently of type of diabetes (St John et al. 2010). Continuous glucose monitoring (CGM) has been usefully proposed to record daily blood glucose fluctuations for a certain period of time, especially in type 1 diabetic patients, under insulin treatments (Adamo et al. 2016). These instruments are fairly advantageous to monitor (fine-tuning insulin doses if necessary) and evaluate both acute and delayed effects of exercise (Allen et al. 2008).

Tele-health and tele-care systems in diabetes mellitus have been progressively developed as far as they now are being largely applied in different clinical context. Information and communication technologies (ICT) management and web solutions have been utilized for several years in patients with good knowledge of hardware and software tools. "Connected" technologies such as smartphone applications, wearable devices and sensors comprise part of a new digital ecosystem of data-driven tools that can link patients with their health-care teams for a fine management of diabetes, especially for patients affected by type 1 diabetes mellitus. These connected technologies are rich sources of physiologic, behavioral, and contextual data that can be integrated and analyzed in "the cloud," ultimately for implementing personalized models of glycemic dynamics.

Likewise for patients with type 2 diabetes mellitus, ICT-based approaches could potentially allow more effective self-management of disease.

Any strategy has to deal with a variety of factors influencing blood glucose responses to exercise: type, intensity, and duration of the effort; pre-exercise conditions (level of training, nutritional status, glycemia); type of insulin or other therapy used; psychological status; level of hydration. Adequate carbohydrate supplementation during and after exercise is one of the most natural measures for sustaining a near-normal glycemic level, along with insulin dosage adjustment.

According to the joint position statement of the *American College of Sports Medicine* and the *American Diabetes Association* (ACSM-ADA 2010; Colberg et al. 2016) (Table 2), persons with type 2 diabetes with no major complications should undertake at least 150 min/week of moderate to vigorous aerobic exercise, at least

Table 2 Exercise recommendations in persons with type 1 and type 2 diabetes mellitus and no major complications, according to ADA/ACSM guidelines

Type 1	Type 2
Aerobic exercise: Daily, to ensure optimal blood glucose control; at 50–85% VO_{2max}, 20–60 min	Aerobic exercise: 3 days/week (with no more than 2 consecutive days between bouts); at 40–60% VO_{2max}, 150 min/week
Resistance exercise: 2–3 days/week on non-consecutive days; at 50–75/80 of 1RM; 5–10 exercises involving the major muscle groups; 10–15 repetitions per set (moderate-intensity, with light weights)	Resistance exercise: 2–3 days/week on non-consecutive days; at 50–75/80 of 1RM; 5–10 exercises involving the major muscle groups; 10–15 repetitions near-to-fatigue per set (progressing over time to heavier weights, 8–10 lifts)
Circuit programs	Supervised training
Timing: Insulin therapy and blood glucose at the time of exercise must be considered. Avoid exercise if FBG > 250 mg/dL + ketosis or FBG > 300 mg/dL without ketosis. Ingest CHO if glucose levels <100 mg/dL	Combined aerobic and resistance exercise training
	Unstructured activity
	Flexibility training and yoga

Abbreviations: *CHO* carbohydrates, *FBG* fasting blood glucose, *min* minutes, *1RM* repetition maximum, $VO_{2\ max}$ maximal oxygen uptake (maximal aerobic capacity)

3 days/week, with no more than 2 days of interruption in the aerobic physical activity. They should also perform moderate to vigorous resistance training at least 2–3 days/week. Supervised, mixed training, or milder forms of physical activities (e.g., yoga) are as well encouraged.

The same position stand outlines general exercise guidelines for people with type 1 diabetes who do not have complications and are in good blood glucose control. Those exercising might ensure metabolic control, avoiding physical activity if fasting blood glucose (FBG) levels are >250 mg/dL with ketosis (caution instead if FBG >300 mg/dL with no ketosis), and supplementing with carbohydrates if blood glucose is <100 mg/dL (and whenever needed during and after physical activity). Yet, in type 1 diabetes subjects, blood glucose monitoring must be thoughtfully guarded before and after physical activity, for adjusting insulin requirements and acquire one's glycemic responses to exercise.

Lifestyle Interventions and Barriers

Several large-scale trials have been successfully conducted to promote lifestyle interventions based on self-monitoring, goal-setting, supervision, and progressive-stage protocols (Balducci et al. 2009; Delahanty and Nathan 2008; Eakin et al. 2010; Malpass et al. 2009; Wadden et al. 2011). These programs address a number of positive behaviors, including increased levels of physical activity, healthy dietary regimens, and weight-loss/maintenance. A wide spectrum of specific equipment has been readily available, like pedometers, informative-educational kit, gym-tools, et cetera. However,

the major barriers to maintain/increase participation in these programs are affective and psychological ones. People with diabetes or obesity feel to be "inadequate" to perform any kind of physical activity; they have low fitness expectations for becoming active; they are daunted by fitness facilities, believing to be not suited-for or to slow down peers in a group exercise. Social support is essential of course; however, strategies should refine self-care behaviors of these people, increasing self-esteem, self-efficacy, and all in all, the individual's perception to be able to overcome barriers related to diabetes management. Greater levels of physical activity are associated with higher levels of self-efficacy, which mirrors one's confidence in the ability to exercise. Some patients with diabetes, notably type 1, are afraid of hypoglycemic events, therefore remaining intimidated from pursuing exercise. CGM and other described technologies may enhance confidence and exercise compliance in individuals with diabetes.

Planning appropriate, tailored, and realistic goals is crucial for getting people with diabetes physically active for a lifetime. To this aim, individuals must be guided by health-care and fitness professionals in setting objectives attainable, gratifying, and capable to modify one's behavior. Thereafter, through measurable stepped-stages in a planned timeline, subjects may incorporate upper levels of intervention. Affective support and encouragement from family, friends, and peers may be most beneficial.

Lastly, supervision of training has been shown to be more effective than self-reported physical activity in adherence to exercise programs. Qualified trainers demonstrated to exert a tremendous impact on glycemic control, blood pressure, and weight management when they can provide high-quality counseling to exercise practitioners overweight/obese or diabetic.

Notes on Type 1 Diabetes Mellitus

The biggest challenge for type 1 diabetic subjects is the glycemic control. Exercise, in fact, guarantees a fair blood glucose regulation only in tight secured conditions, like those assisted with specific devices (see paragraph "General and Novel Strategies with Physical Activity"). Undoubtedly regular exercise results in enhanced insulin sensitivity, glucose metabolism, and CVD prevention even in people with type 1 diabetes (Table 3). However, exercise may have a role beyond its insulin-mimetic action (Codella et al. 2015). A better understanding of the impact of exercise on diversified scenarios (autoimmunity, inflammation) will be of assistance in designing improved exercise prescription also for patients with type 1 diabetes in the clinical arena.

Integrative View

Exercise is an unavoidable treatment in the management of diabetes. A dynamic lifestyle, embracing a proper dietary regimen, is as healthy as well-established in human knowledge for centuries – the sole possible reply to the modern diabetogenic environment.

Table 3 Benefits of exercise in type 1 and type 2 diabetes mellitus

Type 1	Type 2
Improved insulin sensitivity	Improved insulin sensitivity (peripheral and hepatic)
Improved blood lipid profile	Improved blood lipid profile
Increased energy expenditure	Increased energy expenditure
Improved physical fitness	Improved physical fitness
Decreased blood pressure	Decreased blood pressure
Decreased risk of CVD	Decreased risk of CVD
Enhanced psychological well-being	Enhanced psychological well-being
	Improved glycemic control (HbA1c)
	Improved insulin response to oral glucose stimulus

Duality of Interest No potential conflicts of interest relevant to this chapter were reported.

Contribution Statement All authors were responsible for drafting the manuscript and revising it critically for valuable intellectual content. All authors approved the version to be published.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

ACSM-ADA. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Med Sci Sports Exerc. 2010;42 (12):2282–303.

Adamo M, et al. Active subjects with autoimmune type 1 diabetes have better metabolic profiles than sedentary controls. Cell Transpl. 2017;26(1):23–32. https://doi.org/10.3727/096368916X693022. Epub 2016 Sep 20.

Albright A, et al. American College of Sports Medicine position stand. exercise and type 2 diabetes. Med Sci Sports Exerc. 2000;32(7):1345–60.

Allen NA, Fain JA, Braun B, Chipkin SR. Continuous glucose monitoring counseling improves physical activity behaviors of individuals with type 2 diabetes: a randomized clinical trial. Diabetes Res Clin Pract. 2008;80(3):371–9.

Balducci S, et al. Physical activity/exercise training in type 2 diabetes. The role of the Italian diabetes and exercise study. Diabetes Metab Res Rev. 2009;25:S29.

Black LE, Swan PD, Alvar BA. Effects of intensity and volume on insulin sensitivity during acute bouts of resistance training. J Strength Cond Res / Nat Strength Cond Assoc. 2010;24 (4):1109–16. https://doi.org/10.1519/JSC.0b013e3181cbab6d.

Borghouts LB, Wagenmakers AJM, Goyens PLL, Keizer HA. Substrate utilization in non-obese type II diabetic patients at rest and during exercise. Clin Sci (Lond). 2002;103(6):559–66.

Boulé NG, et al. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA. 2001;286(10):1218–27.

Bramble DM, Lieberman DE. Endurance running and the evolution of homo. Nature. 2004;432 (7015):345–52. https://doi.org/10.1038/nature03052.

Bryner RW, et al. Effects of resistance vs. aerobic training combined with an 800 calorie liquid diet on lean body mass and resting metabolic rate. J Am Coll Nutr. 1999;18(2):115–21.

Burstein R, et al. Effect of an acute bout of exercise on glucose disposal in human obesity. J Appl Physiol. 1990;69(1):299–304.

Caro JF, Dohm LG, Pories WJ, Sinha MK. Cellular alterations in liver, skeletal muscle, and adipose tissue responsible for insulin resistance in obesity and type II diabetes. Diabetes/Metab Rev. 1989;5(8):665–89.
Cartee GD, et al. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. Am J Phys. 1989;256:E494–9.
Cheung BMY, et al. Diabetes prevalence and therapeutic target achievement in the United States, 1999 to 2006. Am J Med. 2009;122(5):443–53.
Church TS, et al. Exercise capacity and body composition as predictors of mortality among men with diabetes. Diabetes Care. 2004;27(1):83–8.
Codella R, Luzi L, Inverardi L, Ricordi C. The anti-inflammatory effects of exercise in the syndromic thread of diabetes and autoimmunity. Eur Rev Med Pharmacol Sci. 2015;19(19):3709–22.
Coderre L, Kandror KV, Vallega G, Pilch PF. Identification and characterization of an exercise-sensitive pool of glucose transporters in skeletal muscle. J Biol Chem. 1995;270(46):27584–8.
Colberg SR, et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. Diabetes Care. 2016;39(11):2065–79.
DeFronzo RA, Simonson D, Ferrannini E. Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1982;23(4):313–9.
Delahanty LM, Nathan DM. Implications of the Diabetes Prevention Program and Look AHEAD Clinical Trials for Lifestyle Interventions. J Am Dietetic Assoc. 2008;108(4 Suppl 1):S66–72.
Devlin JT. Effects of exercise on insulin sensitivity in humans. Diabetes Care. 1992;15(11):1690–3.
Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes. 1987;36(4):434–9.
Eakin EG, et al. Living well with diabetes: a randomized controlled trial of a telephone-delivered intervention for maintenance of weight loss, physical activity and Glycaemic control in adults with type 2 diabetes. BMC Public Health. 2010;10:452.
Galbo H, Tobin L, van Loon LJ. Responses to acute exercise in type 2 diabetes, with an emphasis on metabolism and interaction with oral hypoglycemic agents and food intake. Appl Physiol Nutr Metab. 2007;32(3):567–75.
Garetto LP, Richter EA, Goodman MN, Ruderman NB. Enhanced muscle glucose metabolism after exercise in the rat: the two phases. Am J Phys. 1984;246(6 Pt 1):E471–5.
Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annu Rev Med. 1998;49(1):235–61. https://doi.org/10.1146/annurev.med.49.1.235.
Goodyear LJ, et al. Effects of contractile activity on tyrosine phosphoproteins and PI 3-kinase activity in rat skeletal muscle. Am J Physiol. 1995;268(0002-9513(Print)):E987–95.
Hayashi T, Wojtaszewski JF, Goodyear LJ. Exercise regulation of glucose transport in skeletal muscle. Am J Phys. 1997;273(6 Pt 1):E1039–51.
Heath GW, et al. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. J Appl Physiol Respir Environ Exerc Physiol. 1983;55:512–7.
Henriksson J. Effects of physical training on the metabolism of skeletal muscle. Diabetes Care. 1992;15(11):1701–11.
International Diabetes Federation. IDF Diabetes atlas IDF diabetes atlas. 2015. http://www.diabetesatlas.org/resources/2015-atlas.html https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf www.ecuadorencifras.gob.ec.
Kennedy JW, et al. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. Diabetes. 1999;48:1–6.
Kohl HW, Gordon NF, Villegas JA, Blair SN. Cardiorespiratory fitness, glycemic status, and mortality risk in men. Diabetes Care. 1992;15(2):184–92.
Koivisto V, DeFronzo R. Exercise in the Treatment of Type II Diabetes. Acta Endocrinol. 1984;262 (Suppl):107–16.
Kreisman SH, Halter JB, Vranic M, Marliss EB. Combined infusion of epinephrine and norepinephrine during moderate exercise reproduces the Glucoregulatory response of intense exercise. Diabetes. 2003;52(6):1347–54.

Levine JA, et al. Interindividual variation in posture allocation: possible role in human obesity. Science. 2005;307(5709):584–6.
Lund S, Holman GD, Schmitz O, Pedersen O. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. Proc Natl Acad Sci U S A. 1995;92(13):5817–21.
Luzi L. Human evolution and physical exercise: the concept of being 'born to run'. In: Cellular physiology and metabolism of physical exercise. Milano: Springer Milan; 2012. p. 1–7. https://doi.org/10.1007/978-88-470-2418-2_1.
Luzi L, Pizzini G. Born to run: training our genes to cope with ecosystem changes in the twentieth century. Sport Sci Health. 2004;1(1):1–4. https://doi.org/10.1007/s11332-004-0001-0.
Malpass A, Andrews R, Turner KM. Patients with type 2 diabetes experiences of making multiple lifestyle changes: a qualitative study. Patient Educ Couns. 2009;74(2):258–63.
Manders RJF, Van Dijk JWM, Van Loon LJC. Low-intensity exercise reduces the prevalence of hyperglycemia in type 2 diabetes. Med Sci Sports Exerc. 2010;42(2):219–25.
Manetta J, Brun JF, Mercier J, Prefaut C. The effects of exercise training intensification on glucose disposal in elite cyclists. Int J Sports Med. 2000;21(5):338–43.
Marliss EB, Vranic M. Intense exercise has unique effects on both insulin release and its roles in glucoregulation: implications for diabetes. Diabetes. 2002;51:S271.
Minuk HL, et al. Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. Am J Epidemiol. 1981;240(3):458–64.
Myers J, et al. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med. 2002;346(11):793–801.
O'Gorman DJ, et al. Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. Diabetologia. 2006;49(12):2983–92.
Ohlson LO, et al. The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. Diabetes. 1985;34 (10):1055–8.
Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;281(7285):785–9.
Regensteiner JG, et al. Relationship between habitual physical activity and insulin area among individuals with impaired glucose tolerance: the San Luis Valley Diabetes Study. Diabetes Care. 1995;18(4):490–7.
Richter EA, Derave W, Wojtaszewski JFP. Glucose, exercise and insulin: emerging concepts. J Physiol. 2001;535(2):313–22.
Sakamoto K, Goodyear LJ. Invited review: intracellular signaling in contracting skeletal muscle. J Appl Physiol (Bethesda, Md.: 1985). 2002;93:369–83.
Schneider SH, Amorosa LF, Khachadurian AK, Ruderman NB. Studies on the mechanism of improved glucose control during regular exercise in type 2 (non-insulin-dependent) diabetes. Diabetologia. 1984;26(5):355–60.
Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C. Physical activity/exercise and type 2 diabetes. Diabetes Care. 2004;27(10):2518–39.
St John A, Davis WA, Price CP, Davis TM. The value of self-monitoring of blood glucose: a review of recent evidence. J Diabetes Complicat. 2010;24(2):129–41.
Suh S-H, Paik I-Y, Jacobs K. Regulation of blood glucose homeostasis during prolonged exercise. Mol Cells. 2007;23(3):272–9.
Wadden TA, et al. Four-year weight losses in the look AHEAD study: factors associated with long-term success. Obesity. 2011;19(10):1987–98. https://doi.org/10.1038/oby.2011.230/nature06264.
Wahren J, Ekberg K. Splanchnic regulation of glucose production. Annu Rev Nutr. 2007;27: 329–45.
WHO. 894 World Health Organization technical report series Obesity: preventing and managing the global epidemic. Report of a WHO consultation. 2000. http://www.ncbi.nlm.nih.gov/pubmed/11234459.

Winnick JJ, et al. Short-term aerobic exercise training in obese humans with type 2 diabetes mellitus improves whole-body insulin sensitivity through gains in peripheral, not hepatic insulin sensitivity. J Clin Endocrinol Metab. 2008;93(3):771–8.

Wojtaszewski JF, Hansen BF, Ursø B, Richter EA. Wortmannin inhibits both insulin- and contraction-stimulated glucose uptake and transport in rat skeletal muscle. J Appl Physiol (Bethesda, Md.: 1985). 1996;81:1501–9.

Wojtaszewski JFP, et al. Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. J Clin Investig. 1999;104(9):1257–64.

Wojtaszewski JFP, Nielsen JN, Richter EA. Invited review: effect of acute exercise on insulin signaling and action in humans. J Appl Physiol (Bethesda, Md.: 1985). 2002;93(1):384–92.

Treatment with Oral Drugs

19

Cristina Bianchi, Giuseppe Daniele, Angela Dardano, and Stefano Del Prato

Contents

Abstract

Till the turn of the century, treatment of hyperglycemia in Type 2 diabetes was limited to two main classes of oral agents: sulfonylureas and biguanides. In the meantime, better understanding of the pathophysiology of hyperglycemia in Type 2 diabetes has been gained and the identification of several pathogenitic mechanisms has enabled moving from serendipitous discovery – as for sulfonylureas

C. Bianchi · G. Daniele · A. Dardano · S. Del Prato (✉)
Department of Clinical and Experimental Medicine, Section of Metabolic Diseases and Diabetes, University of Pisa, Pisa, Italy
e-mail: c.bianchi@ao-pisa.toscana.it; giuseppe.daniele@unipi.it; angidardano@gmail.com; stefano.delprato@med.unipi.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_19

and metformin – to the development of agents with more targeted modes of action. Current guidelines concur in recommending metformin at diagnosis of diabetes. Selection of the second drug therapy should be made on the basis of an educated process tacking into consideration efficacy, risk of hypoglycemia, effect on body weight, costs of different drugs, as well as patient's characteristics. With more clinical data generated, other features of the available oral agents should be taken into account such as durability, predominant effect on fasting vs. postprandial glucose, as well effects beyond their glucose lowering capacity.

Keywords
Oral antihyperglycemic therapy · Insulin-sensitizers · Biguanides · Thiazolidinediones · Insulin-secretagogues · Sulfonylureas · Meglitinides · Incretin-therapy · Dipeptidyl peptidase-4 inhibitors · α-glucosidase inhibitors · Bile acid sequestrants · Sodium-glucose cotransporter 2 inhibitors · Bromocriptine

Till the turn of the century, treatment of hyperglycemia in Type 2 diabetes (T2DM) was limited to two main classes of oral agents: sulfonylureas and biguanides. The latter originally included buformin, phenformin, and metformin. Only metformin has remained in the diabetes pharmacopeia and currently is the most commonly recommended first-line agent for the treatment of newly diagnosed T2DM. The sulfonylurea class has evolved over the time with several compounds being developed.

Sulfonylureas and metformin, though belonging to two distinct classes, share common features. The first is that they have been used to lower blood glucose much earlier than their mode of action was understood. The second is that they were serendipitous discoveries. Biguanides were originally derived from the *Galega officinalis* or French Lilac, already used in medieval medicine for the treatment of the snake bites, the San Vitus dance, plague, worms, miasma, and dysuria (Bailey and Day 2004). It took much longer before their glucose lowering properties were detected. As such, biguanides represent the first example of pleiotropic agents. Biguanides were synthetized in the 1920s as glucose-lowering agents, but their "pleiotropic" features were not dismissed as they were also used for treating influenza. The glucose lowering potency of phenformin and buformin was greater than that of metformin, but they have been withdrawn in 1970s due to the risk of lactic acidosis. Metformin has remained in the market and the United Kingdom Prospective Diabetes Study (UKPDS) has provided evidence for efficacy, safety, and potential cardiovascular protection (UK Prospective Diabetes Study Group 1998).

As compared to metformin, the history of sulfonylureas is relatively shorter. It starts in the 1940s when the hypoglycemic activity of synthetic sulfur compounds was noticed. Few years later, Marcel Janbon observed hypoglycemia in patients taking para-amino-sulfonamide-isopropyl-thiodiazole for typhoid fever. Then, Loubatieres showed that aryl-SU compounds stimulated insulin secretion from the pancreas and that residual β-cell function was necessary to elicit the glucose-

lowering effect. The first sulfonylurea, tolbutamide, was introduced in 1950 and since then several molecules have been developed. While their ability to stimulate insulin secretion was evident, their intrinsic mechanism of action has remained obscure for decades.

Biguanides and sulfonylureas have been the only oral agents for quite a long time. In the meantime, better understanding of the pathophysiology of hyperglycemia in T2DM has been gained. Development and progression of hyperglycemia is the result of altered β-cell function; glucagon hypersecretion; insulin resistance at the level of the liver, skeletal muscle, and adipose tissue; impaired secretion/action of incretin hormones; altered bile acid metabolism; paradoxical increase of renal glucose reabsorption; defective integration processes at the level of the central nervous system. As described by DeFronzo (2009), there are at least eight different mechanisms underlying the disease. Identification of these mechanisms has enabled moving from serendipitous discovery – as described for sulfonylureas and metformin – to the development of agents with more targeted modes of action (Table 1).

Insulin-Sensitizers

Currently, there are only two classes of drugs targeting insulin action: biguanides and thiazolidinediones, with only one drug per class generally used, i.e., metformin and pioglitazone.

Biguanides

Metformin (dimethylbiguanide) is the only biguanide still available in most countries. Used for the past 60 years in Europe and Canada, metformin was introduced in America in 1995 and it is the drug recommended by almost all guidelines as initial therapy for T2DM.

Pharmacology – Though biguanides are lipophilic and have high membrane binding affinity, at physiologic pH metformin exists as a hydrophilic cation requiring the coordinated action of monoamine transporter (PMAT) and organic cationic transport proteins (OCT) to cross plasma membranes. Recent data have suggested that genetic variants of these proteins could account for individual variability of metformin pharmacokinetics and tolerance (Dujic et al. 2015). Following oral administration, up to 70% of the dose is rapidly absorbed with the remaining amount of the drug lost with feces (Graham et al. 2011). Metformin diffuses widely through plasma and tissues, in particular the liver, kidney, and intestine (Bailey et al. 2008). At tissue level metformin accumulates in mitochondria reaching concentrations that are 1000-fold higher than the one in the extracellular space. Absorbed metformin is eliminated unchanged through the urine mainly via tubular secretion. Nonetheless, impaired glomerular filtration can lead to metformin accumulation in plasma. Metformin is also available as extended release (XR) formulation allowing once daily administration (Jabbour and Ziring 2011).

Table 1 Oral antihyperglycemic therapy for type 2 diabetes mellitus

Class	Compound	Mechanism of action	Benefits	Limitations/ precautions
Biguanide	Metformin	Increases liver and muscle insulin sensitivity Decreases hepatic glucose production	Low risk of hypoglycemia Possible cardiovascular benefit Low cost	Gastro-intestinal adverse effect profile Avoid in severe kidney dysfunction (eGFR <30 ml/ min/1.73 mq)
Sulfonylurea	Glibenclamide/ glyburide Glipizide Gliclazide Glimepiride	Increases insulin secretion by binding to a specific f receptor on β-cell	High efficacy Low cost	Hypoglycemia risk Weight gain Hastens beta-cell dysfunction
Meglitinide	Repaglinide Nateglinide	Increases insulin secretion by binding to a different subunit of the β-cell sulfonylurea receptor	Prandial focus Use in kidney impairment	Hypoglycemia risk Weight gain Mealtime dosing Avoid concomitant use of repaglinide and gemfibrozil
Thiazolidinediones	Pioglitazone Rosiglitazone	Agonists for PPAR-γ which influences the production of a number of gene products involved in glucose and lipid metabolism Increases adipose and muscle insulin sensitivity	Low risk of hypoglycemia Possible cardiovascular benefit	Weight gain Edema Risk of fractures Avoid in NYHA class II-IV
a-Glucosidase inhibitor	Acarbose Miglitol	Delays intestinal carbohydrate absorption by blocking the α-glucosidase enzymes	No systemic absorption Prandial focus	Gastro-intestinal adverse effect profile Mealtime dosing Contraindicated in irritable bowel syndrome

(*continued*)

Table 1 (continued)

Class	Compound	Mechanism of action	Benefits	Limitations/ precautions
DPP-4 inhibitor	Sitagliptin Vildagliptin Saxagliptin Alogliptin Linagliptin	Inhibits DPP-4 enzyme resulting in prolonged active incretin levels with consequent increased insulin synthesis and release and decreased glucagon secretion	Low risk of hypoglycemia Weight neutral Use in kidney impairment (dose adjustment required with the exception of linagliptin)	Avoid in case of previous pancreatitis
Bile acid sequestrant	Colesevelam Colestimide	Unknown	Lipid benefits No systemic absorption	Large pill size/ burden Gastro-intestinal adverse effect profile Avoid with high triglycerides
SGLT-2 inhibitor	Canagliflozin Dapagliflozin Empagliflozin	Reduces reabsorption of filtered glucose from the tubular lumen in the kidney	Low risk of hypoglycemia Weight loss Blood pressure reduction Uric acid reduction Possible cardiovascular benefit	Avoid in moderate to severe kidney dysfunction

Mechanism of action – Metformin enhances insulin-mediated suppression of hepatic glucose production and gluconeogenesis (Natali and Ferrannini 2006). De novo hepatic synthesis of glucose is a main contributor of fasting and postprandial hyperglycemia (Gastaldelli et al. 2001). In the liver, metformin accumulates in mitochondria where it inhibits Complex I (Owen et al. 2000), reduces ATP production, and alters the NAD^+:NADH ratio (El-Mir et al. 2000). Since gluconeogenesis is an energy-dependent process, reduced ATP generation leads to its suppression. Metformin may also restrain gluconeogenesis by inhibiting mitochondrial glycerophosphate (Madiraju et al. 2014). Impaired mitochondrial function activates AMP-activated protein kinase (AMPK), which, in turn, suppresses the expression of gluconeogenesis enzymes (Rena et al. 2017). AMPK can also account for the peripheral insulin sensitizing effect of the drug through transcription and translocation of insulin-dependent glucose transporter GLUT4 (Turban et al. 2012) and activation of glycogen synthase. Moreover, AMPK can modulate lipid metabolism by inhibiting lipolysis, increasing free-fatty acid (FFA)

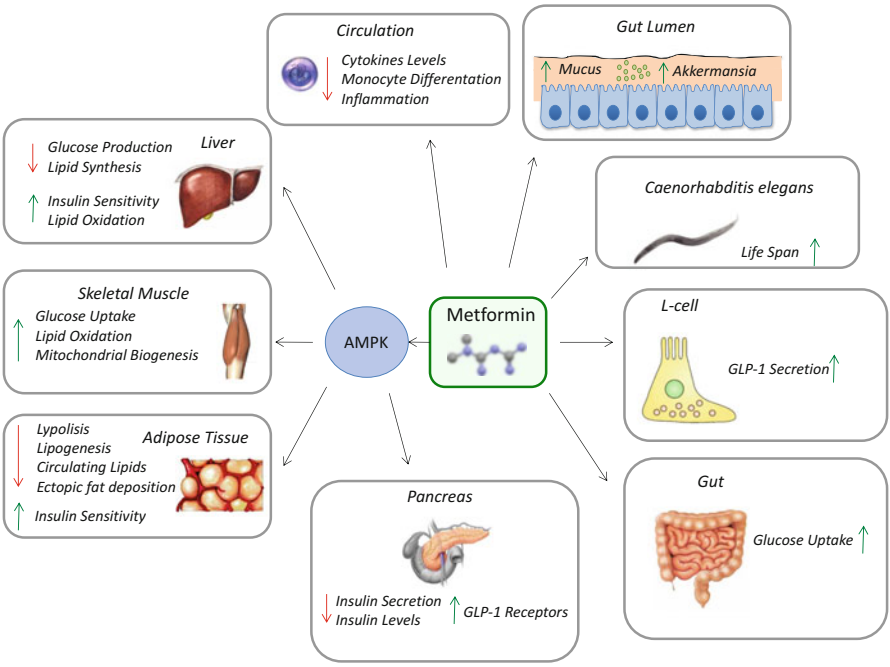


Fig. 1 Synopsis of the effects of metformin. Responses to metformin in target organs/tissues occur through cellular energy sensor AMP-activated protein kinase (AMPK) dependent and independent mechanisms. In the liver metformin regulates hepatic glucose output and leads to improved insulin sensitivity in liver, skeletal muscle, and adipose tissue. Metformin increases GLP-1 receptor expression in pancreatic β-cells. In the intestines, gut metabolism, incretin (GLP-1) secretion, and the microbiome are modified upon metformin use. In the blood metformin reduces monocytes and macrophages differentiation into macrophages as well as pro-inflammatory cytokine secretion

oxidation, reducing fatty acid synthesis, and lowering hepatic secretion of very low-density lipoprotein (VLDL). In the gut, metformin increases glucose utilization and lactate formation, inhibits dipetidyl-peptidase-4 (DPP4) activity (Lindsay et al. 2005), and induces glucagon-like peptide 1 (GLP-1) release. Interestingly, metformin increases GLP-1 receptor expression in pancreatic β-cells (Hur and Lee 2015). Finally, metformin can alter intestinal microbiome, which may contribute to some of the effects of the drug (Fig. 1; Pollak 2017).

Clinical efficacy – Metformin is the most commonly recommended first-line therapy for T2DM. Prescription of metformin was commonly recommended for those not achieving glycemic targets with diet and physical exercise, but the ADA/EASD position statement for treatment of hyperglycemia suggests metformin should be immediately started at diagnosis (Inzucchi et al. 2015). The recommended dose of the drug ranges between 500 and 2500 mg per day, taken in divided doses with meals and starting with the lowest dose to minimize gastrointestinal side effects. Maximal efficacy is usually achieved with 2000 mg, but some subject may benefit of higher dosage, although above this dose there is little improvement in efficacy and increased risk of gastrointestinal

events (Garber et al. 1997). Metformin treatment can reduce fasting plasma glucose by 50–90 mg/dl (3–5 mmol/l) and HbA1c by 1–2% (11–22 mmol/mol) according to baseline levels (Garber et al. 1997). These effects are independent of body weight but require some degree of insulin availability. When used in monotherapy, metformin does not cause body weight gain and carries minimal risk of hypoglycemia. Metformin has more durable efficacy than sulfonylureas though less than the one of rosiglitazone (Kahn et al. 2006). Upon therapeutic failure, all antidiabetes drugs, including insulin, can be added to metformin. Single tablet combination with sulfonylureas, thiazolidinediones, DDP4 inhibitors, and SGLT2 inhibitors are available. Some of these combinations have sound rationale because of their complementary mechanisms of action. For instance, metformin can increase the release of GLP-1 from intestinal L cells that can be preserved by concomitant use of a DPP4 inhibitor. These combinations provide a further 0.6–1.0% HbA1c reduction (Palmer et al. 2016) along with a simplified dosage regimen. In combination with insulin, metformin improves glycemic control with 30% reduction of insulin requirement (Yki-Järvinen et al. 2006).

A modest improvement in lipid profile with lower plasma FFAs and VLDL-triglyceride and increased high-density lipoprotein (HDL) cholesterol has been reported. Metformin can exert antiatherogenic effects including improved insulin sensitivity, reduced tissue plasminogen activator inhibitor 1 (PAI-1) (He et al. 2003), and anti-inflammatory and immunomodulatory action (Pollak 2017). These effects have been claimed to account for the 39% reduction of the risk of myocardial infarction documented in the UKPDS (UK Prospective Diabetes Study Group 1998) though a more recent meta-analysis has casted uncertainty about whether metformin reduces the risk of CV disease in T2DM patients (Griffin et al. 2017). A more recent meta-analysis has reported a significantly lower all-cause mortality in T2DM patients as compared to nondiabetics (hazard ratio (HR) = 0.93, 95%CI 0.88–0.99), and diabetics receiving nonmetformin therapies (HR = 0.72, 0.65–0.80), insulin (HR = 0.68, 0.63–0.75), or sulfonylurea (HR = 0.80, 0.66–0.97) (Fig. 2; Campbell et al. 2017).

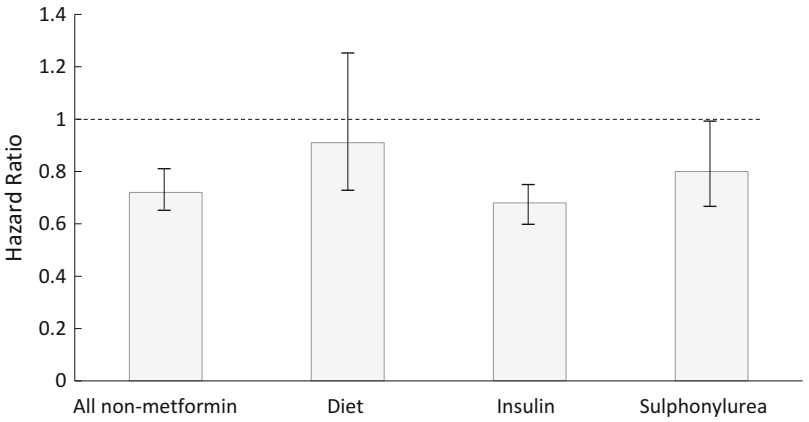


Fig. 2 All-cause mortality in people using metformin as compared to other therapies for diabetes. The hight of the bars represent Hazard Ratio and the line the 95% confidence intervals

Adverse effects – Lactic acidosis is the most severe adverse condition potentially occurring with biguanides. This was particularly true with phenformin, while lactic acidosis is rare with metformin. In a meta-analysis of randomized clinical trials evaluating efficacy and safety of metformin, no cases of lactic acidosis were recorded (Salpeter et al. 2010), while in the real life setting its incidence is as low as 3.3 per 100,000 patient-years (Lipska et al. 2011). Cautiously, current recommendations suggest avoiding use of metformin in patients at risk of lactic acidosis, i.e., those with renal insufficiency, congestive heart failure, or liver disease. Metformin is excreted unchanged in the urine via tubular secretion and has no nephrotoxic effects. Nonetheless caution should be used in patients with impaired kidney function to reduce the risk of accumulation of the drug in the circulation and, therefore, increasing the risk of lactic acidosis. The use of metformin has been contraindicated in subjects serum creatinine >1.5 mg/dl in man and >1.4 in women. Recently, the Food and Drug Administration (FDA) has issued a new labeling recommending starting metformin in patients with an eGFR >45 mL/min/1.73m^2, continuing with assessment of the risks as it falls below 45 mL/min/1.73m^2, and stopping it for eGFR becoming <30 mL/min/1.73m^2. Iodine-containing contrast media may cause acute deterioration of renal function. Therefore, metformin discontinuation prior to contrast imaging procedure is advised. The drug can be re-introduced upon confirmation of no change in eGFR 48 h after the procedure.

Gastrointestinal side effects (nausea, diarrhea, and abdominal pain) are more common occurring up to 20–30% of patients initiated to metformin. Symptoms usually remit with dose reduction and slow up-titration. Metformin should be started at low dose, with gradual escalation to 1 g twice daily at weekly intervals (Garber et al. 1997). A lower prevalence of gastro-intestinal side effect occurs with the use of the XR formulation (Fujioka et al. 2003). Other minor side effects are metallic taste and reduced absorption of vitamin B12 in patients with poor diet (Wulffelé et al. 2003), but deficiency significant enough to cause megaloblastic anemia is uncommon.

Other potential clinical use – Metformin, due to its risk-to-benefit ratio, is the most suitable currently available drug for prevention of diabetes. In the Diabetes Prevention Program (Knowler et al. 2002), metformin administration to subjects with impaired glucose tolerance (IGT) led to a 31% reduction in the conversion to diabetes as compared to placebo, though this was less than the 58% reduction obtained with intensive lifestyle modification.

Metformin has been tested in women with gestational diabetes with no adverse events on the fetus (Rowan et al. 2008). Nonetheless, no official endorsement has been released by regulatory agencies (Lindsay and Loeken 2017). Polycystic ovary syndrome (PCOS) is common in women of reproductive age. In these women, metformin can lower testosterone levels as a result of its insulin sensitizing action (Nestler and Jakubowicz 1997). However, the clinical efficacy of metformin for treatment of PCOS remains questionable due to inconsistent association with improvements in menstrual irregularity or clinical hyperandrogenism and limited effect on fertility and live birth rate. As such, metformin is currently not recommended as a primary treatment for anovulatory infertility (Goodman et al. 2015).

Studies have claimed that metformin can reduce transaminases, liver inflammation, and fibrosis (Mazza et al. 2012) though these results have not been confirmed in a large meta-analysis (Musso et al. 2012). Metformin has been used in patients with HIV-associated lipodystrophy with reduction of plasma insulin but limited effect on glucose and lipid profile (Sheth and Larson 2010).

Finally, metformin therapy is associated with decreased risk of breast, colon, liver, pancreas, prostate, endometrium, and lung cancer (Heckman-Stoddard et al. 2017). In vitro studies have shown an inhibitory effect of the drug on cellular proliferation and several cancer pathways. Metformin is currently under evaluation in trials to ascertain whether it can prevent or slow the progression of different forms of cancer.

Thiazolidinediones

Together with metformin, thiazolidinediones (TZDs) are the only insulin sensitizer currently available. Troglitazone was the first molecule to be introduced in 1997. The drug, however, was soon withdrawn because of severe cases of idiosyncratic hepatotoxicosis. In the meantime, two other molecules were developed: rosiglitazone and pioglitazone. In 2007 a meta-analysis claimed rosiglitazone therapy to be associated with increased risk of cardiovascular events leading to its withdrawal in Europe and restricted use in America. Subsequent meta-analyses and new data have not confirmed such a risk and restriction was lifted by the FDA, but not in Europe.

Pharmacology – Both rosiglitazone and pioglitazone are rapidly and almost completely absorbed following oral intake with a peak concentration 1–2 h after administration and they are largely bound (>99%) to plasma protein. The two molecules are mainly metabolized by CYP2C8 and, to a small degree, by CYP2C9 cytochromes (Scheen 2007). For both drugs, an interaction can occur with rifampicin reducing drug exposure and gemfibrozil, enhancing it. Conversely, rosiglitazone and pioglitazone do not seem to affect the pharmacokinetics of other compounds (Scheen 2007). Liver metabolism of rosiglitazone generates inactive or very weakly active metabolites that are excreted through the kidney. On the contrary, pioglitazone is metabolized to active metabolites that are eliminated in the bile. With normal liver function, the elimination half-life of pioglitazone and rosiglitazone is 5–6 and 3–4 h, respectively. The two active metabolites of pioglitazone have an elimination half-life of 26–28 h, allowing single daily administration. A similar schedule is recommended for rosiglitazone.

Mechanism of action – TZDs are highly selective and potent agonist of Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) receptors. These receptors are highly expressed in the adipose tissue and, to a lesser extent, in skeletal muscle. PPAR-γ activation elicits the formation of a heterodimeric complex with the retinoid-X receptor (RXR) that interacts with a specific nucleotide sequence located in the promoter regions of the PPAR-responsive genes. This leads to the expression of genes involved in glucose and lipid metabolism and energy balance (Fig. 3; Mudaliar and Henry 2001; Bogacka et al. 2004). PPAR-γ activation promotes

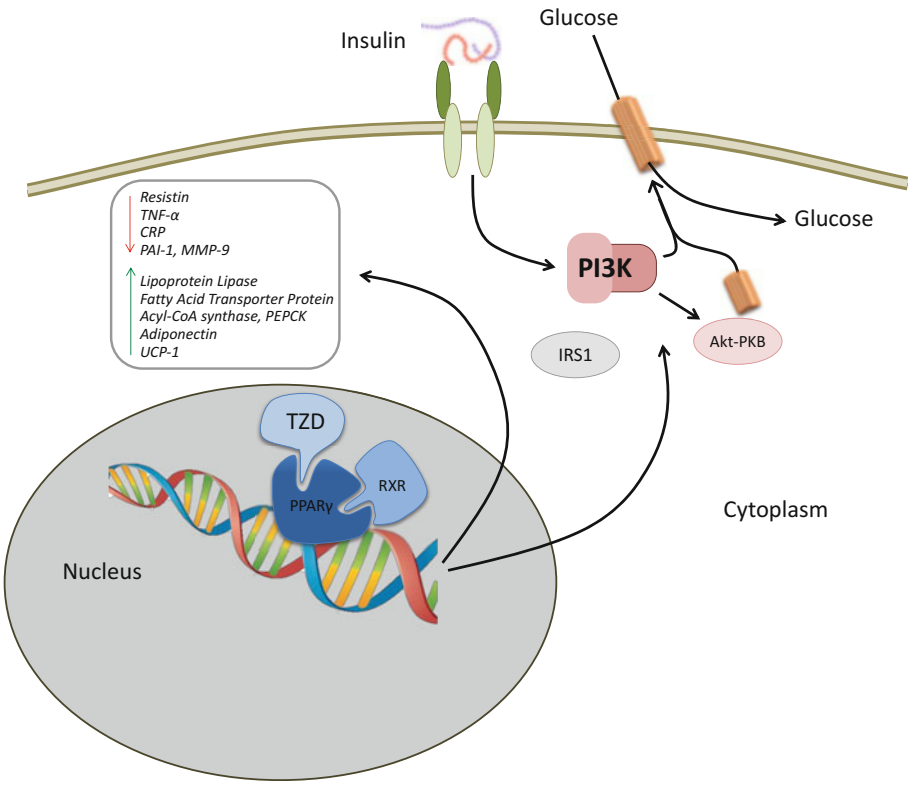


Fig. 3 Schematic representation of cellular mechanism of action of thiazolidinediones (TZD). TZD-mediated PPAR-γ activation elicits the formation of a heterodimeric complex with the retinoid-X receptor (RXR). The complex binds to the peroxisome proliferator response element (PPRE) nucleotide sequence (AGGTCAXAGGTCA) in the promoter regions of certain genes recruiting co-activators and altering the transcriptional activity of these genes and increasing intracellular insulin signaling

differentiation of preadipocytes into mature adipocytes, which possess better insulin sensitivity, lipogenic activity, and lower inflammatory response. This results in a reduction of lipolytic activity and lower circulating levels of FFA and cytokines, improvement of glucose utilization, and reduced hepatic glucose production.

TZDs cause an increase of adiposity. However, because of a more specific differentiating effect on subcutaneous rather than visceral fat (Adams et al. 1997), TZDs redistribute adipose tissue from omental to the subcutaneous compartment (Miyazaki et al. 2002) and reduce ectopic fat in liver and muscle. In vivo, TZDs improve insulin-mediated glucose utilization and increase insulin-mediated suppression of endogenous glucose production (Miyazaki et al. 2001; Pavo et al. 2003). The mechanisms are similar to the ones of metformin, but the effect of TZDs on glucose utilization is more pronounced (Natali and Ferrannini 2006). TZDs may exert a protective effect of β-cell mass and function. PPAR-γ are expressed in the β-cell and

in vitro studies have shown that TZDs can protect from lipotoxicity (Lupi et al. 2004) as well as prevent β-cell loss (Kanda et al. 2010). The combination of improved insulin sensitivity and β-cell protection can account for the characteristic durability of the glucose lowering properties of TZDs (Kahn et al. 2006, 2011).

Clinical efficacy – Treatment with TZDs is associated with 0.5–1.5% HbA1c reduction. As compared to other agents, this reduction is achieved more slowly, though it persists longer (Kahn et al. 2006). Pioglitazone is suitable for once daily administration, usually in the morning, with an initial dose of 15–30 mg that can be uptitrated to 45 mg after 3 months if clinical response is not achieved. Renal dosage adjustment is not necessary. TZDs can be combined with many other glucose-lowering agents with a potentiation of the glucose lowering effect (generally a HbA1c reduction of 0.5–1.0%), although the safety profile can change according to the agent used in the combination. Thus, while metformin can improve the insulin sensitization effect of TZDs, the combination with sulfonylureas or insulin can increase fluid retention and body weight gain (see infra), while the combination with a GLP-1 receptor agonist or SGLT2-inhibitor can limit the typical increase in body weight observed with TZD monotherapy with the latter also limiting fluid retention.

Rosiglitazone tends to increase both LDL- and HDL-cholesterol with no change in their ratio, while pioglitazone has no major effect on cholesterol. Both drugs reduce small and dense, i.e., more atherogenic, LDL particles. Pioglitazone induces greater triglyceride reduction (Goldberg et al. 2005) than rosiglitazone. Moreover, TZDs have a potential antiatherogenic effect as indicated by favorable effects on endothelial function, thrombotic processes, and mitigation of low-grade chronic inflammation (Fig. 4; McGuire and Inzucchi 2008; Erdmann and Wilcox 2010). In the PROactive study (Dormandy et al. 2005), although the primary endpoint (major adverse cardiac events plus peripheral vascular disease) did not reach statistical

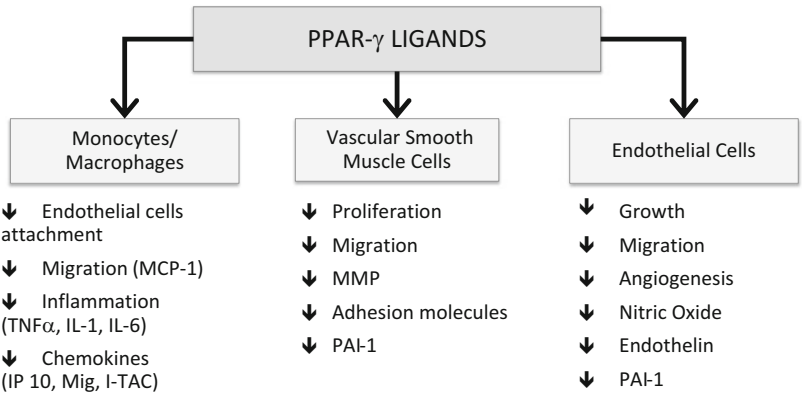


Fig. 4 Pleiotropic effects of TZDs involved in cardiovascular protection. PPAR-γ regulates various inflammatory processes within endothelial cells – e.g., the recruitment of leukocytes, the expression of inducible nitric oxide synthase, modulates cytokine release by monocytes/macrophages, the migration and function of vascular smooth muscle cells, and the process of angiogenesis by endothelial cells

Table 2 Potential benefits and challenges for PPAR-γ agonists

Potential advantages	Potential concerns
Insulin sensitization	Fluid retention
Beta-cell protection	Heart failure
Efficacy on fasting and post-prandial plasma glucose	Macular edema
Durability	Body weight gain
Tolerability in chronic kidney disease	Bone fractures
Cardiovascular protection	

significance (HR 0.90, p = 0.095), the predefined "main secondary endpoint" (i.e., combination of cardiovascular death, nonfatal myocardial infarction and stroke) was significantly reduced with the use of pioglitazone (HR 0.84, p = 0.027). Recently, in the Insulin Resistance Intervention after Stroke (IRIS) trial (Kernan et al. 2016), involving insulin resistant nondiabetic individuals with recent ischemic stroke or TIA, pioglitazone reduced the risk of fatal and nonfatal stroke or myocardial infarction (HR 0.76; 95%CI 0.62–0.93; p = 0.007).

Adverse effects – In spite of the potential cardiovascular benefit, the main concern with the use of TZDs is the risk of congestive heart failure (HF) due to fluid retention (Table 2; Mudaliar et al. 2003). Edema can occur in 5–10% of patients treated with TZDs and the incidence increases when TZDs are used in combination with insulin. TZDs should be used cautiously in NYHA class 1 and 2 patients, while their use is not recommended in those with class NYHA 3 and 4. Fluid retention can contribute to body weight gain and, possibly, worsening of diabetic macular edema. Finally, expansion of plasma volume may result in lower hemoglobin and anemia (Ryan et al. 2006). TZDs are associated with increased risk of bone fracture, due to inhibition of bone formation and increased bone reabsorption (Betteridge 2011). Fractures are more common in women and more commonly involve the lower and the upper distal limb. Caution has been suggested for the use of pioglitazone in patients with abnormal liver tests. On the contrary, there is no contra-indication or need of dose adjustment in those with impaired kidney function. As far as TZDs are used in monotherapy or in combination with drugs that do not stimulate insulin secretion, the risk of hypoglycemia is trivial. An initial concern was raised with respect to risk of bladder cancer with pioglitazone, but the results of a prospective study mandated by the FDA and analyses of large databases (Lewis et al. 2015; Levin et al. 2015) has dismissed it. In spite of that, pioglitazone has been withdrawn in France and Germany.

Other potential clinical use – TZDs are the most powerful drug for prevention of diabetes with reduction of the conversion rate from IGT to overt diabetes as high as 72% with pioglitazone (DeFronzo et al. 2011). In spite of this, no formal indication for the use of these drugs for prevention of diabetes has been approved. In patients with nonalcoholic steatohepatitis (NASH) pioglitazone reduces lipid accumulation, fibrosis, and inflammation (Sanyal et al. 2010). TZD have been used in women with PCOS. Similar to metformin, pioglitazone improves insulin sensitivity and hormonal and clinical signs of hyperandrogenism though there is no clear-cut evidence of improved reproductive outcomes (Du et al. 2012).

Drugs Acting on the β-Cell

Impaired insulin secretion is a key pathogenic defect in the development and progression of T2DM and it results from impaired response to glucose stimulus as well as reduction of β-cell mass. Stimulation of insulin secretion has been, therefore, an early target for treatment. This can be achieved by either direct stimulation of insulin secretion irrespective of prevalent plasma glucose levels as it occurs with sulfonylureas and glinides or in a glucose-dependent manner as it can be done by increasing the availability of GLP-1. As it is readily apparent the main difference between the glucose-independent and the glucose-dependent stimulation of β-cell is the risk of hypoglycemia that is always present with the former and trivial with the latter.

Sulfonylureas

Sulfonylureas have been used for treatment of T2DM for nearly 70 years and they are still widely used. Sulfonylureas have developed from first- (chlorpropamide, tolbutamide) to second-generation (glipizide, gliclazide, glibenclamide, gliquidone, glimepiride) agents with better meal-related insulin secretory response. First generation sulfonylureas are rarely used nowadays.

Pharmacology – All agents have a characteristic basic aryl sulfonylurea molecule. Substitutions at para position on the benzene ring and the nitrogen residue of the urea moiety characterize the different agents. First-generation sulfonylureas (chlorpropamide and tolbutamide) have a straight aliphatic side chain at NH_2 terminus, while the second-generation ones have a complex structure at the benzene ring and a ring structure at the amino terminus. Pharmacokinetic proprieties differ among individual sulfonylureas (Table 3). They are all well absorbed and reach a peak plasma concentration in 2–4 h. Onset of action is fastest for glipizide with glimepiride, whereas gliclazide, tolbutamide, glibenclamide, and chlorpropamide exhibit progressively less rapid onset. Gliclazide is also available as a modified release (MR) preparation suitable for once-daily administration. All sulfonylureas are highly (90–99%) bound to plasma proteins resulting in potential competition with other protein-bound agents such as warfarin, sulfonamides, and salicylates. They are metabolized by the liver and metabolites with variable activity and route of excretion are generated. Chlorpropamide is more hydrophilic and is partly excreted unchanged by the kidney. Glibenclamide is degraded into active metabolites, while those of glipizide and gliclazide are inactive. The biological effect of sulfonylureas can exceed their plasma half-life due to receptor interaction and degradation into active metabolites. The half-life of these agents is prolonged in case of renal failure. Variants of the genes encoding the K^+-ATP channel (*KCNJ11* and *ABCC8*) can alter the response to sulfonylureas (Winkler and Gerô 2011).

Mechanism of action – Sulfonylureas binding to the ATP-dependent K^+ channel (SUR1/Kir6.2) on pancreatic β-cells induce the closure of K^+-ATP channels, favoring local plasma membrane depolarization and opening of voltage-dependent L-type

Table 3 Pharmacologic characteristics of the most commonly used sulfonylureas

Molecule	Daily dose (mg)	Duration of action (hours; h)	Metabolism and elimination	SUR affinity and selectivity
Glibenclamide	2.5–15 mg (once/three times a day)	Intermediate to long (5–7 h)	Completely metabolized (mainly by oxidation) in the liver Kidney ≈ 50% Bile ≈ 50%	SUR1 SUR2 A and B receptors
Gliclazide	80–160 mg (once/twice a day)	Intermediate (10 h)	Almost completely metabolized in the liver Kidney ≈ 80–90% Bile ≈ 10–20%	SUR1 (high affinity and strong selectivity)
Gliclazide modified-release formulation	30–120 mg (once a day)	Intermediate (10 h)	Liver Kidney ≈ 80–90% Bile ≈ 10–20%	SUR1 (high affinity and strong selectivity)
Glipizide	2.5–20 mg (once/three times a day)	Short to intermediate (2–4 h)	Completely metabolized (mainly by oxidative hydroxylation) in the liver Kidney ≈ 80% Bile ≈ 20%	SUR1 SUR-2 A and B receptors
Gliquidone	30–90 mg (once/three times a day)	Short to intermediate (3 to 4 h)	Fully metabolized by the liver (hydroxylation and demethylation) Bile ≈ 95%	SUR1(high selectivity) SUR-2 A and B (low affinity) receptors
Glimepiride	2–6 mg (once/three times a day)	Intermediate (5 to 8 h)	Completely biotransformed by oxidative metabolism (CYP2C9) in the liver Kidney ≈ 60% Bile ≈ 40%	SUR1 SUR-2 A and B receptors

Ca^{++} channel with subsequent increase of Ca^{++} influx and cytosolic free Ca^{++} concentration. Higher intracellular Ca^{++} levels activate Ca^{++}-dependent signaling proteins that control the contractility of microtubules and microfilaments responsible for the exocytosis of the insulin granules. Since this mechanism is independent of glucose levels, use of sulfonylurea can increase the risk of hypoglycemia (Fig. 5; Sola et al. 2015).

Clinical efficacy – Sulfonylureas are commonly used as second- or third-line agents. Average and maximal reduction in plasma glucose is similar for all of them. When used as monotherapy, sulfonylureas lower fasting plasma glucose by 20–40 mg/dl (1–2 mmol/mol) and HbA1c by 1–2% (11–22 mmol/mol) (Hirst et al. 2013). The glucose lowering effect is usually rapid, but tends to wane over time. As compared to metformin and rosiglitazone, glibenclamide had the least

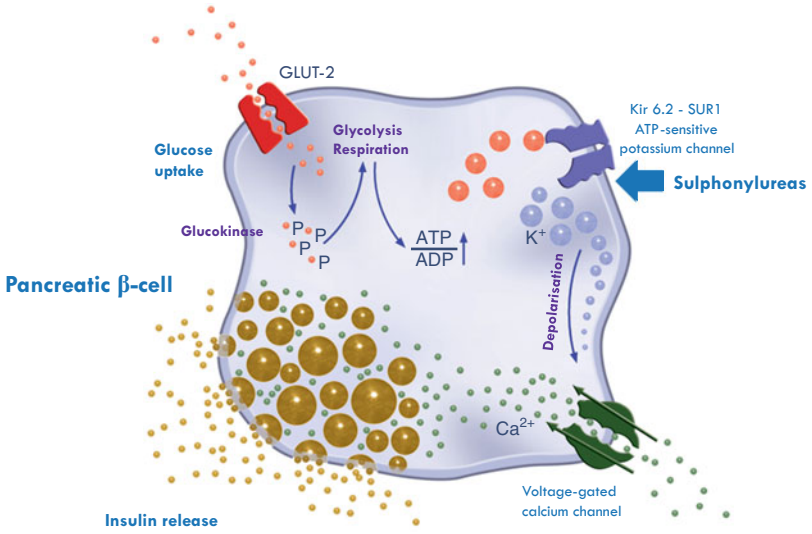


Fig. 5 Schematic representation of the mode of action of sulfonylureas. Sulfonylureas increase endogenous insulin secretion by binding to the extracellular domain of the ATP-dependent K+ channel on pancreatic β-cells and triggering a cascade of intracellular events, which lead to insulin secretion. Glucose enters the pancreatic β-cell via the GLUT-2 transporter and is phosphorylated via glucokinase, leading to changes in the ATP/ADP ratio. Under hyperglycemic conditions, glucose uptake and the consecutive change in the ATP/ADP ratio activates ATP-dependent K^+ channels that facilitate membrane depolarization of the β-cell. Membrane depolarization then triggers $Ca2^+$ influx from extracellular stores that stimulate insulin release from the granules in the β-cell. Sulfonylureas bind directly to the extracellular domain of the ATP-dependent K^+ channel and activate it. In this way, they trigger membrane depolarization of the β-cell with consecutive insulin release, independent of the actual glucose concentration

durable effect with greater rate of treatment failure (Garber et al. 1997). The efficacy of sulfonylureas largely depends on the degree of residual β-cell function. Since the latter is known to decrease over the time, a progressive loss of response to sulfonylureas can be expected. Moreover, some desensitization can occur with chronic or repeated stimulation by these agents (Ball et al. 2000). In vitro data have suggested that sulfonylureas could activate β-cell apoptosis (Maedler et al. 2005), although no clear in vivo evidence is available. Overall, secondary failure occurs in 5–10% of sulfonylurea-treated patients per annum. Sulfonylureas are commonly used in combination with other oral and injectable glucose-lowering agents. Because of the risk of hypoglycemia, treatment should be started with the lowest dose and uptitrated at 2- to 4-week interval according to glucose response. Sulfonylureas have trivial effect on lipid profile and may slightly increase blood pressure. Their effect on cardiovascular risk is a matter of discussion. A cross-reactivity with cardiovascular ATP-dependent K^+ channels (SUR2A/Kir6.2) and inhibition of "ischemic preconditioning" has been claimed (Meier et al. 2004). However, sulfonylureas have different affinity for the ATP-dependent K^+ channel on cardiac

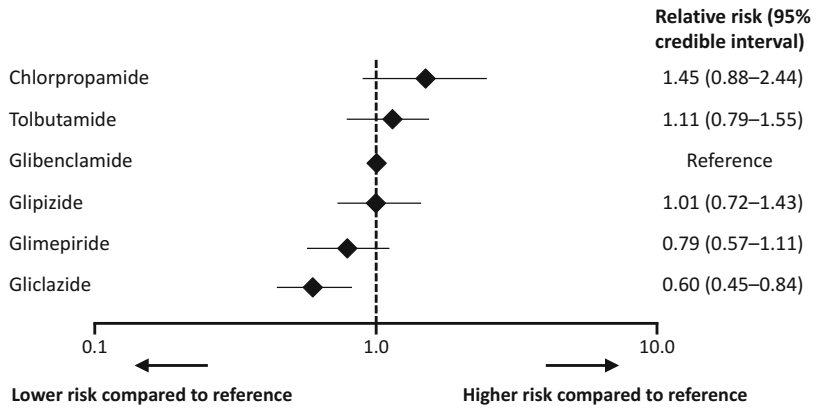


Fig. 6 Cardiovascular-related mortality for individual sulfonylureas. Data are pooled relative risks and 95% credible intervals calculated by network meta-analysis of direct and indirect evidence from 13 studies (Simpson et al. 2015)

muscle (Loubani et al. 2005; Mocanu et al. 2001), and those with greater selectivity for the pancreatic SUR1 receptor are considered to be safer (Fig. 6; Simpson et al. 2015) as it has been also shown with gliclazide in the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) (ADVANCE Collaborative Group 2008). Recent meta-analyses have generated conflicting results with some showing increased risk (Monami et al. 2013; Phung et al. 2013) and others finding no effect on CV events (Varvaki Rados et al. 2016). More recently, the TOSCA.IT study showed no difference in CV risk in patients randomized to treatment with sulfonylureas or pioglitazone added on top of metformin (Vaccaro et al. 2017). Sulfonylureas are the drug of choice in *HNF1A*- and *HNF4A*-MODY patients (Hattersley and Patel 2017).

Adverse effects – Use of sulfonylureas is associated with weight gain (1–4 kg) and risk of hypoglycemia. Hypoglycemia is usually mild occurring in ~20% of patients with <1% per annum experiencing severe hypoglycemia. This more commonly happens with chlorpropamide and glibenclamide. Irregular eating habits, aging, drug interactions, renal or liver disease, and presence of diabetic complications are risk factors for hypoglycemia. Combination therapy with other glucose lowering agents can further increase the risk of hypoglycemia. Treatment of hypoglycemia, particularly if due to long-acting sulfonylureas, can require hospitalization because to its typical persistence and the risk of relapse after glucose administration due to the potentiation of sulfonylurea-stimulated insulin secretion. Other infrequent side effects include skin reactions such as erythema multiforme, exfoliative dermatitis and, more rarely, photosensitivity. Occasionally, they can cause abnormalities in liver function tests. As mentioned, the action of nonselective sulfonylureas on vascular and cardiac muscle K^+channels have been a matter of concern. Tolbutamide and glibenclamide have been shown to inhibit ischemic preconditioning while gliclazide and glimepiride are safer on this count (Simpson et al. 2015).

Meglitinides

This class comprises repaglinide (introduced in 1998) and the structurally related D-phenylalanine analogue nateglinide (introduced in 2001).

Pharmacology – Meglitinides or glinides are rapidly absorbed (0.5–1 h) and rapidly cleared from the plasma (half-life <1 hr) after hepatic metabolism (Malaisse 2003). As such, their administration requires frequent administration, usually 15 min before each meal. Repaglinide is metabolized by the liver by cytochrome P450 (CYP3A4) with generation of inactive metabolites largely (90%) eliminated throughout the biliary tract. Therefore, drugs inhibiting cytochrome P450 (e.g., azoles, erythromycin) as well as those inducing its transcription (e.g., rifampin, barbiturates, carbamazepine, phenytoin) can alter the therapeutic efficacy of the drug. Only 2% of repaglinide is eliminated as such allowing its use in patients with renal insufficiency with no need for dose adjustment, whereas a slower titration schedule is recommended in those with liver disease. Nateglinide is metabolized mainly via CYP2C9 and CYP3A4 isoenzymes of cytochrome P450 and eliminated primarily by the kidney. Twenty percent of nateglinide dose is eliminated unmodified in the bile and 10% in the urine. Nateglinide is extensively bound to plasma proteins (98%) and has a relatively small volume of distribution. Onset of action is slightly faster and duration shorter as compared to repaglinide.

Mechanism of action – Glinides also work by closing ATP-dependent K^+ channels on the β-cell membrane binding a site of SUR-1 distinct from the sulfonylurea one (McLeod 2004; Owens et al. 2000). Binding to ATP-dependent K^+ channels induces insulin secretion via the same pathway described for sulfonylureas. Due to their pharmacokinetic properties, they have a more rapid onset of action and a shorter half-life resulting in a more physiologic insulin response largely restricted around mealtime (Schmitz et al. 2002), thereby reducing the risk of severe hypoglycemia (Hu et al. 2001).

Clinical efficacy – These medications mainly lower postprandial hyperglycemia ensuring a 0.6–1% (10–15 mmol/mol) HbA1c reduction, particularly in combination with metformin (Gerich et al. 2005). Because of prevalent meal effect, glinides are used in individuals with greater postprandial glucose excursion or irregular meal ingestion. Therapy should be started with low dose and up-titrated according to individual targets. Though monotherapy is indicated (at least in some countries), these agents are more commonly used in combination with insulin sensitizers. Variants of genes involved in drug metabolism, such as CYP2C9, CYP2C8, and SLCO1B1 as well as T2DM susceptibility genes (KCNQ1, PAX4 and BETA2) may influence efficacy and tolerability of glinides (Chen et al. 2015). There is no evidence for effects of repaglinide on CV risk. In the Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) trial, conducted in subjects with IGT and CV disease or CV risk factors, nateglinide therapy did not reduce the risk of diabetes or CV events (NAVIGATOR Study Group et al. 2010).

Adverse effects – The incidence of hypoglycemia is relatively low, due to the drug's short duration of action (Hu et al. 2001). Also modest weight gain can occur with glinide monotherapy. Sensitivity reactions are uncommon.

Drugs Acting on the Intestine

The gut contributes in several manners to glucose homeostasis. Hormones (incretins) are released at the time of nutrient ingestion that can amplify and accelerate the signal on the β-cell to secrete insulin. Among incretins, GLP-1 is crucial for prompt stimulation of insulin secretion and suppression of glucagon release. The effects of GLP-1 on islet function are glucose-dependent and, therefore, self-imitating allowing avoidance of over- and undershooting in plasma glucose concentration. Moreover, GLP-1 plays a key role in the maintenance of β-cell mass. The hormone, however, has a short half-life (1–2 min) being promptly degraded by the DPP-4 enzyme. Given the importance of ensuring sufficient GLP-1 in the circulation, blocking its degradation has become a therapeutic target (Meier 2012). The rate of absorption of carbohydrate determines the rate of increase of plasma glucose levels after the ingestion of a meal as clearly shown by lower postprandial glucose peaks with the ingestion of slowly absorbable carbohydrates and high-fiber diet.

Dipeptidyl Peptidase-4 Inhibitors

DPP4 inhibitors, also known as "gliptins," work by blocking the activity of the enzyme DPP-4 responsible for degradation of GLP1 (Meier 2012). The prolonged persistence of biologically active GLP1 enhances glucose-dependent stimulation of insulin secretion and glucagon suppression. The first DPP-4 inhibitor made available for clinical use was sitagliptin in 2006, followed by vildagliptin (2008), saxagliptin (2008) and, more recently, by linagliptin (2011) and alogliptin (2013).

Pharmacology – DPP-4 inhibitors share many properties but also have pharmacokinetics differences (Table 4). DPP-4 inhibitors are absorbed rapidly with onset of activity in <10 min after administration achieving t_{max} within 2 h. Sitagliptin and alogliptin are characterized by a rather long half-life, allowing once-daily administration. Linagliptin has higher binding to proteins (>80% at the therapeutic dose) also resulting in long half-life. In contrast, vildagliptin has shorter half-life and is administered twice daily. Saxagliptin has a rather short half-life but, due to generation of active metabolite, also can be given once daily. In contrast metabolism of sitagliptin and vildagliptin leads to formation of inactive metabolites, whereas alogliptin undergoes little metabolism. Since the kidney eliminates most DPP-4 inhibitors, a dose reduction is required for patients with moderate to severe renal impairment, with the exception of linagliptin, which is eliminated mainly via the bile requiring no dose adjustment in people with renal impairment (Ramirez et al. 2013). Recommendations for DPP4-inhibitors use in severe hepatic impairment vary due to relatively limited clinical experience in such patients. Drug–drug interactions between DPP-4 inhibitors and other medications are minimal; only saxagliptin may have drug–drug interactions via cytochrome P450 requiring dose reduction if co-administered with potent CYP3A4 inhibitors.

Mechanism of action – DPP-4 is a member of a family of proteases that includes DDP-8, DPP-9, and fibroblast activation protein (FAP). DPP-4 cleaves the

Table 4 Pharmacologic characteristics of DPP-4 inhibitors

DPP-4 Inhibitor	Chemistry	Compound $t_{1/2}$	Dosing	DPP-4 inhibition[a]	Metabolism	Elimination route
Sitagliptin	β-Amino acid-based	8–24 h	100 mg qd	Max~97%; >80% 24 h postdose	Not appreciably metabolized	Renal (~80% unchanged as parent)
Vildagliptin	Cyanopyrrolidine	$1_{1/2}$–$4_{1/2}$ h	50 mg bid	Max~95%; >80% 24 h postdose	Hydrolyzed to inactive metabolite (P_{450} enzyme independent)	Renal (22% as parent, 55% as primary metabolite)
Saxagliptin	Cyanopyrrolidine	2–4 h (parent) 3–7 h (metabolite)	5 md qd	Max~80%; >70% 24 h postdose	Hepatically metabolized to active metabolite (via P_{450} 3A4/5)	Renal (12–29% as parent, 21–52% as metabolite)
Alogliptin	Modified pyrimidinedione	12–21 h	25 mg qd	Max~90%; >75% 24 h postdose	Not appreciably metabolized	Renal (>70% unchanged as parent)
Linagliptin	Xanthine-based	10–40 h	5 mg qd	Max~80%; >70% 24 h postdose	Not appreciably metabolized	Biliary (>70% unchanged as parent); <6% via kidney

[a]DPP-4 activity measured in human plasma ex vivo; not corrected for sample dilution in the assay

N-terminal dipeptide from peptides that have either an alanine or a proline residue penultimate to the N-terminus. DPP-4 inhibitors prevent aminopeptidase activity of DPP-4 (Sedo and Malík 2001) reducing inactivation of GLP-1 (and GIP) which persists at higher concentration in the circulation (Flatt et al. 2008; Verspohl 2009). The increase of GLP-1 levels is less than the one achieved with GLP1-receptor agonists and not sufficient to elicit satiety, to slow gastric emptying, and to cause nausea. Nonetheless, the increased availability of GLP-1 enhances nutrient-induced insulin secretion and suppresses glucagon secretion. Besides this canonical mechanism of action, alternative mechanism have been claimed to account for the glucose lowering action of these drugs, including a direct effect on hepatic glucose metabolism. The glucose-dependent concerted hormonal effect accounts for low risk of hypoglycemia. DPP4-inhibitors predominantly lower postprandial hyperglycemia, but because of a carryover effect and overnight glucagon suppression a reduction of fasting and interprandial glycemia occurs as well (Verspohl 2009; Deacon 2011).

Clinical efficacy – In spite of different pharmacokinetics and pharmacodynamics (Table 1), DPP4-inhibitors have similar antihyperglycemic properties. As monotherapy and in combination with other agents, they reduce HbA1c by ~0.7–1.0%

(8–11 mmol/mol) depending on baseline levels, with reductions of up to ~2% in subjects with elevated HbA1c. Corresponding reduction in postprandial and fasting plasma glucose are ~54 mg/dl (~3 mmol/l) ~18–27 mg/dl (~1–1.5 mmol/l), respectively. DPP-4 inhibitor monotherapy generally results in smaller HbA1c reductions than metformin, but they are overall equivalent to sulfonylureas and TZDs as add-on therapy to metformin (Karagiannis et al. 2012). DPP-4 inhibitors are recommended as monotherapy in patients in whom metformin cannot be used; in such cases they are preferred agents since they do not cause hypoglycemia and are weight neutral. As add-on to metformin they provide a rational combination targeting insulin resistance and gluconeogenesis as well as islet dysfunction. Interestingly, the combination is associated with lower incidence of gastrointestinal side effects as compared to metformin monotherapy (Reasner et al. 2011). Because of greater efficacy, some guidelines (e.g., AACE/ACE) recommend this as initial combination therapy in patients with elevated HbA1c levels at diagnosis (Rodbard et al. 2009; Garber et al. 2013). An ongoing trial (VERIFY) investigates the effect of early combination even in patients with lower HbA1c at presentation (Del Prato et al. 2014). The sulfonylurea/DPP-4 inhibitor combination gives additional glycemic efficacy but greater risk of hypoglycemia. Thus, lower sulfonylurea dosage should be used at the time of combination with a DPP4-inhibitor (de Heer and Holst 2007). The pioglitazone/DPP-4 inhibitors combination reduces HbA1c more than with either agent alone (Rosenstock et al. 2006) thanks to complementary mechanisms of action. Adding a DPP-4 inhibitor to insulin can improve glycemic control with no increase in hypoglycemia (Kothny et al. 2013) and some insulin-sparing (Barnett et al. 2013). The DPP-4 cleaves substrates other than GLP-1 such as neuropeptide Y, substance P, SDF-1α, cytokines, and chemokines modulating their circulating concentrations and actions. DPP-4 inhibition can alter these processes with effects that may be relevant with respect to the CV risk, diabetic nephropathy, and retinopathy (Wu et al. 2014; Mori et al. 2014; Tani et al. 2013; Gonçalves et al. 2014; Ott et al. 2014; Fig. 7). In spite of the activation of potential cardiovascular benefits, the cardiovascular outcomes trials (Scirica et al. 2013; Green et al. 2015; White et al. 2013) have provided evidence for safety but not reduced CV risk and suggested a beneficial effect on albuminuria independent of glucose control (Avogaro and Fadini 2014). In all cases, DPP4-inhibitors provide an effective and safe approach of diabetic subjects at any stage of impaired kidney function (Russo et al. 2013).

Adverse effects – DPP-4 inhibitors have a good safety profile often indistinguishable from that of placebo (Monami et al. 2011; Deacon and Holst 2013). The glucose-dependent action on pancreatic hormone secretion confers a low risk of hypoglycemia, unless DPP-4 inhibitors are administered with sulfonylurea or insulin.

Since DPP-4 is the CD26 T-cell activation antigen, initial concern was raised, but neither CD26 knockout mice nor DPP-4- inhibitors have shown significant untoward immune-related effects (Karagiannis et al. 2012). The selectivity of DPP-4 inhibition represents an important feature of the class because inhibition of related enzymes such as DPP-8 and DPP-9 was associated with blood dyscrasia and skin lesions at least in some species.

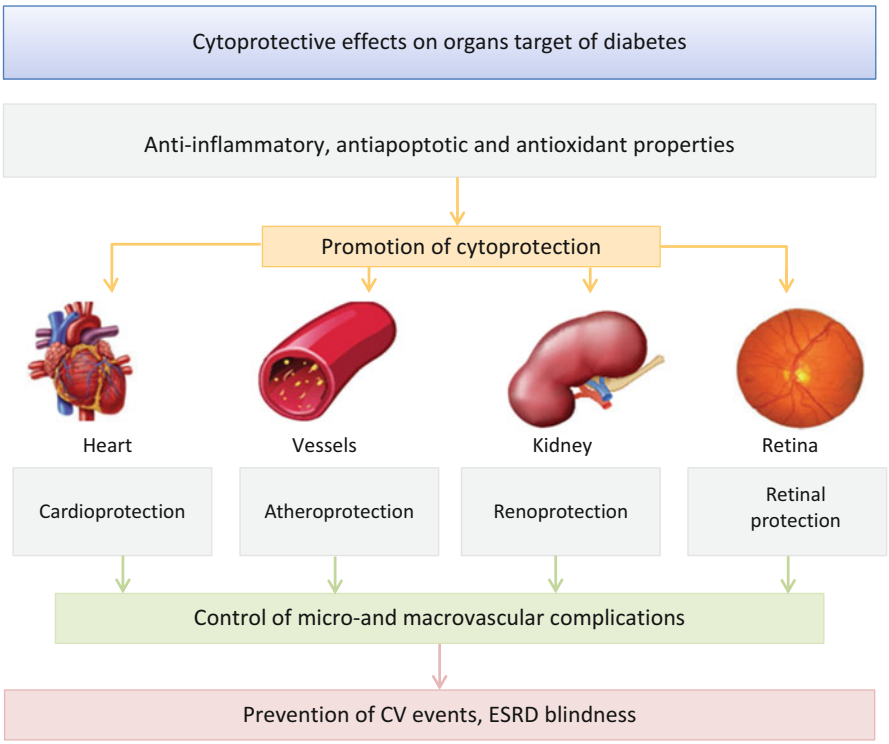


Fig. 7 Putative cytoprotective effects of dipeptidyl peptidase-4 inhibitors on organs/tissues targeted by diabetes, including the heart, vessels, kidney, and retina, that are associated with serious diabetic complications

Upon introduction of DPP4-inhibitors in the market, there has been much debate about potential risk of acute pancreatitis and pancreatic cancer. Current evidence does not support increased incidence of both events (Monami et al. 2011). Regulatory authorities in the USA and Europe have carried out independent reviews of all available data (Egan et al. 2014) finding no evidence to support a causal relationship between incretin therapies and pancreatitis (Fig. 8). In the CV outcome trials with DPP-4 inhibitors a low incidence of acute pancreatitis was recorded with small nonstatistically significant imbalances in the number of events, being numerically higher with DPP-4 inhibitors than placebo (Scirica et al. 2013; Green et al. 2015; White et al. 2013; Avogaro and Fadini 2014; Meier and Nauck 2014). Event rates for pancreatic cancer were even lower, and numerically lower in DPP-4 inhibitor recipients (Raz et al. 2014). Nevertheless, appropriate caution is recommended and DPP-4 inhibitors should be stopped if pancreatitis is suspected, and alternative therapy should be preferred for people with a history of pancreatitis.

The DPP-4 inhibitors have undergone comprehensive investigation of their CV safety showing that they do not increase CV events in individuals with T2DM and CV disease or at high risk for CV disease. Saxagliptin was associated with a small,

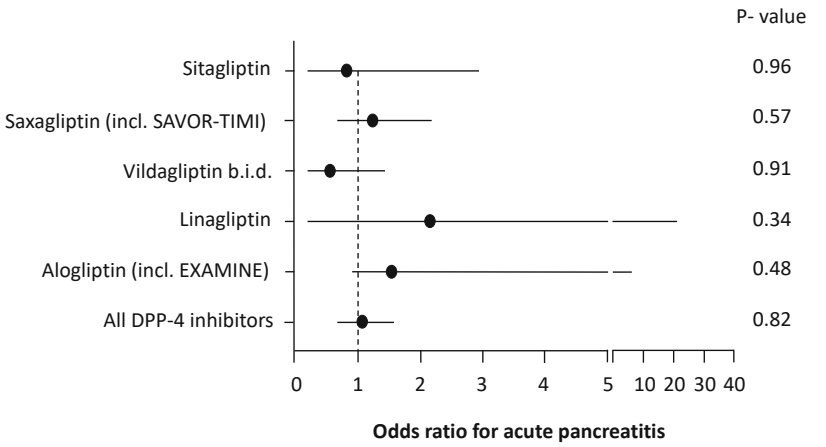


Fig. 8 Risk of acute pancreatitis (Odd Ratios, 95% Confidence Interval) comparing treatment with DPP4 inhibitors versus placebo or active glucose-lowering medications from pooled results of phase III clinical trials. b.i.d, twice daily. (Adapted from Meier and Nauck 2014)

but statistically significant 27% increase in hospitalization for heart failure compared with placebo with no increase in mortality. This risk was greater in patients with preexisting heart failure, elevated baseline B-type natriuretic peptide levels or chronic kidney disease (Scirica et al. 2014). A retrospective post hoc analysis of the EXAMINE data (Zannad et al. 2015) showed a similar, albeit nonsignificant, trend (3.1 vs. 2.9%). There was no indication of any increase in heart failure-related hospitalizations in TECOS (McGuire et al. 2016). More recently, the FDA has issued a warning for saxagliptin and alogliptin concerning potential risk of heart failure.

α-Glucosidase Inhibitors

The rate of absorption of carbohydrate from the gut is a main factor controlling plasma glucose excursions after the ingestion of a meal. Reducing the rate of absorption, as it can be done with slowly absorbable carbohydrate and/or high-fiber diets, can reduce postprandial glucose elevation. A similar effect can be obtained by inhibiting α-glucosidase enzymes responsible for degradation and absorption of complex carbohydrates ingested with meals. To this purpose specific α-glucosidase inhibitors have been developed late in the 1970s and introduced in the diabetes pharmacopeia in the early 1990s.

Pharmacology – α-Glucosidase inhibitors are poorly absorbed as they are locally degraded in the gut by amylase and the intestinal flora. The small proportion (<2%) of drug appearing in the systemic circulation is eliminated through the kidney. The available α-glucosidase inhibitors have slightly different affinity for the various amylases (Bischoff 1994).

Mechanism of action – α-Glucosidase inhibitors (acarbose, miglitol, and voglibose) block the α-glucosidase enzymes (maltase, isomaltase, sucrase, and gluco-amylase) in the small intestinal brush border and delay the cleavage of disaccharides and absorption of glucose (Clissold and Acarbose 1988). As a consequence of delayed absorption, more glucose is delivered to the distal portion of the intestine where it can stimulate the release of GLP-1. Overall, administration of α-glucosidase inhibitors reduces plasma insulin concentration after the ingestion of a meal due to slower appearance of glucose in the systemic circulation. In order to be effective, the drug may be present in the gut and, therefore, α-glucosidase inhibitors must be taken before each meal.

Clinical efficacy – The main effect of the α-glucosidase inhibitors is reduction of postprandial glucose in the range of 20–60 mg/dl (2–3 mmol/l) (Laar et al. 2005) though, with chronic treatment, an average reduction in fasting plasma glucose of about 20 mg/dl (1 mmol/l) is achieved. The concomitant reduction in HbA1c averages 0.5–0.8%. There is no much information about differential efficacy of the three α-glucosidase inhibitors with only one study showing acarbose to be more potent than voglibose (Matsumura et al. 2009). The glucose lowering efficacy of acarbose is less than that the one of sulfonylureas and metformin (Chiasson et al. 1994; Coniff et al. 1995; Bayraktar et al. 1996). α-Glucosidase inhibitors do not cause weight gain or hypoglycemia and may slightly reduce triglyceride levels (Ogawa et al. 2004). A reduction in cardiovascular events was found in an intervention trial with acarbose in IGT subjects (Chiasson et al. 2003). Similarly, a significant reduction in cardiovascular events was found in a meta-analysis of seven randomized clinical trials in T2DM subjects (Hanefeld et al. 2004). This has not been confirmed in a Cochrane systematic review, and meta-analysis of 41 studies involving exposure to α-glucosidase inhibitors in monotherapy for $\geq$12 weeks in T2DM patients found no evidence of any effect on morbidity or mortality (Laar et al. 2005) nor in a large intervention trials performed in Chinese individuals with IGT (Holman et al. 2017).

Adverse effects – Hypoglycemia is uncommon unless α-glucosidase inhibitors are used in combination with drugs with greater hypoglycemic potency. Gastrointestinal side effects (abdominal pain, flatulence, and diarrhea) are common though generally mild, and they can be minimized by starting with a low dose (50 mg/day acarbose) followed by gradual up-titration. These effects usually vanish with time (4–8 weeks), but can lead to discontinuation in some cases. Pneumatosis cystoides intestinalis is a rare condition characterized by gas accumulation in the submucosa of the bowel wall. The drugs are contraindicated in patients with inflammatory bowel disease, malabsorption syndromes, colonic ulceration, or partial intestinal occlusion.

Other potential clinical use – Acarbose has been tested for T2DM prevention in IGT patients. In the STOP-NIDDM trial, acarbose treatment was associated with a 25% risk reduction of conversion to T2DM (Chiasson et al. 2002). A larger study carried out in Chinese subjects with IGT has confirmed a preventative effect of T2DM but no effect on CV risk (Hanefeld et al. 2004). Acarbose has been also used for postprandial reactive hypoglycemia (Peter 2003) as well as for hypoglycemia associated with dumping syndrome (Fujita et al. 2012) and subsequent to bariatric surgery (Valderas et al. 2012).

Bile Acid Sequestrants

Bile acid sequestrants have been used for the treatment of dyslipidemia for decades. In addition to their ability to lower LDL cholesterol, they have been shown to improve glycemic control in T2DM patients (Fonseca et al. 2010). Colesevelam was approved for treatment of dyslipidemia in 2000, and in 2008 it was also approved for controlling hyperglycemia in adult T2DM subjects in the United States. Colestimide is approved in Japan for the same indication.

Pharmacology – Pharmacokinetic data are limited, as colesevelam is not systemically absorbed. Animal studies indicate that absorption is not altered by chronic use. Interactions between colesevelam and other drugs within the intestinal tract are theoretically minimal due to the polymer structure of colesevelam, compared with other bile acid sequestrants. However, colesevelam has been shown to reduce intestinal absorption of certain medications including some glucose lowering agents (Weitzman et al. 2009).

Mechanism of action – The exact mechanism of how bile acid sequestrants lower glucose levels is unknown. Sequestration of bile acids can activate bile acid receptor-1 (TGR5) and farnesoid receptor X (FRX) leading to suppression of hepatic glucose production. Colesevelam could also increase delivery of bile acid to distal portion of the intestine where they could stimulate L cell and increase GLP-1 secretion (Hansen et al. 2014).

Clinical efficacy – Colesevelam, added on top of existing glucose lowering therapy, reduces HbA1c by 0.50% (Handelsman 2011) with low risk of hypoglycemia and neutral effect on body weight. Along with glucose lowering effect, colesevelam improves lipid profile reducing total cholesterol and non-HDL cholesterol though an increase of triglycerides has been noted (Fonseca et al. 2008).

Adverse effects – As per all bile acid sequestrants, constipation, dyspepsia, and nausea are the most common adverse effects associated with the use of colesevelam. The medication should be used with caution in patients with *gastroparesis* and not used in patients with triglyceride concentrations >500 mg/dL, a history of bowel obstruction, or previous hypertriglyceridemia-induced pancreatitis. According to the manufacturer's package insert, this agent should be administered at least 4 h apart from the time of administration of other drugs known to have reduced absorption or side effect when co-administered with colesevelam (e.g., phenytoin, warfarin, cyclosporine, levothyroxine).

Drugs Acting on the Kidney

Experimental evidence in rodents as well as human data indicates that the renal threshold for glucose reabsorption is increased by ~20% in the diabetic condition (Farber et al. 1951) accounting for a paradoxical increase in glucose reabsorption in spite of prevalent hyperglycemia. Renal glucose reabsorption is largely mediated by the activity of the Sodium-Glucose Co-transporter 1 and 2 (SGLT1 and SGLT2) located in the proximal tubule. For glucose levels below the tubular threshold, the

SGLT-2 isoform, located in the S1 segment of the proximal tubule, is responsible for 90% of glucose reabsorption with the remaining 10% reabsorbed downstream by SGLT-1. The former is mainly expressed in the kidney, while the second is abundant in the gut where it participates to absorption of dietary sugars. Early experimental studies with phlorizin, a SGLT2 and 1 inhibitor, showed that phlorizin-induced glycosuria reduced hyperglycemia via an insulin-independent mechanism suggesting inhibition of glucose reabsorption as a therapeutic target (Rossetti et al. 1987a, b).

Sodium-Glucose Co-transporter 2 Inhibitors

Phlorizin has low oral bioavailability and it causes gastrointestinal side effects. Subsequent research led to development of highly selective SGLT2 inhibitors. Currently, three SGLT2 inhibitors (canagliflozin, dapagliflozin, and empagliflozin) are available with three additional agents (ipragliflozin, luseogliflozin, and tofogliflozin) marketed in Japan. Dual SGLT2 and SGLT1 inhibitors are currently in their clinical development (Lapuerta et al. 2015).

Pharmacokinetics – After oral administration, the three SGLT2 inhibitors have similar peak plasma concentrations (t_{max} 1–2 h), half-life ($t_{1/2}$ 13 h), and binding to plasma protein (>90%). Dapagliflozin and related metabolites are primarily eliminated via urinary excretion with less than 2% excreted as unchanged parental molecule. Canagliflozin is metabolized in the liver via O-glucuronidation into two inactive O-glucuronide metabolites. Parental molecule and metabolites are eliminated with feces (70–75%) and the remaining metabolites with urine. Empagliflozin is eliminated in feces (41.2%) and urine (54.4%). SGLT2 inhibitors show no clinically significant propensity to drug-to-drug interactions (Table 5; Scheen 2014).

Mechanism of action – SGLT2 inhibitors reduce the maximum tubular transport rate (*Tm*) of glucose resulting in glycosuria at lower plasma glucose concentration (Wright 2001; Lee et al. 2007; Hummel et al. 2011) and excretion in the urine of 30–50% of the glucose filtered through the glomerulus (50–80 gr/day) (Liu et al. 2012; DeFronzo et al. 2013). The glucose lowering effect of these drugs is independent of β-cell function or insulin sensitivity accounting for low risk of hypoglycemia (Zhang et al. 2010; Nauck 2014). Because of calorie loss and osmotic diuresis driven by glycosuria, treatment with SGLT2 inhibitors is associated with sustained reduction in body weight and blood pressure. Body weight loss, however, is less than expected, most likely due to compensatory increased calorie intake (Ferrannini et al. 2015). Interesting, reduction of blood pressure is not associated with increased heart rate. Canagliflozin have a mild inhibitory effect on SGLT1 that could contribute to its glucose lowering effect. Chronic improvement of glucose control can relieve glucose toxicity, which, in turn, may result in an improvement of beta-cell function (Merovci et al. 2015) and insulin sensitivity (Merovci et al. 2016). The overall glucose-lowering efficacy can be, to some extent, hampered by concomitant increase in glucagon levels (Merovci et al. 2014; Ferrannini et al. 2014), which, along with

Table 5 Main pharmacologic characteristics of SGLT2 inhibitors (Modified from 143)

	Dapagliflozin	Empagliflozin	Canagliflozin
Molecular formula	$C_{21}H_{25}ClO_6$	$C_{23}H_{27}ClO_7$	$C_{24}H_{25}FO_5S$
Molecular class	C-glycoside	C-glycoside	C-glycoside
Dose (mg)	5 and 10	10 and 25	100 and 300
Administration	Oral	Oral	Oral
Mean absolute oral bioavailability (%)	78	Not available	≈65
T_{max} (h)	1–2	1–2	1–2
Maximum plasma concentration; C_{max} (ng/ml)	94/158	102/227	1069/2939
AUC (ng.h/ml)	324/628	786/1725	6871/20972
Plasma binding protein (%)	91	Not available	99 (mainly to albumin)
Elimination half-life; $t_{½}$ (h)	12.2/12.9	13.1/10.2	10.6/13.1
Steady-state volume of distribution (L)	118	Not available	119
Mean systemic clearance (mL/min)	207	Not available	192
Renal clearance (mL/min)	≈5	≈40	≈1–2
Metabolism	Mainly hepatic	Dual renal and hepatic	Mainly hepatic
Route of elimination	75% in urine (parent drug and inactive metabolites) and 21% in feces	11–19% unchanged in urine	Biliary excretion (60%) and urine (32%); <1% unchanged in urine
CYP-mediated metabolism	No	ADME not yet reported	Minimal (7% CYP3A4); weak inhibition of CYP2B6, CYP2C8, CYP2C9 and CYP3A4
Glucose excretion (g/day)	18–62	70–90	≈70

lower insulin levels and increased lipid mobilization, may increase ketone body formation (Fig. 9).

Efficacy of SGLT2 inhibitors depends on plasma glucose levels and rate of glomerular filtration. In T2DM patients with moderate renal impairment, SGLT2 inhibition is associated with less urinary glucose excretion than in patients with normal renal function and glucose-lowering efficacy also is reduced (Ferrannini and Solini 2012; Kohan et al. 2014; Yale et al. 2014). SGLT2 inhibitors have no detrimental effects on renal function (DeFronzo et al. 2012; Devineni et al. 2012; Kohan et al. 2016; Barnett et al. 2014).

Clinical efficacy – SGLT2-inhibitors can be successfully used in monotherapy, and due to their mechanisms of action independent of insulin secretion and action, they can be combined with any other existing treatment. SGLT2-inhibitors cause a rapid reduction in both fasting and postprandial plasma glucose levels with a reduction of HbA1c of 0.6–1.2% (7–13 mmol/mol). These effects are more sustained and durable than the one obtained with sulfonylureas (Del Prato et al. 2015) and are attained with lower risk of hypoglycemia unless SGLT2 inhibitors used in combination with sulfonylureas or insulin. In combination with metformin, SGLT2 inhibitors are associated with 2–4 kg body weight reduction, largely accounted for reduction of adipose tissue both at the subcutaneous and visceral level. In combination with insulin, SGLT2 inhibitors improve glycemic control with no further increase in the risk of hypoglycemia and prevent some of the body weight gain typically found with insulin therapy (Wilding et al. 2014). Blood pressure is also decreased (systolic 3–5 mmHg) as an effect of osmotic diuresis and potentiation of other blood pressure lowering agents (Table 6). Although current labeling of SGLT2 inhibitors contraindicates their use in patients with an eGFR <45 ml/min/1.73 m^2, recent experimental and clinical observations suggest that these agents may exert a potential nephroprotective effect (Fioretto et al. 2016). SGLT2 inhibitors also lower serum uric acid level (Davies et al. 2015) and exert a mixed effect on lipid profile with reduction of triglycerides and some increase in LDL and HDL cholesterol without affecting their ratio. The recent CV outcome trials with empagliflozin (EMPA-REG OUTCOME) (Zinman et al. 2015) and canagliflozin (CANVAS) (Neal et al. 2017) have provided evidence for a CV protection. In particular, in the EMPA-REG OUTCOME, empagliflozin reduced the risk of death from CV causes (38% relative risk reduction), hospitalization for heart failure (35% relative risk reduction), and death from any cause (32%) (Zinman et al. 2015). Similarly, in the CANVAS Program the rate of the primary outcome (a composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke) was lower with canagliflozin than with placebo (HR 0.86, 95% CI 0.75–0.97) (Neal et al. 2017). Currently, the FDA and EMA have approved empagliflozin for reduction of CV risk in T2DM patients with prior CV events, and a similar recommendation has been formulated in official guidelines.

Adverse effects – Urinary tract and genital tract infections are the most common side effect associated with SGLT2 inhibitors (Wu et al. 2016) occurring at greater incidence in women (in particular mycotic vulvo-vaginitis) than in men. No predisposing risk factors of UTIs or genital infections have been identified. These events tend to be self-limiting and to respond to usual treatment. Volume depletion due to drug-induced osmotic diuresis is a potential adverse event particularly in older patients, in those taking antihypertensive agents and with moderate renal impairment (Johnsson et al. 2016). Adequate hydration, particularly at the start of therapy, should be always considered due to initial marked glycosuria and fluid loss, particularly in case of high plasma glucose levels. The FDA has issued a warning for risk of fracture associated with use of dapagliflozin and canagliflozin. Evidence for a direct effect on bone metabolism is uncertain, and it has been suggested the risk of bone fracture may be rather the consequence of falls due to orthostatic hypotension.

Table 6 Potential benefits and challenges for SGLT2 inhibitors

Pros	Cons
Glucose-lowering efficacy	Increased risk of mild/moderate genital mycotic infection
Durability	Bone fracture
Versatility (mono or combination therapy, including insulin)	Euglycemic ketoacidosis
Low hypoglycemia risk	Lower-limb amputations
Novel mechanism of action (insulin-independent)	Volume-related adverse events
Improvements in insulin sensitivity and beta cell function	Acute kidney failure
Weight reduction	Relatively ineffective for low GFR
Hemodynamic effects (blood pressure reduction)	Costly
Generally safe and well tolerated	
Cardiovascular benefit	
Renal benefit	

A recent meta-analysis has not lent support to a damaging effect of SGLT2 inhibitors on bone metabolism (Tang et al. 2016), and additional analysis is needed for any firm conclusion to be drawn.

An increased risk of lower limb amputation (primarily at the level of the toe or metatarsal) has been documented in patients in the CANVAS and CANVAS-R trials taking canagliflozin (Neal et al. 2017). The mechanism responsible for this risk is still unclear, and it could be related to a reduction of circulating volume and increased blood viscosity (Table 6).

In the early stage of the clinical program of dapagliflozin, a nonstatistically significant imbalance for male bladder cancer and female breast cancer was observed. This association has not been confirmed in large CV outcomes trials (Zinman et al. 2015; Neal et al. 2017). A meta-analysis (Tang et al. 2017) found no increased risk of overall cancer (OR 1.14 [95% CI 0.96, 1.36]), though an increased risk for bladder cancer emerged along with reduction of the risk for tumors of the gastro-intestinal tract with the use of canagliflozin (OR 0.15 [95% CI 0.04, 0.60]). Overall, data are inconclusive requiring larger studies and longer observation.

Cases of euglycemic ketoacidosis have been reported during treatment with SGLT2 inhibitors (Burke et al. 2017), inducing regulatory authorities to issue a warning (Food and Drug Administration 2015). Overall, the incidence of DKA in clinical trials with SGLT2 inhibitors in T2DM patients is low (Erondu et al. 2015; Rosenstock and Ferrannini 2015) and the majority of the cases occurred in T1DM patients (currently not an indication) or vulnerable insulin-treated T2DM patients in whom SGLT2 inhibitors should be used with extreme caution (Rosenstock and Ferrannini 2015; Monami et al. 2017; Fig. 9) although it has been claimed that DKA may not be limited to any particular demographic or comorbid subpopulation

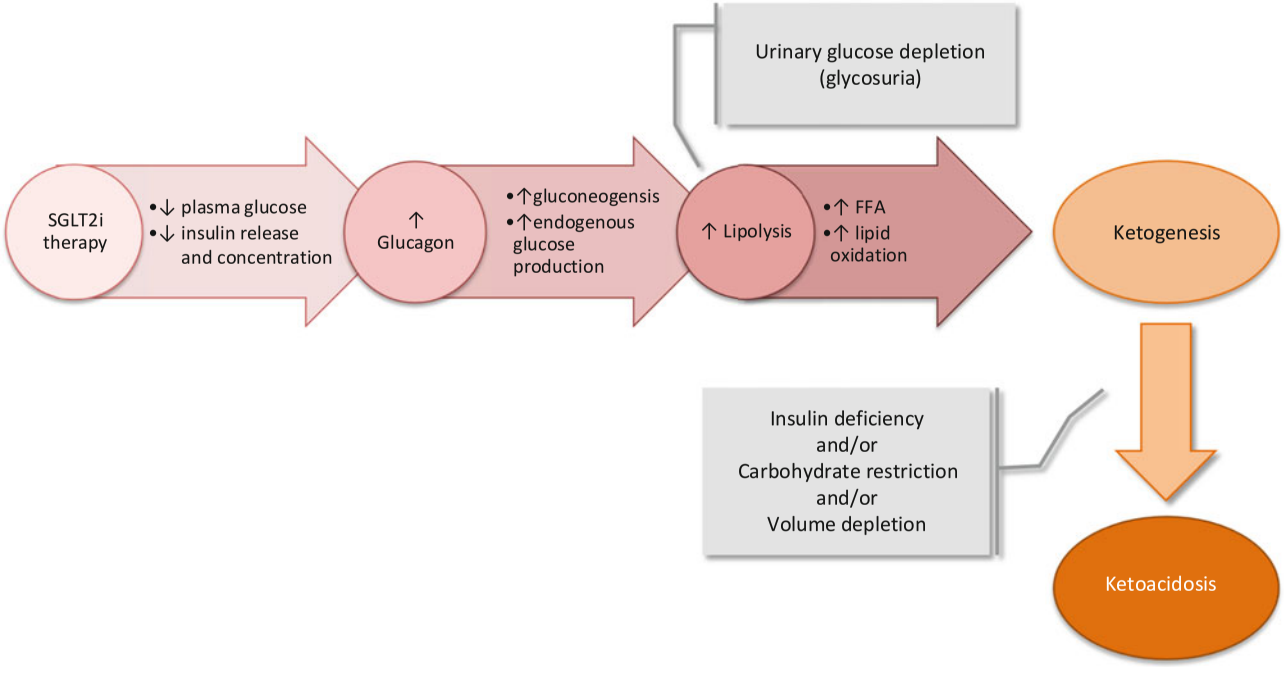


Fig. 9 Schematic representation of the pathophysiologic mechanisms responsible for SGLT2 inhibitor related euglycemic ketoacidosis. SGLT2 inhibitors decrease plasma glucose levels and insulin while increasing glucagon concentrations increase. The hormonal shift accounts for activation of gluconeogenesis in the liver, increased endogenous glucose production, stimulated lipolysis, and enhanced lipid oxidation. Increased FFA availability results in mild activation of ketogenesis, which under stress condition or marked insulinopenia can evolve toward ketoacidosis. The concomitant SGLT2 inhibitors mediated glycosuria account for paradoxical euglycemia

and could occur at any duration of SGLT2 inhibitors use (Fadini et al. 2017). Practical recommendation is not to initiate a SGLT2 inhibitor in patients with a history of DKA and to withdraw the treatment in the occasion of stress conditions (severe infection, major surgical procedures, etc.) and to restore it as soon as the critical condition has been overcome.

Drugs Acting on the Central Nervous System

The central nervous system (CNS) plays a major control in integrating hormonal and metabolic effects (Thorens 2011) making it an intriguing though complex treatment target. Agents have been developed to interfere with mechanisms regulating satiety and energy balance for treatment of obesity. Agents like the 5HT2 receptor agonist lorcaserin and bupropion have shown a glucose lowering effect. Rimonabant, an agonist of the cannabinoid CB1 receptor, has been shortly used in T2DM before withdrawal owing to increase suicidal risk (Thomas et al. 2014). Currently, the only drug licensed in the USA acting at the central level is bromocriptine.

Bromocriptine

Pharmacology – Bromocriptine is an agonist of the dopamine D2 receptor largely used for the treatment of pituitary tumors and, in a different pharmacological formulation, Parkinson disease. A low-dose quick-release (QR) formulation is available in America as glucose lowering agent. This formulation is characterized by rapid absorption, high protein binding, and rapid removal through cytochrome CYP3A4 followed by bile elimination.

Mode of action – Bromocriptine resets the CNS sympathetic and dopaminergic tone (Cincotta 2002) restoring circadian cycle of glucose homeostasis. Systemic or intracerebral administration of bromocriptine decreases hepatic glucose production, gluconeogenesis, lipolysis, and improves insulin sensitivity (Luo et al. 1999). Early morning administration of QR-bromocriptine in T2DM subjects reduces prolactin levels during the day, restores dopaminergic activity, and lowers plasma glucose (Cincotta et al. 1999).

Clinical efficacy – QR-bromocriptine is administered early in the morning and as add-on therapy reduces HbA1c, compared with placebo, by 0.5–0.7% (5–8 mmol/mol) and fasting plasma glucose by 15–20 mg/dl (0.8–1.0 mmol/l) with neutral effects on postprandial glucose. The risk of hypoglycemia is low and QR-bromocriptine has no effect on body weight and lipid profile (Liang et al. 2015). A study has reported fewer CV outcomes (Gaziano et al. 2010).

Adverse effects – Treatment with QR-bromocriptine can cause nausea and vomiting. The drug is contraindicated in patients with psychotic disorders as they can be exacerbated by bromocriptine, and in nursing women because of inhibition of lactation. Caution should be used in patients on antihypertensive agents as it can cause orthostatic hypotension.

Drug Therapy Management

In the past 20 years or so, more oral agents with more targeted mechanisms of action have been made available and more will come both in term of novel mechanisms of action as well as novel targets (Fig. 10; Tahrani et al. 2011). The possibility to target-specific pathogenic mechanisms should allow a better-tailored approach to address individual needs and reduce side effects. Addressing in a more precise manner the pathogenic mechanisms of hyperglycemia can be expected to result in a more sustained glycemic control, i.e., reducing the risk of developing long-term diabetic complications. However, the greater the number of available pharmacologic therapies, the greater the need for guidance for their appropriate use. Current guidelines concur in recommending metformin at diagnosis of diabetes together with lifestyle modification with the suggestion that if target glycemic control (HbA1c) is not reached within 3 months a second agent should be considered. Selection of the second drug therapy should be made on the basis of an educated process tacking into consideration efficacy, risk of hypoglycemia, effect on body weight, costs of different drugs (Table 1) (Inzucchi et al. 2015), as well as patient's characteristics (age, phenotype, presence or absence of complications or co-morbidities, duration of the disease, proneness to hypoglycemia, etc.) (Pozzilli et al. 2010) and individual preferences, habits, educational level... (Raz et al. 2013). With more clinical data generated, other features of the available oral agents should be taken into account such as durability, predominant effect on fasting versus postprandial glucose, as well effects beyond their glucose lowering capacity. In the past 10 years or so, oral diabetes medications have been tested with respect to their effect on cardiovascular outcomes. Large studies in high CV risk T2DM patients have provided evidence for safety for DPP4 inhibitors (Scirica et al. 2013; Green et al. 2015; White et al. 2013) and gliclazide (ADVANCE Collaborative Group 2008) and reduction of CV risk for SGLT2 inhibitors (Davies et al. 2015; Zinman et al. 2015) and pioglitazone (Dormandy et al. 2005; Kernan et al. 2016). In T2DM patients at lower CV risk, it is still unclear which drug may convey specific benefit (Vaccaro et al. 2017). Similarly, more data on renal safety and potential kidney protection are now available. All this information can contribute in improving the individualized treatment algorithm (Avogaro et al. 2016).

The availability of drugs with more targeted mode of action and complementary mechanisms also can allow a more rational combination therapy with simultaneous correction of more than one pathogenic mechanism and better efficacy-to-safety ratio (Bianchi et al. 2017). In line with this view, concomitant use of metformin to improve insulin action on the liver, pioglitazone to enhance insulin sensitivity in peripheral tissues, and an injectable GLP-1 receptor agonist to sustain insulin secretion and suppress glucagon release initiated at the time of T2DM diagnosis has been shown to provide better and more durable glycemic control with less risk of hypoglycemia than the traditional stepwise approach with initial treatment with metformin, escalation with addition of a sulfonylurea, and intensification with basal insulin (Abdul-Ghani et al. 2015). Ongoing studies will provide information on the efficacy and safety of early oral combination therapy. The *Vildagliptin*

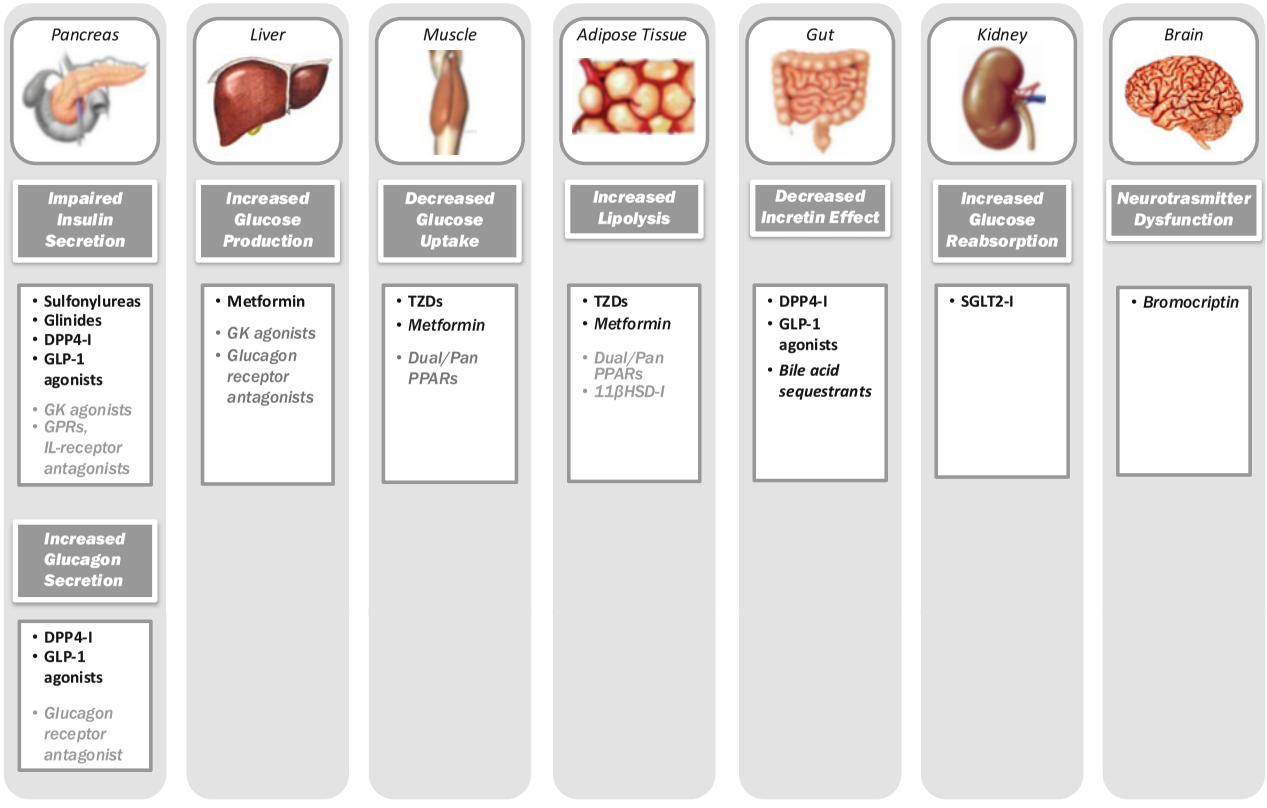


Fig. 10 Summary of the current target for antihyperglycemic therapy for type 2 diabetes. The figure summarizes, for each target organ/mechanism the available pharmacological agents (in black) and the potential future ones (in gray). Drugs not available in Europe are shown in italics (Bianchi et al. 2017)

Efficacy in combination with metfoRmIn For earlY treatment of T2DM (VERIFY) trial is a 5 years study designed to evaluate the effect of early combination therapy of metformin and a DPP4 inhibitor (Del Prato et al. 2014) on durability of glycemic effect while the *Glycemia Reduction Approaches in DiabEtes* (GRADE) will compare the long-term effectiveness of major glucose lowering medications added on top of metformin (Nathan et al. 2013). Both these approaches identify metformin as a common component of treatment medications, but, even considering only the main classes of glucose lowering agents, the number of possible permutation is large enough to generate perplexities about how to select the initial combination. A more precise characterization of the patient could help in defining the choice of the oral agent(s) and the time for its (their) introduction. In the future, precision medicine, pharmacogenetics, and development of biomarkers for each of the mechanisms causing progression of the disease may allow a more precise selection of the drugs and their best combination for each person with diabetic (Lyssenko et al. 2016).

References

Abdul-Ghani MA, Puckett C, Triplitt C, Maggs D, Adams J, Cersosimo E, DeFronzo RA. Initial combination therapy with metformin, pioglitazone and exenatide is more effective than sequential add-on therapy in subjects with new-onset diabetes. Results from the Efficacy and Durability of Initial Combination Therapy for Type 2 Diabetes (EDICT): a randomized trial. Diabetes Obes Metab. 2015;17:268–75.

Adams M, Montague CT, Prins JB, Holder JC, Smith SA, Sanders L, Digby JE, Sewter CP, Lazar MA, Chatterjee VK, O'Rahilly S. Activators of peroxisome proliferator-activated receptor gamma have depot-specific effects on human preadipocyte differentiation. J Clin Invest. 1997;100:3149–53.

ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med. 2008;358:2560–72.

Avogaro A, Fadini GP. The effects of Dipeptidyl Peptidase-4 inhibition on microvascular diabetes complications. Diabetes Care. 2014;37:2884–94.

Avogaro A, Fadini GP, Sesti G, Bonora E, Del Prato S. Continued efforts to translate diabetes cardiovascular outcome trials into clinical practice. Cardiovasc Diabetol. 2016;15:111.

Bailey CJ, Day C. Metformin: its botanical background. Pract Diab Int. 2004;21:115–7.

Bailey CJ, Wilcock C, Scarpello JH. Metformin and the intestine. Diabetologia. 2008;51(8):1552–3.

Ball AJ, Flatt PR, McClenaghan NH. Desensitization of sulphonylurea- and nutrient-induced insulin secretion following prolonged treatment with glibenclamide. Eur J Pharmacol. 2000;408:327–33.

Barnett AH, Charbonnel B, Li J, Donovan M, Fleming D, Iqbal N. Saxagliptin add-on therapy to insulin with or without metformin for type 2 diabetes mellitus: 52-week safety and efficacy. Clin Drug Investig. 2013;33:707–17.

Barnett AH, Mithal A, Manassie J, Jones R, Rattunde H, Woerle HJ, Broedl UC, EMPA-REG RENAL trial investigators. Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2014;2:369–84.

Bayraktar M, Van Thiel DH, Adalar N. A comparison of acarbose versus metformin as an adjuvant therapy in sulfonylurea-treated NIDDM patients. Diabetes Care. 1996;19:252–4.

Betteridge DJ. Thiazolidinediones and fracture risk in patients with type 2 diabetes. Diabet Med. 2011;28:759–71.

Bianchi C, Daniele G, Dardano A, Miccoli R, Del Prato S. Early combination therapy with oral glucose-lowering agents in type 2 diabetes. Drugs. 2017;77:247–64.
Bischoff H. Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest. 1994;24(Suppl 3):3–10.
Bogacka I, Xie H, Bray GA, Smith SR. The effect of pioglitazone on peroxisome proliferator-activated receptor-gamma target genes related to lipid storage in vivo. Diabetes Care. 2004;27:1660–7.
Burke KR, Schumacher CA, Harpe SE. SGLT2 inhibitors: a systematic review of diabetic ketoacidosis and related risk factors in the primary literature. Pharmacotherapy. 2017;37:187–94.
Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. Ageing Res Rev. 2017;40:31–44.
Chen M, Hu C, Jia W. Pharmacogenomics of glinides. Pharmacogenomics. 2015;16:45–60.
Chiasson JL, Josse RG, Hunt JA, Palmason C, Rodger NW, Ross SA, et al. The efficacy of acarbose in the treatment of patients with non-insulindependent diabetes mellitus. A multicenter controlled clinical trial. Ann Intern Med. 1994;121:928–35.
Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, STOP-NIDDM Trail Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002;359:2072–7.
Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA. 2003;290:486–94.
Cincotta AH. Hypothalamic role in the insulin resistance syndrome. In: Hansen B, Shaffrir E, editors. Insulin resistance syndrome. London: Taylor & Francis; 2002. p. 271–312.
Cincotta AH, Meier AH, Cincotta MJ. Bromocriptine improves glycaemic control and serum lipid profile in obese Type 2 diabetic subjects: a new approach in the treatment of diabetes. Expert Opin Investig Drugs. 1999;8:1683–707.
Clissold SP, Edwards C. Acarbose. A preliminary review of its pharmacodynamics and pharmacokinetic properties, and therapeutic potential. Drugs. 1988;35:214–43.
Coniff RF, Shapiro JA, Robbins D, Kleinfield R, Seaton TB, Beisswenger P, et al. Reduction of glycosylated hemoglobin and postprandial hyperglycemia by acarbose in patients with NIDDM. A placebo-controlled dose-comparison study. Diabetes Care. 1995;18:817–24.
Davies MJ, Trujillo A, Vijapurkar U, Damaraju CV, Meininger G. Effect of canagliflozin on serum uric acid in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2015;17:426–9.
de Heer J, Holst JJ. Sulfonylurea compounds uncouple the glucose dependence of the insulinotropic effect of glucagon-like peptide 1. Diabetes. 2007;56:438–43.
Deacon CF. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obes Metab. 2011;13:7–18.
Deacon CF, Holst JJ. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: comparison, efficacy and safety. Expert Opin Pharmacother. 2013;14:2047–58.
DeFronzo RA, Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58:773–95.
DeFronzo RA, Tripathy D, Schwenke DC, Banerji M, Bray GA, Buchanan TA, Clement SC, Henry RR, Hodis HN, Kitabchi AE, Mack WJ, Mudaliar S, Ratner RE, Williams K, Stentz FB, Musi N, Reaven PD, ACT NOW Study. Pioglitazone for diabetes prevention in impaired glucose tolerance. N Engl J Med. 2011;364:1104–15.
DeFronzo RA, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. Diabetes Obes Metab. 2012;14:5–14.
DeFronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, Pfister M, Morrow LA, Leslie BR, Boulton DW, Ching A, LaCreta FP, Griffen SC. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. Diabetes Care. 2013;36:3169–76.
Del Prato S, Foley JE, Kothny W, Kozlovski P, Stumvoll M, Paldánius PM, Matthews DR. Study to determine the durability of glycaemic control with early treatment with a vildagliptin-metformin combination regimen vs. standard-of-care metformin monotherapy-the VERIFY trial: a randomized double-blind trial. Diabet Med. 2014;31:1178–84.

Del Prato S, Nauck M, Durán-Garcia S, Maffei L, Rohwedder K, Theuerkauf A, Parikh S. Long-term glycaemic response and tolerability of dapagliflozin versus a sulphonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. Diabetes Obes Metab. 2015;17:581–90.

Devineni D, Morrow L, Hompesch M, Skee D, Vandebosch A, Murphy J, Ways K, Schwartz S. Canagliflozin improves glycaemic control over 28 days in subjects with type 2 diabetes not optimally controlled on insulin. Diabetes Obes Metab. 2012;14:539–45.

Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet. 2005;366:1279–89.

Du Q, Wang YJ, Yang S, Wu B, Han P, Zhao YY. A systematic review and meta-analysis of randomized controlled trials comparing pioglitazone versus metformin in the treatment of polycystic ovary syndrome. Curr Med Res Opin. 2012;28:723–30.

Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of organic cation transporter 1 with intolerance to metformin in type 2 diabetes: a GoDARTS study. Diabetes. 2015;64:1786–93.

Egan AG, Blind E, Dunder K, et al. Pancreatic safety of incretin-based drugs–FDA and EMA assessment. N Engl J Med. 2014;370:794–7.

El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem. 2000;275:223–8.

Erdmann E, Wilcox R. Pioglitazone and mechanisms of CV protection. QJM. 2010;103:213–28.

Erondu N, Desai M, Ways K, Meininger G. Diabetic ketoacidosis and related events in the Canagliflozin type 2 diabetes clinical program. Diabetes Care. 2015;38:1680–6.

Fadini GP, Bonora BM, Avogaro A. SGLT2 inhibitors and diabetic ketoacidosis: data from the FDA Adverse Event Reporting System. Diabetologia. 2017;60(8):1385–9. https://doi.org/10.1007/s00125-017-4301-8.

Farber SJ, Berger EY, Earle DP. Effect of diabetes and insulin of the maximum capacity of the renal tubules to reabsorb glucose. J Clin Invest. 1951;30:125–9.

Ferrannini E, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol. 2012;8:495–502.

Ferrannini E, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, Broedl UC, Woerle HJ. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest. 2014;124:499–508.

Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy balance after sodium-glucose Cotransporter 2 inhibition. Diabetes Care. 2015;38:1730–5.

Fioretto P, Zambon A, Rossato M, Busetto L, Vettor R. SGLT2 inhibitors and the diabetic kidney. Diabetes Care. 2016;39(Suppl 2):S165–71.

Flatt PR, Bailey CJ, Green BD. Dipeptidyl peptidase IV (DPP IV) and related molecules in type 2 diabetes. Front Biosci. 2008;13:3648–60.

Fonseca VA, Rosenstock J, Wang AC, Truitt KE, Jones MR. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. Diabetes Care. 2008;31:1479–84.

Fonseca VA, Handelsman Y, Staels B. Colesevelam lowers glucose and lipid levels in type 2 diabetes: the clinical evidence. Diabetes Obes Metab. 2010;12:384–92.

Food and Drug Administration. Safety Alert on Canagliflozin, issued on Oct 10th 2015. http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm461876.htm. Last accessed on 3 Aug 2016; http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/SGLT2_inhibitors/human_referral_prac_000052.jsp&mid=WC0b01ac05805c516f.

Fujioka K, Pans M, Joyal S. Glycemic control in patients with type 2 diabetes mellitus switched from twice-daily immediate-release metformin to a once-daily extended-release formulation. Clin Ther. 2003;25:515–29.

Fujita Y, Tamada D, Kozawa J, Kobayashi Y, Sasaki S, Kitamura T, Yasuda T, Maeda N, Otsuki M, Okita K, Iwahashi H, Kaneto H, Funahashi T, Imagawa A, Shimomura I. Successful treatment

of reactive hypoglycemia secondary to late dumping syndrome using miglitol. Intern Med. 2012;51:2581–5.
Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med. 1997;103:491–7.
Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, Dagogo-Jack S, Davidson MB, Einhorn D, Garvey WT, Grunberger G, Handelsman Y, Hirsch IB, Jellinger PS, McGill JB, Mechanick JI, Rosenblit PD, Umpierrez GE, Davidson MH. American Association of Clinical Endocrinologists' comprehensive diabetes management algorithm 2013 consensus statement – executive summary. Endocr Pract. 2013;19:536–57.
Gastaldelli A, Toschi E, Pettiti M, Frascerra S, Quiñones-Galvan A, Sironi AM, Natali A, Ferrannini E. Effect of physiological hyperinsulinemia on gluconeogenesis in nondiabetic subjects and in type 2 diabetic patients. Diabetes. 2001;50:1807–12.
Gaziano JM, Cincotta AH, O'Connor CM, Ezrokhi M, Rutty D, Ma ZJ, Scranton RE. Randomized clinical trial of quick-release bromocriptine among patients with type 2 diabetes on overall safety and cardiovascular outcomes. Diabetes Care. 2010;33:1503–8.
Gerich J, Raskin P, Jean-Louis L, Purkayastha D, Baron MA. PRESERVE-β: two-year efficacy and safety of initial combination therapy with nateglinide or glyburide plus metformin. Diabetes Care. 2005;28:2093–9.
Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA, Tan MH, Khan MA, Perez AT, Jacober SJ, GLAI Study Investigators. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care. 2005;28:1547–54.
Gonçalves A, Marques C, Leal E, Ribeiro CF, Reis F, Ambrósio AF, Fernandes R. Dipeptidyl peptidase-IV inhibition prevents blood-retinal barrier breakdown, inflammation and neuronal cell death in the retina of type 1 diabetic rats. Biochim Biophys Acta. 2014;1842:1454–63.
Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E, American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE), Androgen Excess and PCOS Society (AES). American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society Disease State Clinical Review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome – part 1. Endocr Pract. 2015;21:1291–300.
Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 2011;50:81–98.
Green JB, Bethel MA, Armstrong PW, et al. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2015;373:232–42.
Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. Diabetologia. 2017;60:1620. Epub ahead of print.
Handelsman Y. Role of bile acid sequestrants in the treatment of type 2 diabetes. Diabetes Care. 2011;34(Suppl 2):S244–50.
Hanefeld M, Cagatay M, Petrowitsch T, Neuser D, Petzinna D, Rupp M. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. Eur Heart J. 2004;25:10–6.
Hansen M, Sonne DP, Knop FK. Bile acid sequestrants: glucose-lowering mechanisms and efficacy in type 2 diabetes. Curr Diab Rep. 2014;14:482.
Hattersley AT, Patel KA. Precision diabetes: learning from monogenic diabetes. Diabetologia. 2017;60:769–77.
He G, Pedersen SB, Bruun JM, Lihn AS, Richelsen B. Metformin, but not thiazolidinediones, inhibits plasminogen activator inhibitor-1 production in human adipose tissue in vitro. Horm Metab Res. 2003;35:18–23.

Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford LG. Repurposing metformin for the prevention of cancer and cancer recurrence. Diabetologia. 2017. https://doi.org/10.1007/s00125-017-4372-6. [Epub ahead of print].

Hirst JA, Farmer AJ, Dyar A, Lung TW, Stevens RJ. Estimating the effect of sulfonylurea on HbA1c in diabetes: a systematic review and meta-analysis. Diabetologia. 2013;56:973–84.

Holman RR, Coleman RL, Chan JCN, Chiasson JL, Feng H, Ge J, Gerstein HC, Gray R, Huo Y, Lang Z, McMurray JJ, Rydén L, Schröder S, Sun Y, Theodorakis MJ, Tendera M, Tucker L, Tuomilehto J, Wei Y, Yang W, Wang D, Hu D, Pan C, ACE Study Group. Effects of acarbose on cardiovascular and diabetes outcomes in patients with coronary heart disease and impaired glucose tolerance (ACE): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2017. https://doi.org/10.1016/S2213-8587(17)30309-1. [Epub ahead of print].

Hu S, Wang S, Dunning BE. Glucose-dependent and glucose-sensitizing insulinotropic effect of nateglinide: comparison to sulfonylureas and repaglinide. Int J Exp Diabetes Res. 2001;2:63–72.

Hummel CS, Lu C, Loo DD, Hirayama BA, Voss AA, Wright EM. Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. Am J Physiol Cell Physiol. 2011;300:C14–21.

Hur KY, Lee MS. New mechanisms of metformin action: focusing on mitochondria and the gut. J Diabetes Invest. 2015;6:600–9.

Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38:140–9.

Jabbour S, Ziring B. Advantages of extended-release metformin in patients with type 2 diabetes mellitus. Postgrad Med. 2011;123:15–23.

Johnsson K, Johnsson E, Mansfield TA, Yavin Y, Ptaszynska A, Parikh SJ. Osmotic diuresis with SGLT2 inhibition: analysis of events related to volume reduction in dapagliflozin clinical trials. Postgrad Med. 2016;128:346–55.

Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G, ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med. 2006;355:2427–43.

Kahn SE, Lachin JM, Zinman B, Haffner SM, Aftring RP, Paul G, Kravitz BG, Herman WH, Viberti G, Holman RR, ADOPT Study Group. Effects of rosiglitazone, glyburide, and metformin on β-cell function and insulin sensitivity in ADOPT. Diabetes. 2011;60:1552–60.

Kanda Y, Shimoda M, Hamamoto S, Tawaramoto K, Kawasaki F, Hashiramoto M, Nakashima K, Matsuki M, Kaku K. Molecular mechanism by which pioglitazone preserves pancreatic beta-cells in obese diabetic mice: evidence for acute and chronic actions as a PPARgamma agonist. Am J Physiol Endocrinol Metab. 2010;298:E278–86.

Karagiannis T, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. BMJ. 2012;344:e1369.

Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, Guarino PD, Lovejoy AM, Peduzzi PN, Conwit R, Brass LM, Schwartz GG, Adams HP Jr, Berger L, Carolei A, Clark W, Coull B, Ford GA, Kleindorfer D, O'Leary JR, Parsons MW, Ringleb P, Sen S, Spence JD, Tanne D, Wang D, Winder TR, IRIS Trial Investigators. Pioglitazone after ischemic stroke or transient ischemic attack. N Engl J Med. 2016;374:1321–31.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346:393–403.

Kohan DE, Fioretto P, Tang W, List JF. Long-term study of patients with type 2 diabetes and moderate renal impairment shows that dapagliflozin reduces weight and blood pressure but does not improve glycemic control. Kidney Int. 2014;85:962–71.

Kohan DE, Fioretto P, Johnsson K, Parikh S, Ptaszynska A, Ying L. The effect of dapagliflozin on renal function in patients with type 2 diabetes. J Nephrol. 2016;29:391–400.
Kothny W, Foley J, Kozlovski P, Shao Q, Gallwitz B, Lukashevich V. Improved glycemic control with vildagliptin added to insulin, with or without metformin, in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2013;15:252–7.
Lapuerta P, Zambrowicz B, Strumph P, Sands A. Development of sotagliflozin, a dual sodium-dependent glucose transporter 1/2 inhibitor. Diab Vasc Dis Res. 2015;12:101–10.
Lee YJ, Lee YJ, Han HJ. Regulatory mechanisms of Na(+)/glucose cotransporters in renal proximal tubule cells. Kidney Int Suppl. 2007;106:S27–35.
Levin D, Bell S, Sund R, Hartikainen SA, Tuomilehto J, Pukkala E, Keskimäki I, Badrick E, Renehan AG, Buchan IE, Bowker SL, Minhas-Sandhu JK, Zafari Z, Marra C, Johnson JA, Stricker BH, Uitterlinden AG, Hofman A, Ruiter R, de Keyser CE, MacDonald TM, Wild SH, McKeigue PM, Colhoun HM, Scottish Diabetes Research Network Epidemiology Group, Diabetes and Cancer Research Consortium. Pioglitazone and bladder cancer risk: a multi-population pooled, cumulative exposure analysis. Diabetologia. 2015;58:493–504.
Lewis JD, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, Ehrlich SF, Mamtani R, Bilker W, Vaughn DJ, Nessel L, Van Den Eeden SK, Ferrara A. Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. JAMA. 2015;314:265–77.
Liang W, Gao L, Li N, Wang B, Wang L, Wang Y, Yang H, You L, Hou J, Chen S, Zhu H, Jiang Y, Pan H. Efficacy and safety of Bromocriptine-QR in type 2 diabetes: a systematic review and meta-analysis. Horm Metab Res. 2015;47:805–12.
Lindsay RS, Loeken MR. Metformin use in pregnancy: promises and uncertainties. Diabetologia. 2017. https://doi.org/10.1007/s00125-017-4351-y. [Epub ahead of print].
Lindsay JR, Duffy NA, McKillop AM, Ardill J, O'Harte FP, Flatt PR, Bell PM. Inhibition of dipeptidyl peptidase IV activity by oral metformin in type 2 diabetes. Diabet Med. 2005;22:654–7.
Lipska KJ, Bailey CJ, Inzucchi SE. Use of metformin in the setting of mild-to-moderate renal insufficiency. Diabetes Care. 2011;34:1431–7.
Liu JJ, Lee T, DeFronzo RA. Why do SGLT2 inhibitors inhibit only 30–50% of renal glucose reabsorption in humans? Diabetes. 2012;61:2199–204.
Loubani M, Powler A, Standen NB, Galinanes M. The effect of gliclazide and glibenclamide on preconditioning of the human myocardium. Eur J Pharmacol. 2005;515:142–9.
Luo S, Liang Y, Cincotta AH. Intracerebroventricular administration of bromocriptine ameliorates the insulin-resistant/glucose intolerant state in hamsters. Neuroendocrinology. 1999;69:160–6.
Lupi R, Del Guerra S, Marselli L, Bugliani M, Boggi U, Mosca F, Marchetti P, Del Prato S. Rosiglitazone prevents the impairment of human islet function induced by fatty acids: evidence for a role of PPARgamma2 in the modulation of insulin secretion. Am J Physiol Endocrinol Metab. 2004;286:E560–7.
Lyssenko V, Bianchi C, Del Prato S. Personalized therapy by phenotype and genotype. Diabetes Care. 2016;39(Suppl 2):S127–36.
Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ, Camporez JP, Lee HY, Cline GW, Samuel VT, Kibbey RG, Shulman GI. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510:542–6.
Maedler K, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY. Sulfonylurea induced beta-cell apoptosis in cultured human islets. J Clin Endocrinol Metab. 2005;90:501–6.
Malaisse WJ. Pharmacology of the meglitinide analogs: new treatment options for type 2 diabetes mellitus. Treat Endocrinol. 2003;2:401–14.
Matsumura M, Monden T, Miyashita Y, Kawagoe Y, Shimizu H, Nakatani Y, Domeki N, Yanagi K, Ikeda S, Kasai K. Effects of changeover from voglibose to acarbose on postprandial triglycerides in type 2 diabetes mellitus patients. Adv Ther. 2009;26(6):660.
Mazza A, Fruci B, Garinis GA, Giuliano S, Malaguarnera R, Belfiore A. The role of metformin in the management of NAFLD. Exp Diabetes Res. 2012;2012:716404.

McGuire DK, Inzucchi SE. New drugs for the treatment of diabetes mellitus: Part I: Thiazolidinediones and their evolving cardiovascular implications. Circulation. 2008;117:440–9.
McGuire DK, Van de Werf F, Armstrong PW, Standl E, Koglin J, Green JB, Bethel MA, Cornel JH, Lopes RD, Halvorsen S, Ambrosio G, Buse JB, Josse RG, Lachin JM, Pencina MJ, Garg J, Lokhnygina Y, Holman RR, Peterson ED, Trial Evaluating Cardiovascular Outcomes With Sitagliptin (TECOS) Study Group. Association between Sitagliptin use and heart failure hospitalization and related outcomes in type 2 diabetes mellitus: secondary analysis of a randomized clinical trial. JAMA Cardiol. 2016;1:126–35.
McLeod JF. Clinical pharmacokinetics of nateglinide: a rapidly-absorbed, short-acting insulinotropic agent. Clin Pharmacokinet. 2004;43:97–120.
Meier JJ. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. Nat Rev Endocrinol. 2012;8:728–42.
Meier JJ, Nauck MA. Risk of pancreatitis in patients treated with incretin-based therapies. Diabetologia. 2014;57:1320–4.
Meier JJ, Gallwitz B, Schmidt WE, et al. Is impairment of ischaemic preconditioning by sulfonylurea drugs clinically important? Heart. 2004;90:9–12.
Merovci A, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D, Xiong J, Perez Z, Norton L, Abdul-Ghani MA, DeFronzo RA. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124:509–14.
Merovci A, Mari A, Solis C, Xiong J, Daniele G, Chavez-Velazquez A, Tripathy D, Urban McCarthy S, Abdul-Ghani M, DeFronzo RA. Dapagliflozin lowers plasma glucose concentration and improves β-cell function. J Clin Endocrinol Metab. 2015;100:1927–32.
Merovci A, Abdul-Ghani M, Mari A, Solis-Herrera C, Xiong J, Daniele G, Tripathy D, DeFronzo RA. Effect of Dapagliflozin with and without Acipimox on insulin sensitivity and insulin secretion in T2DM males. J Clin Endocrinol Metab. 2016;101:1249–56.
Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahankali S, Ferrannini E, Cusi K, Mandarino LJ, DeFronzo RA. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. Diabetes Care. 2001;24:710–9.
Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab. 2002;87:2784–91.
Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. Circulation. 2001;103:3111–6.
Monami M, Dicembrini I, Martelli D, Mannucci E. Safety of dipeptidyl peptidase-4 inhibitors: a meta-analysis of randomized clinical trials. Curr Med Res Opin. 2011;27(suppl 3):57–64.
Monami M, Genovese S, Mannucci E. Cardiovascular safety of sulfonylureas: a meta-analysis of randomized clinical trials. Diabetes Obes Metab. 2013;15:938–53.
Monami M, Nreu B, Zannoni S, Lualdi C, Mannucci E. Effects of SGLT-2 inhibitors on diabetic ketoacidosis: a meta-analysis of randomised controlled trials. Diabetes Res Clin Pract. 2017;130:53–60.
Mori H, Okada Y, Arao T, Tanaka Y. Sitagliptin improves albuminuria in patients with type 2 diabetes mellitus. J Diabetes Investig. 2014;5:313–9.
Mudaliar S, Henry RR. New oral therapies for type 2 diabetes mellitus: the glitazones or insulin sensitizers. Annu Rev Med. 2001;52:239–57.
Mudaliar S, Chang AR, Henry RR. Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications. Endocr Pract. 2003;9:406–16.
Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia. 2012;55:885–904.
Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. Diabetologia. 2006;49:434–41.

Nathan DM, Buse JB, Kahn SE, Krause-Steinrauf H, Larkin ME, Staten M, Wexler D, Lachin JM, GRADE Study Research Group. Rationale and design of the glycemia reduction approaches in diabetes: a comparative effectiveness study (GRADE). Diabetes Care. 2013;36:2254–61.

Nauck MA. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Des Devel Ther. 2014;8:1335–80.

NAVIGATOR Study Group, Holman RR, Haffner SM, McMurray JJ, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang FT, Charbonnel B, Chow CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of nateglinide on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362:1463–76.

Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR, CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377:644–57.

Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab. 1997;82:4075–9.

Ogawa S, Takeuchi K, Ito S. Acarbose lowers serum triglyceride and postprandial chylomicron levels in type 2 diabetes. Diabetes Obes Metab. 2004;6:384–90.

Ott C, Raff U, Schmidt S, Kistner I, Friedrich S, Bramlage P, Harazny JM, Schmieder RE. Effects of saxagliptin on early microvascular changes in patients with type 2 diabetes. Cardiovasc Diabetol. 2014;13:19.

Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000;348:607.

Owens DR, Luzio SD, Ismail I, Bayer T. Increased prandial insulin secretion after administration of a single preprandial oral dose of repaglinide in patients with type 2 diabetes. Diabetes Care. 2000;23:518–23.

Palmer SC, Mavridis D, Nicolucci A, Johnson DW, Tonelli M, Craig JC, Maggo J, Gray V, De Berardis G, Ruospo M, Natale P, Saglimbene V, Badve SV, Cho Y, Nadeau-Fredette AC, Burke M, Faruque L, Lloyd A, Ahmad N, Liu Y, Tiv S, Wiebe N, Strippoli GF. Comparison of clinical outcomes and adverse events associated with glucose-lowering drugs in patients with type 2 diabetes: a meta-analysis. JAMA. 2016;316:313–24.

Pavo I, Jermendy G, Varkonyi TT, Kerenyi Z, Gyimesi A, Shoustov S, Shestakova M, Herz M, Johns D, Schluchter BJ, Festa A, Tan MH. Effect of pioglitazone compared with metformin on glycemic control and indicators of insulin sensitivity in recently diagnosed patients with type 2 diabetes. J Clin Endocrinol Metab. 2003;88:1637–45.

Peter S. Acarbose and idiopathic reactive hypoglycemia. Horm Res. 2003;60:166–7.

Phung OJ, Schwartzman E, Allen RW, Engel SS, Rajpathak SN. Sulphonylureas and risk of cardiovascular disease: systematic review and meta-analysis. Diabet Med. 2013;30:1160–71.

Pollak M. The effects of metformin on gut microbiota and the immune system as research frontiers. Diabetologia. 2017;60:1662. [Epub ahead of print].

Pozzilli P, Leslie RD, Chan J, De Fronzo R, Monnier L, Raz I, Del Prato S. The A1C and ABCD of glycaemia management in type 2 diabetes: a physician's personalized approach. Diabetes Metab Res Rev. 2010;26:239–44.

Ramirez G, Morrison AD, Bittle PA. Clinical practice considerations and review of the literature for the use of DPP-4 inhibitors in patients with type 2 diabetes and chronic kidney disease. Endocr Pract. 2013;19:1025–34.

Raz I, Riddle MC, Rosenstock J, Buse JB, Inzucchi SE, Home PD, Del Prato S, Ferrannini E, Chan JC, Leiter LA, Leroith D, Defronzo R, Cefalu WT. Personalized management of hyperglycemia

in type 2 diabetes: reflections from a Diabetes Care Editors' Expert Forum. Diabetes Care. 2013;36:1779–88.
Raz I, Bhatt DL, Hirshberg B, et al. Incidence of pancreatitis and pancreatic cancer in a randomized controlled multicenter trial (SAVOR-TIMI 53) of the dipeptidyl peptidase-4 inhibitor saxagliptin. Diabetes Care. 2014;37:2435–41.
Reasner C, Olansky L, Seck TL, Williams-Herman DE, Chen M, Terranella L, Johnson-Levonas AO, Kaufman KD, Goldstein BJ. The effect of initial therapy with the fixed-dose combination of sitagliptin and metformin compared with metformin monotherapy in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2011;13:644–52.
Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60:1577. [Epub ahead of print].
Rodbard HW, Jellinger PS, Davidson JA, Einhorn D, Garber AJ, Grunberger G, Handelsman Y, Horton ES, Lebovitz H, Levy P, Moghissi ES, Schwartz SS. Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: an algorithm for glycemic control. Endocr Pract. 2009;15:540–59.
Rosenstock J, Ferrannini E. Euglycemic diabetic ketoacidosis: a predictable, detectable, and preventable safety concern with SGLT2 inhibitors. Diabetes Care. 2015;38:1638–42.
Rosenstock J, Brazg R, Andryuk PJ, Lu K, Stein P. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Clin Ther. 2006;28:1556–68.
Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest. 1987a;79:1510–5.
Rossetti L, Shulman GI, Zawalich W, DeFronzo RA. Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. J Clin Invest. 1987b;80:1037–44.
Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, MiG Trial Investigators. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358:2003–15.
Russo E, Penno G, Del Prato S. Managing diabetic patients with moderate or severe renal impairment using DPP-4 inhibitors: focus on vildagliptin. Diabetes Metab Syndr Obes. 2013;6:161–70.
Ryan EH Jr, Han DP, Ramsay RC, Cantrill HL, Bennett SR, Dev S, Williams DF. Diabetic macular edema associated with glitazone use. Retina. 2006;26:562–70.
Salpeter SR, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev. 2010;4:CD002967.
Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR, NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362:1675–85.
Scheen AJ. Pharmacokinetic interactions with thiazolidinediones. Clin Pharmacokinet. 2007;46:1–12.
Scheen AJ. Drug-drug interactions with sodium-glucose cotransporters type 2 (SGLT2) inhibitors, new oral glucose-lowering agents for the management of type 2 diabetes mellitus. Clin Pharmacokinet. 2014;53(4):295–304. https://doi.org/10.1007/s40262-013-0128-8.
Schmitz O, Lund S, Andersen PH, Jonler M, Porksen N. Optimizing insulin secretagogue therapy in patients with type 2 diabetes: a randomized double-blind study with repaglinide. Diabetes Care. 2002;25:342–6.
Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I. SAVOR-TIMI 53 steering committee and investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med. 2013;369:1317–26.

Scirica BM, Braunwald E, Raz I, et al. Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. Circulation. 2014;130:1579–88.

Sedo A, Malík R. Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? Biochim Biophys Acta. 2001;1550:107–16.

Sheth SH, Larson RJ. The efficacy and safety of insulin-sensitizing drugs in HIV-associated lipodystrophy syndrome: a meta-analysis of randomized trials. BMC Infect Dis. 2010;10:183.

Simpson SH, Lee J, Choi S, Vandermeer B, Abdelmoneim AS, Featherstone TR. Mortality risk among sulfonylureas: a systematic review and network meta-analysis. Lancet Diabetes Endocrinol. 2015;3:43–51.

Sola D, Rossi L, Carnevale Schianca GP, Maffioli P, Bigliocca M, Mella R, Corlianò F, Fra GP, Bartoli E, Derosa G. Sulfonylureas and their use in clinical practice. Arch Med Sci. 2015;11:840–8.

Tahrani AA, Bailey CJ, Del Prato S, Barnett AH. Management of type 2 diabetes: new and future developments in treatment. Lancet. 2011;378:182–97.

Tang HL, Li DD, Zhang JJ, Hsu YH, Wang TS, Zhai SD, Song YQ. Lack of evidence for a harmful effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors on fracture risk among type 2 diabetes patients: a network and cumulative meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2016;18:1199–206.

Tang H, Dai Q, Shi W, Zhai S, Song Y, Han J. SGLT2 inhibitors and risk of cancer in type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. Diabetologia. 2017. https://doi.org/10.1007/s00125-017-4370-8. [Epub ahead of print].

Tani S, Nagao K, Hirayama A. Association between urinary albumin excretion and low-density lipoprotein heterogeneity following treatment of type 2 diabetes patients with the dipeptidyl peptidase-4 inhibitor, vildagliptin: a pilot study. Am J Cardiovasc Drugs. 2013;13:443–50.

Thomas KH, Martin RM, Potokar J, Pirmohamed M, Gunnell D. Reporting of drug induced depression and fatal and non-fatal suicidal behaviour in the UK from 1998 to 2011. BMC Pharmacol Toxicol. 2014;15:54.

Thorens B. Brain glucose sensing and neural regulation of insulin and glucagon secretion. Diabetes Obes Metab. 2011;13(Suppl 1):82–8.

Turban S, Stretton C, Drouin O, Green CJ, Watson ML, Gray A, Ross F, Lantier L, Viollet B, Hardie DG, Marette A, Hundal HS. Defining the contribution of AMP-activated protein kinase (AMPK) and protein kinase C (PKC) in regulation of glucose uptake by metformin in skeletal muscle cells. J Biol Chem. 2012;287:20088–99.

UK Prospective Diabetes Study Group. Effect of intensive blood glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352:854–65.

Vaccaro O, Masulli M, Nicolucci A, Bonora E, Del Prato S, Maggioni AP, Rivellese AA, Squatrito S, Giorda CB, Sesti G, Mocarelli P, Lucisano G, Sacco M, Signorini S, Cappellini F, Perriello G, Babini AC, Lapolla A, Gregori G, Giordano C, Corsi L, Buzzetti R, Clemente G, Di Cianni G, Iannarelli R, Cordera R, La Macchia O, Zamboni C, Scaranna C, Boemi M, Iovine C, Lauro D, Leotta S, Dall'Aglio E, Cannarsa E, Tonutti L, Pugliese G, Bossi AC, Anichini R, Dotta F, Di Benedetto A, Citro G, Antenucci D, Ricci L, Giorgino F, Santini C, Gnasso A, De Cosmo S, Zavaroni D, Vedovato M, Consoli A, Calabrese M, di Bartolo P, Fornengo P, Riccardi G, Thiazolidinediones Or Sulfonylureas Cardiovascular Accidents Intervention Trial (TOSCA.IT) study group, under the mandate of the Italian Diabetes Society. Effects on the incidence of cardiovascular events of the addition of pioglitazone versus sulfonylureas in patients with type 2 diabetes inadequately controlled with metformin (TOSCA.IT): a randomised, multicentre trial. Lancet Diabetes Endocrinol. 2017. https://doi.org/10.1016/S2213-8587(17)30317-0.

Valderas JP, Ahuad J, Rubio L, Escalona M, Pollak F, Maiz A. Acarbose improves hypoglycaemia following gastric bypass surgery without increasing glucagon-like peptide 1 levels. Obes Surg. 2012;22:582–6.

Van De Laar FA, Lucassen PL, Akkermans RP, Van De Lisdonk EH, Rutten GE, Van Weel C. Alpha-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care. 2005;28:154–63.

Varvaki Rados D, Catani Pinto L, Reck Remonti L, Bauermann Leitão C, Gross JL. The association between sulfonylurea use and all-cause and cardiovascular mortality: a meta-analysis with trial sequential analysis of randomized clinical trials. PLoS Med. 2016;13:e1001992.

Verspohl EJ. Novel therapeutics for type 2 diabetes: incretin hormone mimetics (glucagon-like peptide-1 receptor agonists) and dipeptidyl peptidase-4 inhibitors. Pharmacol Ther. 2009;124: 113–38.

Weitzman SP, Ginsburg KC, Carlson HE. Colesevelam hydrochloride and lanthanum carbonate interfere with the absorption of levothyroxine. Thyroid. 2009;19:77–9.

White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F, EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med. 2013;369: 1327–35.

Wilding JP, Woo V, Rohwedder K, Sugg J, Parikh S, Dapagliflozin 006 Study Group. Dapagliflozin in patients with type 2 diabetes receiving high doses of insulin: efficacy and safety over 2 years. Diabetes Obes Metab. 2014;16:124–36.

Winkler G, Gerô L. Pharmacogenetics of insulin secretagogue antidiabetics. Orv Hetil. 2011;152: 1651–60.

Wright EM. Renal Na(+)-glucose cotransporters. Am J Physiol Renal Physiol. 2001;280(1):F10–8.

Wu S, Hopper I, Skriba M, Krum H. Dipeptidyl-peptidase-4 inhibitors and cardiovascular outcomes: meta-analysis of randomized clinical trials with 55,141 participants. Cardiovasc Ther. 2014;32:147–58.

Wu JH, Foote C, Blomster J, Toyama T, Perkovic V, Sundström J, Neal B. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2016;4:411–9.

Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, Donker AJ, Stehouwer CD. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med. 2003;254:455–63.

Yale JF, Bakris G, Cariou B, Nieto J, David-Neto E, Yue D, Wajs E, Figueroa K, Jiang J, Law G, Usiskin K, Meininger G, DIA3004 Study Group. Efficacy and safety of canagliflozin over 52 weeks in patients with type 2 diabetes mellitus and chronic kidney disease. Diabetes Obes Metab. 2014;16:1016–27.

Yki-Järvinen H, Kauppinen-Mäkelin R, Tiikkainen M, Vähätalo M, Virtamo H, Nikkilä K, Tulokas T, Hulme S, Hardy K, McNulty S, Hänninen J, Levänen H, Lahdenperä S, Lehtonen R, Ryysy L. Insulin glargine or NPH combined with metformin in type 2 diabetes: the LANMET study. Diabetologia. 2006;49:442–51.

Zannad F, Cannon CP, Cushman WC, et al. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. Lancet. 2015;385:2067–76.

Zhang L, Feng Y, List J, Kasichayanula S, Pfister M. Dapagliflozin treatment in patients with different stages of type 2 diabetes mellitus: effects on glycaemic control and body weight. Diabetes Obes Metab. 2010;12(6):510.

Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE, EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373:2117–28.

Treatment with GLP-1 Receptor Agonists 20

Sten Madsbad and Jens J. Holst

Contents

S. Madsbad (✉)
Department of Endocrinology, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark

The NNF Center for Basic Metabolic Research and the Department of Medical Sciences, Panum Instituttet, University of Copenhagen, Copenhagen, Denmark
e-mail: sten.madsbad@regionh.dk

J. J. Holst
The NNF Center for Basic Metabolic Research and the Department of Medical Sciences, Panum Instituttet, University of Copenhagen, Copenhagen, Denmark
e-mail: jjholst@sund.ku.dk

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_20

Abstract

The GLP-1 RAs have become popular because of their efficacy and durability in relation to glycemic control and their low risk of hypoglycemia in combination with weight loss in most patients. GLP-1 RAs mimic the effects of native GLP-1, which increases insulin secretion, inhibits glucagon secretion, increases satiety, and slows gastric emptying. Notably, the insulinotropic and glucagonostatic effects are glucose dependent, and therefore the risk of hypoglycemia is very low during treatment with a GLP-1 RA. The effect on gastric emptying is primarily observed with the short-acting GLP-1 RAs, since significant tachyphylaxis for this effect develops after few days' treatment with the long-acting GLP-1 RAs. The postprandial glucose control mediated by the short-acting GLP-1 RA seems to be primarily explained through the delaying effect on gastric emptying rather than the effect on insulin and glucagon secretion. In addition, GLP-1 RAs reduce blood pressure during chronic treatment, increase pulse rate, and reduce postprandial triglyceride concentrations. Studies have suggested that GLP-1 receptor agonists might have neuroprotective effects.

The most common adverse events are nausea and other gastrointestinal discomfort. The drawbacks of the GLP-1 RAs include the subcutaneous administration, the gastrointestinal side effects, and the cost.

Several GLP-1 RAs are now licensed for the treatment of type 2 diabetes. However, the intra-class difference raises challenges in relation to individual treatment. In the present chapter, the individual GLP-1 RAs will be presented followed by a head-to-head comparison of GLP-1 RAs. Thereafter, the adverse events and the cardiovascular effects of GLP-1 RAs including the cardiovascular endpoint trials with GLP-1 RAs will be discussed. The efficacy and safety of fixed combination of basal insulin and a GLP-1 RA will be reviewed. The use of GLP-1 RAs in the treatment of patients with type 1 diabetes or in treatment of obesity will also be examined.

Keywords
GLP-1RA · Type 2 diabetes · Type 1 diabetes · Obesity, fixed combination · Head-to-head comparison · Adverse events · Cardiovascular effects

The use of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) has expanded the treatment options for type 2 diabetes (T2DM) over the last decade (Garber et al. 2016; Inzucchi et al. 2015). The GLP-1 RAs have become popular because of their efficacy and durability in relation to glycemic control and their low risk of hypoglycemia in combination with weight loss in most patients (Ostergaard et al. 2016; Meier 2012). GLP-1 RAs mimic the effects of native GLP-1, which increases insulin secretion, inhibits glucagon secretion, increases satiety, and slows gastric emptying (Ostergaard et al. 2016; Meier 2012). Notably, the insulinotropic and glucagonostatic effects are glucose dependent, and therefore the risk of hypoglycemia is very low during treatment with a GLP-1 RA, unless it is combined with sulfonylurea or insulin (Ostergaard et al. 2016; Meier 2012; Nauck et al. 1993). The effect on gastric emptying is primarily observed with the short-acting GLP-1 RAs, since significant tachyphylaxis for this effect develops after few days' treatment with the long-acting GLP-1 RAs (Jelsing et al. 2012; Meier et al. 2003). The postprandial glucose control mediated by the short-acting GLP-1 RA seems to be primarily explained through the delaying effect on gastric emptying rather than the effect on insulin and glucagon secretion (Meier et al. 2003). In addition, GLP-1 RAs reduce blood pressure during chronic treatment, increase pulse rate, and reduce postprandial triglyceride concentrations (Drucker 2016; Hermansen et al. 2013; Kumarathurai et al. 2017a). The potential effect of GLP-1 on cardiovascular function is an area of major interest and will be discussed in detail later in this chapter. Whether treatment with a GLP-1 RA may protect the beta-cell mass through beta-cell regeneration and inhibition of apoptosis and thereby reduce or halt the progression of type 2 diabetes has been debated (Kielgast et al. 2009). In one study, beta-cell function was evaluated after 3 years of treatment with a short-acting GLP-1 RA (exenatide), and during this period there was no deterioration, but the same was true in the control group subjected to intensive insulin therapy (Bunck et al. 2011). In the LEADER study of the cardiovascular safety of liraglutide, hemoglobin A1c levels remained almost unchanged over a period of 5 years, perhaps reflecting some protective action on the beta cells (Marso et al. 2016a). Studies in rodent models of Parkinson's and Alzheimer's diseases and mouse models of ischemic stroke have suggested that GLP-1 receptor agonist might have neuroprotective effects and prevent memory impairment (McClean et al. 2011; Harkavyi et al. 2008; Teramoto et al. 2011). However, studies in humans have not supported the use of GLP-1 RA in cerebral diseases (Calsolaro and Edison 2015), except for one clinical trial of 48 weeks, which suggested that exenatide once weekly had positive effects in Parkinson's disease, which was sustained beyond the period of exposure (Athauda et al. 2017). Whether exenatide affects the underlying disease pathophysiology or the result simply is secondary to long-lasting metabolic improvement effects is uncertain.

The most common adverse events are nausea and other gastrointestinal discomfort (Ostergaard et al. 2016; Meier 2012). The drawbacks of the GLP-1 RAs include the subcutaneous administration, the gastrointestinal side effects, and the cost (Ostergaard et al. 2016).

As a drug class, the GLP-1 RAs have proven efficacy for lowering HbA1c and decreasing weight in T2D, with a reduced risk of hypoglycemia compared with insulin or sulfonylureas (Garber et al. 2016; Inzucchi et al. 2015; Ostergaard et al. 2016). These characteristics underlie the inclusion of GLP-1 RAs in various clinical practice guidelines. Their use as dual therapy with metformin after first-line metformin and as triple therapy (in combination with metformin and a sulfonylurea/thiazolidinedione/insulin) is part of the European Association for the Study of Diabetes/American Diabetes Association recommendations (Inzucchi et al. 2015). Glucagon-like peptide-1 receptor agonists are recommended as monotherapy, dual therapy, and triple therapy by the American Association of Clinical Endocrinologists/American College of Endocrinology guidelines (Garber et al. 2016).

In the present chapter, the individual GLP-1 RAs will be presented followed by a head-to-head comparison of GLP-1 RAs. Thereafter, the adverse events and the cardiovascular effects of GLP-1 RAs including the cardiovascular endpoint trials with GLP-1 RAs will be discussed. The efficacy and safety of fixed combination of basal insulin and a GLP-1 RA will be reviewed. The use of GLP-1 RAs in the treatment of patients with type 1 diabetes or in treatment of obesity will also be examined. Lastly, some future aspects of GLP-1-based therapy will be presented. A thorough review of all trials with GLP-1 RAs in type 2 and type 1 diabetes up to 2016 can be found in Ostergaard et al. (2016), Dejgaard et al. (2016a), and Frandsen et al. (2016).

Characteristics of GLP-1 Receptor Agonists

For therapeutic purposes, continuous subcutaneous administration of native GLP-1 is necessitated because of its extremely short plasma half-life (1–2 min) but has limited therapeutic value (Zander et al. 2002). Therefore, several GLP-1 RAs have been developed with an extended duration of action achieved by various changes of the molecular structure compared with the native peptide (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016).

Six GLP-1 RAs are currently (2017) approved in Europe and the USA. GLP-1 RAs differ substantially in their molecular structures and sizes, chemical and physiological properties, and duration of action (Table 1; Madsbad 2016). Exenatide (Byetta®), administered twice daily (BID), and lixisenatide (Lyxumia®), administered once daily (QD), are short-acting agents based on the structure of the lizard peptide exendin-4. Liraglutide (Victoza®) is based on the GLP-1 structure, classified as long-acting, and is administered QD, while the very long-acting agents, including exenatide long-acting release (LAR) (Bydureon®), albiglutide (Eperzan® and Tanzeum®), and dulaglutide (Trulicity®), are administered once weekly (QW) (Madsbad 2016). A number of important studies have been reported using another

Table 1 Comparative characteristics of the GLP-1 RAs

	Exenatide BID	Exenatide QW	Liraglutide	Lixisenatide	Albiglutide	Dulaglutide	Taspoglutide
Percentage amino acid sequence similarity to native GLP-1	53%	53% [	97%	≈50%*	95%	90%	93%
Properties of the drug	Resistant to DPP-4 cleavage, largely due to the substitution of alanine in position 2 by glycine	Encapsulated in biodegradable polymer microspheres	C-16 fatty acid confers albumin binding and heptamer formation	Based on exenatide but is modified by the deletion of one proline residue and addition of six lysine residues at the C-terminal	GLP-1 dimer fused to albumin	The GLP-1 portion of the molecule is fused to an IgG4 molecule, limiting renal clearance and prolonging activity	Modifications designed to hinder cleavage by DPP-4 and by serine proteases and also allows greater receptor binding
Half-life	2.4 h	Half-life is unpublished but steady-state concentrations at 6–7 weeks	11–15 h	2.7–4.3 h	6–8 days	≈5 days	165 h
T_{max}	2.1 h	2.1–5.1 h during the first 48 h	≈9–12 h	1.25–2.25 h	72–96 h	24–72 h	4, 6, and 8 h at 1, 8, and 30 mg doses, respectively
Clearance	9.1 l/h	Unpublished	1.2 l/h	21.2–28.5 l/h	67 ml/h	0.75 mg and 1.5 mg at steady state was 0.073 and 0.107 l/h, respectively	Unpublished

(*continued*)

Table 1 (continued)

	Exenatide BID	Exenatide QW	Liraglutide	Lixisenatide	Albiglutide	Dulaglutide	Taspoglutide
Antibody formation	In head-to-head studies, antibodies were more common, and titers were higher with exenatide OW compared with exenatide BID Antibodies did not correlate with rates of reported AEs		From six phase 3 studies, 8.7 and 8.3% of participants had low-titer antibodies to liraglutide 1.2 and 1.8 mg, respectively, after 26 weeks	Antibodies developed in: 56–60% of participants treated with 20 μg OD 43% of participants treated with 10 μg OD and 71% treated with 20 μg BID	Antibodies developed in 3.7% of participants treated with albiglutide	Dulaglutide anti-drug antibodies in 1% of participants and dulaglutide neutralizing anti-drug antibodies in 1% of patients	Detected in 49% of participants

AE adverse event, *BID* twice daily, *DPP-4* dipeptidyl peptidase-4, *GLP-1* glucagon-like peptide-1, *GLP-1 RA* GLP-1 receptor agonist, *IgG4* immunoglobulin 4, *OD* once daily, *OW* once weekly, T_{max} time to maximum plasma concentration

agonist for weekly use, namely, semaglutide, which is structurally related to liraglutide (Lau et al. 2015; Nauck et al. 2016a).

One method to extend the half-life of GLP-1 has been to make it resistant to degradation by DPP-4 by changing the penultimate N-terminal amino acid (Gallwitz et al. 2000). However, the intact hormone is still subject to renal elimination, which alone results in a half-life of 4–5 min (Deacon et al. 1998; Meier et al. 2004). Therefore, other approaches to prolong half-life have been based on reversible or irreversible binding to albumin (liraglutide, semaglutide, and albiglutide), whereby renal filtration is prevented (Meier 2012). Dulaglutide is conjugated with the Fc fragment of IgG to extend the duration of action (Meier 2012). The GLP-1 RA can also be coupled to biodegradable polymer microspheres resulting in a protracted release of the peptide from a subcutaneous depot as in exenatide-LAR (Bydureon) (Meier 2012).

Taspoglutide once weekly was halted in development due to serious hypersensitivity reactions and GI adverse events (AEs) during clinical trials (due to an inexpedient prolongation technique, resulting in an unsuitable plasma profile). Semaglutide once weekly is not yet approved for the treatment of people with type 2 diabetes but is expected in 2017. Therefore, the available data for these two compounds are included here to give a full picture of the GLP-1 RA family.

The different durations of action largely explain the variations among GLP-1 RAs with respect to their impact on fasting plasma glucose (FPG), 24-h glucose profiles, and postprandial plasma glucose (PPG) levels (Kapitza et al. 2013; Meier et al. 2015). Delayed gastric emptying, for example, is more strongly associated with short-acting than longer-acting GLP-1 RAs (Figs. 1 and 2), and this probably explains the greater effects on PPG observed with short-acting GLP-1 RAs. Conversely, the greater half-lives of the longer-acting compounds allow for enhanced effects on the average 24-h glucose level, including FPG (Kapitza et al. 2013; Meier et al. 2015). Longer-acting GLP-1 RAs affect gastric motility to a limited extent. Instead, longer-acting GLP-1 RAs exert more of their effect via the pancreas, increasing insulin secretion and inhibiting glucagon secretion (Kapitza et al. 2013; Meier et al. 2015).

The chemical and pharmacokinetic differences between GLP-1 RAs are also reflected in their varying efficacy with regard to HbA1c reduction and weight loss, their differing tolerability profiles, and potential for immunogenicity (Ostergaard et al. 2016; Meier 2012; Madsbad et al. 2011; Madsbad 2016; Kapitza et al. 2013; Meier et al. 2015). It is important to understand these specific characteristics to make the appropriate choice of GLP-1 RA for the individual patient. Head-to-head clinical trials are the best way to evaluate the differences in efficacy and tolerability, and a number of such studies have been conducted with GLP-1 RAs in T2D, but first the eight GLP-1 RAs will be discussed. The GLP-1 RA family is presented in Fig. 1, and the differences in molecular structure, chemical and physiological properties, and durations of action are summarized in Table 1.

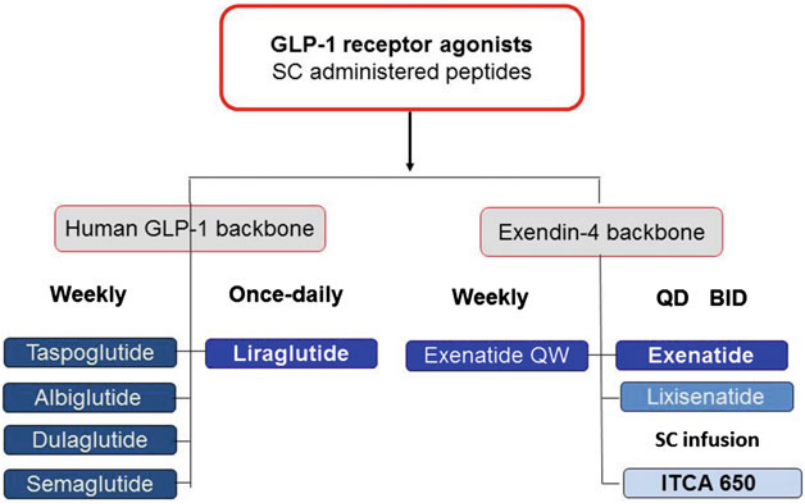


Fig. 1 Shows the glucagon-like peptide-1 receptor agonists, which have already been approved, except for taspoglutide, which was halt in phase 3 development and ITCA 650, which is in phase 3 development. The agonists are subdivided in relation to whether the backbone of the compound is human GLP-1 or exenatide and in relation to the frequency of administration (once weekly, or once daily or twice daily). ITCA is a mini-pump, which infused exenatide for 3–12 months per pump (Fig. 2)

Exenatide Twice Daily

Exenatide (Byetta®), which is a 39-amino-acid peptide, was the first GLP-1 RA introduced to the market (2005). Exenatide BID is derived from the saliva of the Gila monster and is 53% homologous to native GLP-1 with respect to the first 30 amino acids (the sequence of the remaining 9 has no human homologies) (Kolterman et al. 2005). Exenatide BID is indicated as adjunct to diet and exercise in patients with type 2 diabetes. It can be used in monotherapy or in combination with oral antidiabetic agents including basal insulin. After injection, the duration of action is about 8–10 h, and peak levels are achieved 2–3 h after injection (Kolterman et al. 2005). Injection should be administered 20–60 min prior to two main meals at least 6 h apart. The delayed gastric emptying after breakfast and dinner is the main mechanism by which exenatide improves postprandial glucose excursions. Exenatide has only minor effect on lunch glucose excursions. The increase in insulin secretion and reduction in glucagon secretion, which result in a decreased hepatic glucose production, also contributes to the improved glucose metabolism (Cervera et al. 2008), but the effect on fasting plasma glucose is less than that of the long-acting GLP-1 RAs (Buse et al. 2009). Initial dose is 5 μg, increasing to 10 μg BID. Exenatide is not recommended in patients with severe renal impairment (eGFR<30 ml/min). The phase 3 studies are discussed in details in Inzucchi et al. (2015). Exenatide BID has demonstrated similar efficacy as glimepiride or pioglitazone with a reduction of

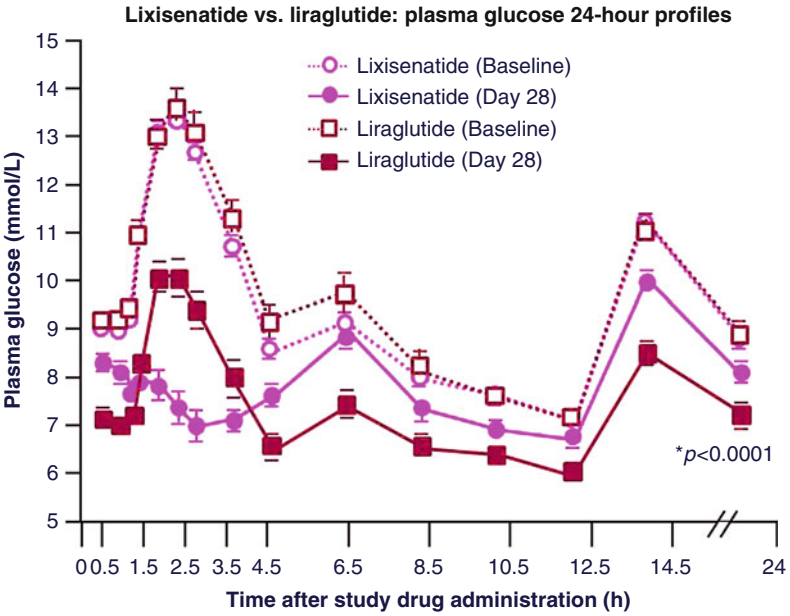


Fig. 2 Mean 24-h postprandial plasma glucose at baseline and after 28 days treatment with the short-acting lixisenatide once daily compared with the longer-acting once-daily liraglutide. With lixisenatide postprandial glucose is lower during breakfast, while during liraglutide treatment plasma glucose is lower from lunch and during the rest of the 24 h

0.8–1.5% in HbA1c and induces a weight loss ranging from 1 to 4 kg (Ostergaard et al. 2016). Compared with basal insulin, the reduction in HbA1c is similar or greater with exenatide BID (Ostergaard et al. 2016). Nausea occurs initially in 30–60% of patients with vomiting in about 15–20% (Ostergaard et al. 2016). The gastrointestinal side effects are often transient. Antibodies against exenatide have been detected in 40–60% of the patients, but in the majority of the patients, their presence does not seem to impair efficacy of exenatide (Buse et al. 2011; Drucker et al. 2008). In patients with very high titers of antibodies, the reduction in HbA1c was smaller compared with patients without antibodies (Buse et al. 2011; Drucker et al. 2008). Apparently, the antigenicity of exenatide has not lead to major clinical complications so far.

Lixisenatide Once Daily

Lixisenatide is identical to exendin-4 but has a proline deletion in position 38 and is extended with six lysine residues at the C-terminus (Ratner et al. 2010). The half-life is 2–3 h, and peak plasma concentrations are achieved 1.5–2.5 h after injection, similar to exenatide, but lixisenatide is nevertheless approved for s.c. administration once daily (Ratner et al. 2010). The dose is 10 μg increasing to 20 μg after 2 weeks.

The efficacy of lixisenatide has been tested in monotherapy and in combination therapy and has been compared with placebo and exenatide BID (Bolli et al. 2014; Fonseca et al. 2012; Rosenstock et al. 2013a, 2014a; Pinget et al. 2013). Compared with exenatide, the mean change in HbA1c was −0.79% for lixisenatide versus −0.96% for exenatide BID (Rosenstock et al. 2013a). Both agents induced weight loss (from 94.5 to 91.7 kg and from 96.7 to 92.9 kg with lixisenatide and exenatide, respectively) (Rosenstock et al. 2013a). Incidence of adverse events (AEs) was similar for lixisenatide and exenatide. Lixisenatide has been added on to insulin in Asian people, and after 24 weeks, the HbA1c changes were −0.77% and +0.11% in the lixisenatide and placebo groups (Seino et al. 2012). A weight loss of 0.4 kg was observed in the lixisenatide group, while a weight gain of 0.1 kg was found in the placebo group. In another 24 weeks study, the reductions in HbA1c were −0.6% and −0.3% and in body weight −1.8 versus −0.5 kg in the lixisenatide and placebo groups, respectively (Riddle et al. 2013). Because of the short action, the effect on fasting plasma glucose is less than with the long-acting GLP-1 RAs (Nauck et al. 2016b). In most of the trials, body weight decreased significantly with lixisenatide compared with placebo. The cardiovascular endpoint trial ELIXA with lixisenatide will be discussed later.

Liraglutide Once Daily

The amino acid sequence of liraglutide shows 97% identity with that of native human GLP-1, and liraglutide has a half-life of approximately 13 h; therefore, it is suitable for subcutaneous administration once daily (Agerso et al. 2002). The peptide differs from GLP-1 owing to a Lys34Arg amino acid substitution and addition of glutamate residue and a 16-carbon free fatty acid to Lys26, modifications that promote non-covalent binding to plasma albumin (Knudsen et al. 2000). Consequently about 99% of the liraglutide molecules are bound to albumin, ensuring a rather constant, high plasma level after once-daily administration (Knudsen et al. 2000).

Dose-finding studies resulted in the doses of 0.6 mg, 1.2 mg, and 1.8 mg being moved forward to the clinical phase 3 development program "Liraglutide Effect and Action in Diabetes" (LEAD™), completed in 2007 (Ostergaard et al. 2016; Madsbad 2009). Treatment is initiated with 0.6 mg for 1 week and then titrated to the standard dose of 1.2 mg, which can be escalated to 1.8 mg once daily (Ostergaard et al. 2016; Madsbad 2009).

In the phase 3 program, the HbA1c reduction was 1.1–1.8% with only minor differences between 1.2 and 1.8 mg, but there was little effect on postprandial glucose excursions during chronic therapy, probably because of the tachyphylaxis with respect to gastric emptying (Ostergaard et al. 2016; Madsbad 2009). The reduction in mean body weight was in the range of 2–3 kg in the LEAD studies (Ostergaard et al. 2016; Madsbad 2009). The effect on body weight seems to be dose dependent, but the greatest mean weight loss of 4.5 kg was observed in subjects with a BMI > 35 kg/m2 and when liraglutide was combined with metformin (Ostergaard et al. 2016; Madsbad 2009). Liraglutide reduced systolic blood pressures by about

2–7 mm HG, and increases in pulse rate of 2–4 beat per minutes were reported (Ostergaard et al. 2016; Madsbad 2009). As discussed later, liraglutide therapy has been associated with reduced risk of cardiovascular events and mortality (Marso et al. 2016a). Nausea was reported by 20–40% of the patients and vomiting in 5–10%, but both were generally transient and could be reduced by slow up-titration (Ostergaard et al. 2016; Madsbad 2009). The number of patients developing antibodies against liraglutide is about 3–10% (Ostergaard et al. 2016; Madsbad 2009).

Liraglutide has been compared with all oral antidiabetic agents and with basal insulin glargine and showed better efficacy with respect to both reduction in HbA1c and weight loss (Ostergaard et al. 2016; Madsbad 2009). As discussed later, liraglutide has also been compared with lixisenatide, exenatide BID, exenatide QW, and dulaglutide QW (Ostergaard et al. 2016; Madsbad 2016, 2009; Nauck et al. 2016b).

Exenatide Once Weekly

Exenatide administered once weekly (QW) (2 mg/dose) was marketed in Europe in 2011 and in the USA in 2012. The drug, in a fixed dose of 2 mg, is encapsulated in biodegradable microspheres (0.06 mm in diameter), allowing the drug to be slowly released through diffusion and microsphere breakdown gradually over 10 weeks (Drucker et al. 2008; Mann and Raskin 2014). The microspheres are reconstituted in a premeasured aqueous solution before injection. The plasma concentration continues to rise for weeks after treatment initiation, and steady-state levels are obtained after 6–7 weeks (Drucker et al. 2008; Mann and Raskin 2014). The gradual release from the formulation eliminates the need for slow up-titration. The main results of the phase 3 DURATION 1–6 trials are discussed in Ostergaard et al. (2016) and Brunton and Davidson (2016). Exenatide QW has been compared with exenatide BID, liraglutide, insulin glargine, and oral antidiabetic agents, and the HbA1c reduction ranges between 1.3% and 1.9% (Ostergaard et al. 2016; Brunton and Davidson 2016). In a head-to-head comparison, the HbA1c reduction was significantly greater (1.9% vs. 1.5%) with exenatide QW compared with exenatide BID, primarily explained by a greater reduction in plasma glucose during nighttime, while postprandial glucose excursions were more reduced with exenatide BID (Drucker et al. 2008). The increase in morning pulse rate was also greater with exenatide QW compared with exenatide BID (Drucker et al. 2008). The reduction in weight did not differ between the short- and long-acting exenatide. More patients developed antibodies against exenatide QW than against exenatide BID (74% vs. 43%), but only in few patients did the antibodies seem to affect efficacy in relation to HbA1c reduction (Drucker et al. 2008). Compared with liraglutide 1.8 mg once daily, the reduction was less (1.48% vs. 1.28%), and patients treated with liraglutide lost more weight than exenatide QW-treated patients (Buse et al. 2013). More patients experienced nausea with liraglutide, while serious adverse events were more often reported with exenatide QW (Buse et al. 2013). Compared with insulin glargine, the reduction in HbA1c was greater with exenatide QW (1.5% vs. 1.3%), and most patients

experienced weight loss in contrast to weight gain during treatment with insulin glargine (Diamant et al. 2014). Hypoglycemia occurred more often with insulin glargine. In a recent trial exenatide once weekly was compared with dapagliflozin (DURATION-8) as add-on to metformin, and after 28 weeks the reduction in HbA1c was 1.6% and 1.4%, respectively, while the reduction was 2.0% in the combined exenatide plus dapagliflozin group (Frias et al. 2016). Weight loss was greater with dapagliflozin compared with exenatide once weekly (2.2 kg vs. 1.5 kg) compared with 3.4 kg in the combined group (Frias et al. 2016).

Because of the consistency of the injection suspension, injections of exenatide previously required a rather large-bore needle (23 gauge, 0.64 mm), and a convenient device was not available. Today, exenatide QW is available in a new, prefilled single-dose pen device, which simplifies reconstitution of the drug. Injection site reactions including erythema, pruritus, and nodules are being reported by about 10–15% of patients (Brunton and Davidson 2016). The most frequent gastrointestinal side effects are the expected: nausea, vomiting, and diarrhea, which, however, occur less frequently than with exenatide BID (Drucker et al. 2008). No cases of pancreatic cancer were reported in the DURATION trials, and exenatide QW was not associated with an increased risk of pancreatitis (Brunton and Davidson 2016). The cardiovascular safety of exenatide QW will be discussed later in relation to the EXSCEL trial.

Albiglutide Once Weekly

Albiglutide is composed of two DPP-4-resistant GLP-1 molecules arranged in tandem and fused to human albumin, which consequently leads to a plasma half-life of 5–8 days, allowing QW dosing. Maximal concentration is observed 3–5 days after s.c. injection (Young et al. 2014). An amino acid substitution (alanine to glycine at residue no 2 from the N-terminus) in the GLP-1 dimer makes it resistant to DPP-4 degradation (Young et al. 2014). Otherwise the two GLP-1 moieties are 97% homologous to native GLP-1 (Young et al. 2014). Albiglutide is a large molecule and is thus relatively inaccessible to the central nervous system; this quality may have implications for gastrointestinal tolerability of the drug and for weight loss. The European Medicines Agency (EMA) and US Food and Drug Administration (FDA) approved albiglutide in 2014. Studies comparing different dosing regimens have suggested that a dose of 30 mg once weekly might be optimal. For patients unable to reach glycemic goal, escalating to 50 mg weekly is appropriate and results in further improvement in glycemic control. The efficacy and safety of albiglutide were tested in the phase 3 HARMONY 1–8 program, and the main results are presented in Ostergaard et al. (2016), Madsbad et al. (2011), Madsbad (2016), and Blair and Keating (2015). The reduction in HbA1c and weight has been less, and rates of gastrointestinal side effects are also reduced compared to other GLP-1 RAs (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016; Blair and Keating 2015). In the HARMONY 4 study, albiglutide was compared with insulin glargine and gave a reduction of HbA1c of 0.66 versus 0.81 for albiglutide and insulin

glargine, respectively, with an additional small weight loss in the albiglutide group (Ostergaard et al. 2016; Weissman et al. 2014). Compared with liraglutide in the HARMONY 7 trial, the reduction in HbA1c was 0.78% for albiglutide and 0.99% for liraglutide, and liraglutide was also associated with a greater weight loss (Pratley et al. 2014). More gastrointestinal side effects were reported with liraglutide (Pratley et al. 2014). In the HARMONY 6 trial, including patients taking basal insulin, albiglutide add-on was tested versus thrice-daily prandial insulin lispro (Rosenstock et al. 2014b). After 26 weeks, the reduction in HbA1c was 0.82% with albiglutide and 0.66% with lispro, and the weight changes were −0.73 kg and +0.81 kg, respectively (Rosenstock et al. 2014b).

Albiglutide has been associated with up to a 20% incidence of injection site reaction, and antibodies against albiglutide developed in up to 5.5% of patients but had no obvious effect on the efficacy of albiglutide (Blair and Keating 2015). On July 26, 2017, GlaxoSmithKline (GSK) in a press release announced that they plan to discontinue the manufacturing and sale of albiglutide by July 2018.

Dulaglutide Once Weekly

Dulaglutide is a GLP-1 RA constructed by two GLP-1 analogues linked to a human IgG4-Fc heavy chain (Barrington et al. 2011). The association with the IgG4-Fc heavy chain prevents renal clearance (Barrington et al. 2011; Glaesner et al. 2010), and the molecule is resistant to DPP-4 degradation because of amino acid substitutions at position 2 of the GLP-1 parts; additional substitutions are present at positions 8 and 22 (Barrington et al. 2011; Glaesner et al. 2010). The half-life is approximately 5 days, making it suitable for QW administration (Barrington et al. 2011; Glaesner et al. 2010). Dulaglutide was approved in the USA and in Europe in 2014. Dulaglutide is administered as 0.75 mg once weekly, which can be escalated to 1.5 mg once weekly (Barrington et al. 2011). Steady-state concentration is obtained after 2–4 weeks (Barrington et al. 2011; Jendle et al. 2016). Dulaglutide is available as a prefilled pen syringe ready for injection. Across the clinical studies, about 1.6% of dulaglutide-treated patients developed antibodies, which did not reduce the glucose-lowering effect (Jendle et al. 2016). Injection site reactions (rash and erythema) were reported in 0.5% of the patients (Jendle et al. 2016). Dulaglutide's efficacy and safety has been tested in a variety of phase 3 trials known as the AWARD-studies, and the findings are reviewed in Jendle et al. (2016). Dulaglutide has been found to reduce HbA1c more than sitagliptin, metformin, and exenatide BID, while weight reduction and gastrointestinal side effects did not differ between dulaglutide and exenatide (Jendle et al. 2016). Dulaglutide 1.5 mg reduced HbA1c (−0.9% vs. −0.62%) more than insulin glargine (Jendle et al. 2016). In the AWARD-6 trial comparing dulaglutide with liraglutide, the HbA1c reduction was 1.42% with dulaglutide and 1.36 for liraglutide, while patients treated with liraglutide experienced a significantly greater weight loss (3.61 vs. 2.90 kg). The incidence of adverse events did not differ between the two groups (Jendle et al. 2016; Dungan et al. 2014). In AWARD-10 dulaglutide 1.5 mg and 0.75 mg or placebo were add-on to SGLT-2 inhibitor with or without metformin for 24 weeks. The reduction in HbA1c was

for 1.5 mg −1.34 % (−14.7 mmol/mol) and for 0.75 mg −1.21% (−13.2 mmol/mol) compared with −0.54% (−5.9 mmol/mol) for placebo (Ludvig B et al. Lancet Diabetes Endocrinol 2018, Febr 23 epub ahead of print). Head- to head comparison between dulaglutide and semaglutide is discussed below under semaglutide.

Taspoglutide Once Weekly

The GLP-1 receptor agonist, taspoglutide, has 93% homology with the native hormone and contains two α-aminoisobutyric acid substitutions replacing Ala^8 and Gly^{35} of hGLP-1 (7–36)NH_2 (Dong et al. 2011). Taspoglutide is fully resistant to DPP-4 degradation, while protraction is provided by a sustained release formulation (Dong et al. 2011). Its biological actions have been shown to be similar to those of native GLP-1, but after a single dose, a glucose-lowering effect was found for up to 2 weeks.

Taspoglutide was evaluated in seven clinical trials in the T-emerge program using 10 and 20 mg once weekly (Madsbad et al. 2011). Both doses of taspoglutide reduced HbA1c more than exenatide BID (difference 0.33% for 20 mg) with comparable weight loss but with unacceptable levels of nausea/vomiting, injection site reactions, and systemic allergic reactions (Madsbad et al. 2011). Vomiting occurred in most cases on the day of injection in the taspoglutide groups and in the majority already after the first injection. In other trials, taspoglutide reduced HbA1c more than sitagliptin but had similar effects as pioglitazone and insulin glargine (Madsbad et al. 2011). In September 2010 the T-emerge program was halted because a potential association between hypersensitivity reactions and anti-drug antibodies was suggested, and taspoglutide is not expected to come to the market (Madsbad et al. 2011).

Semaglutide Once Weekly

Semaglutide was developed from liraglutide by increasing the albumin affinity and securing full stability against metabolic degradation. The fatty acid moiety and its linking to GLP-1 were the key features securing high albumin affinity and GLP-1 receptor (GLP-1R) potency and also resulted in a prolonged exposure and action of the GLP-1 analogue (Lau et al. 2015). Like liraglutide, semaglutide has an amino acid substitution at position 34 (Lys-Arg) and is derivatized at lysine 26 (Lau et al. 2015). An additional substitution at position 8 (Ala- > Aib) secures DPP-4 resistance. The GLP-1R affinity of semaglutide is similar to that of liraglutide, while the albumin affinity is improved (Lau et al. 2015). The plasma half-life is reported to be 165 h in human (Kapitza et al. 2015). Semaglutide is currently in late phase 3 clinical testing, and the first six trials have been presented in public. In a 12 weeks phase 2 study, semaglutide reduced HbA1c by impressive 1.7% from a baseline of 8.1% and body weight up to 4.8 kg, which was greater than with liraglutide 1.8 mg QD (Nauck et al. 2016a). Semaglutide doses of 0.5 mg and 1.0 mg with a 4-week dose escalation were selected for the SUSTAIN phase 3 program (Nauck et al. 2016a). In SUSTAIN-1, semaglutide

0.5 mg and 1.0 mg in patients with type 2 diabetes reduced HbA1c from a baseline of 8.1% by 1.4% and 1.5% compared with placebo after 30 weeks, and about 73% reached a HbA1c below 7.0% and 60% below 6.5% (Sorli et al. 2017). Weight loss was 2.8 and 3.6 kg greater than with placebo, respectively (Sorli et al. 2017). In the 56 weeks SUSTAIN-2 trial, semaglutide 0.5 mg and 1.0 mg reduced HbA1c by 1.3% and 1.6% versus 0.5% with sitagliptin (baseline, 8.1%). Weight losses were 4.3 kg, 6.1 kg, and 1.9 kg, respectively (Ahren et al. 2017). In the SUSTAIN-3, trial semaglutide was compared with exenatide QW (Ahmann Aj et al. Diabetes Care 2018; 41: 258–66). After 56 weeks, semaglutide 1.0 mg reduced HbA1c by 1.5% from a baseline HbA1c of 8.3%, compared with 0.9% with exenatide QW, and 67% versus 40% reached a HbA1c $<$ 7.0%, respectively. Weight losses were 5.6 kg and 1.9 kg, respectively. Gastrointestinal adverse events occurred in 42% and 33%, and injection site reactions were reported by 1.2% and 22%, respectively. In SUSTAIN-4, semaglutide was compared with insulin glargine in insulin-naïve patients. After 30 weeks, the reduction in HbA1c was 1.2%, 1.6%, and 0.8% from a baseline of 8.2% with 0.5 mg and 1.0 mg of semaglutide and insulin glargine, respectively (Aroda et al. 2017). Weight loss was 3.5 kg and 5.2 kg versus a weight gain of 1.2 kg with insulin glargine (Aroda et al. 2017). Risk of hypoglycemia was also reduced with semaglutide. Efficacy and safety of semaglutide versus placebo as add-on to basal insulin was investigated in SUSTAIN-5. After 30 weeks (baseline HbA1c 8.4%) 61% and 79% versus 11% with 0.5 mg, 1.0 mg, or placebo had achieved a HbA1c below 7.0%. Weight losses were 3.7 kg, 6.4 kg, and 1.4 kg, respectively. The cardiovascular endpoint study SUSTAIN-6 will be discussed later in this chapter (Marso et al. 2016b). The SUSTAIN-7 trial is a head-to-head comparison between semaglutide and dulaglutide as add-on to metformin during 40 weeks (Pratley RE et al. Lancet Diabetes Endocrinol 2018 Jan 31, Epub ahead of print). Patients in the 0.5 mg semaglutide group had a reduction in HbA1c of 1.5% against a 1.1% reduction in the 0.75 mg dulaglutide group. Additionally, 1.0 mg of semaglutide reduced HbA1c by 1.8% compared with a decrease by 1.4% among patients treated with 1.5 mg dulaglutide. Those on 0.5 mg semaglutide lost on average 4.6 kg of body weight compared to 2.3 kg with 0.75 mg dulaglutide. The higher doses led to losses of 6.5 kg and 3.0 kg, respectively. The side effects including changes in retinopathy did not differ between the two GLP-1 RAs.

Semaglutide has not yet been approved for treatment of type 2 diabetes. Overall, semaglutide seems at least as effective and possibly more potent than the other GLP-1 RAs. Safety profile of semaglutide did not differ from those reported with other GLP-1 RAs (Marso et al. 2016b; Sorli et al. 2017). In the SUSTAIN 6 trial semaglutide was associated with a significant increase in the risk of diabetic retinopathy (Marso et al. 2016b). In a post-hoc analyses of the SUSTAIN 6 data the increase in diabetic retinopathy was attributed to the magnitude and rapidly of HbA1c reduction during the first 16 weeks of treatment in patients who had pre-existing diabetic retinopathy and poor glycemic control at baseline, and who were treated with insulin (Vilsbøll T et al. Diabet Obes Metab 2018, 20: 889–97). In the SUSTAIN 1-5 trials there were no imbalance in diabetic retinopathy with semaglutide versus placebo (Vilsbøll T et al. Diabet Obes Metab 2018; 20: 889–97). Semaglutide is also in development as an obesity drug.

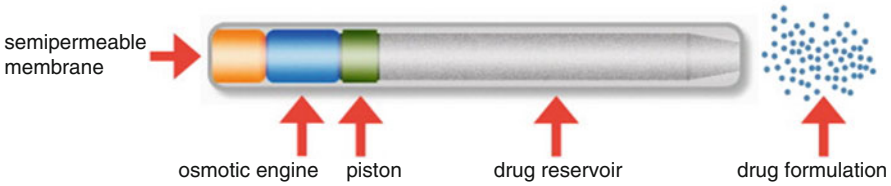


Fig. 3 ITCA 650 utilizes a novel drug delivery technology to provide continuous and controlled subcutaneous delivery of exenatide for as long as 1 year of treatment at a precise and predetermined rate. Initiating treatment with ITCA 650 involves the subcutaneous placement of a matchstick-sized osmotic mini-pump done during a short office procedure that can be performed by a physician, physician's assistant, or other licensed practitioner. ITCA 650 consists of a cylindrical titanium alloy reservoir with external dimensions of 4 mm in diameter by 44 mm in length. The reservoir is capped at one end by a controlled-rate, semipermeable membrane and capped at the other end by a diffusion moderator through which drug formulation is released from the drug reservoir. The drug formulation, piston, and osmotic engine are contained inside the cylinder. ITCA 650 releases drug at a predetermined rate based on the principle of osmosis. Water from the extracellular space enters through the semipermeable membrane directly into the osmotic engine that expands to drive the piston at a slow and consistent rate of travel

Intarcia (ITCA) 650

A new interesting concept is ITCA 650, which provides a constant and continuous subcutaneous delivery of exenatide via an osmotic mini-pump (the size of a matchstick, see Fig. 3) for treatment of patients with type 2 diabetes (Henry et al. 2013a, b, 2014). In the phase 3 FREEDOM program, which also includes a cardiovascular endpoint study, more than 5000 patients with type 2 diabetes are enrolled (Henry et al. 2013a, b, 2014). In the trials, the mini-pumps first delivered for 3 months a 20 mcg/day introductory dose, followed by a 60 mcg/day 6-month maintenance dose. A 12-month mini-pump with a 60 mcg/day delivery is in development with the goal to deliver exenatide with yearly renewal of the pump. In FREEDOM 2, ITCA 650 (baseline HbA1c about 8.6%) reduced HbA1c 1.5% versus 0.8% with sitagliptin. Weight changes were −4.0 kg and 1.3 kg, respectively. ITCA 650 treatment has also been shown to be superior to exenatide BID (Henry et al. 2013a). The adverse events were gastrointestinal as with other GLP-1 RAs, and placement and removal of ITCA were well-tolerated (Henry et al. 2013a, b, 2014).

Safety and Adverse Events of GLP-1 RAs

Gastrointestinal

As discussed above the most frequently observed AEs with GLP-1 RAs are nausea, vomiting, and diarrhea (Ostergaard et al. 2016; Frandsen et al. 2016; Madsbad et al. 2011; Madsbad 2016, 2009; Bettge et al. 2017). They are usually described as gastrointestinal, although they are more likely to be due to interaction with receptors in the central nervous system. Importantly, they can be reduced by slow up-titration

of the dose. In most patients these adverse events are transient, and less than 5% of the patients discontinued treatment in clinical trials (except for taspoglutide), although higher rates may be seen in clinical practice (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016; Bettge et al. 2017; Cefalu et al. 2014).

Concerns have been raised with respect to potential pancreatic side effects associated with GLP-1 RAs (Egan et al. 2014), but in the four cardiovascular endpoint studies so far available, there was no increased risk of pancreatitis or pancreas cancer (Marso et al. 2016a, b; Pfeffer et al. 2015; Holman et al. 2017). In 2014, FDA and European Medicines Agency (EMA) reviewed studies with over 28.000 patients and concluded that no evidence existed suggesting a causal association between use of GLP-1 RAs and pancreatitis or pancreas cancer (Egan et al. 2014). There are limited published data on the effects of GLP-1 RAs on pancreatic enzymes. In a 26-week study, serum amylase and lipase levels increased with lixisenatide and liraglutide, more so with liraglutide (Nauck et al. 2016b). Notably, the increased enzyme levels are not associated with or predict subsequent development of acute pancreatitis (which occurs with increased frequency in patients with T2DM).

Thyroid

In rodent models, GLP-1 RAs stimulate the release of calcitonin and during long-term exposure may lead to hyperplasia and adenoma formation and with high doses cancer (Bjerre et al. 2010). In humans the C-cells express a very low number of GLP-1 receptors compared to rodents, and the GLP-1 RAs do not stimulate release of calcitonin (Bjerre et al. 2010; Hegedus et al. 2011). In addition, there is no evidence of a causal relationship between GLP-1 RAs and thyroid tumors in humans (Hegedus et al. 2011). In the phase 3 trials and the cardiovascular endpoint trials, there were no cases of medullary thyroid carcinoma in the exposed patients (Hegedus et al. 2011). In a resent post-hoc anlayses of the LEADER trial There was no evidence of a difference in calcitonin concentrations between the liraglutide and placebo groups, and no C-cell malignancies occurred in the liraglutide group (Hegedus L et al. Diabetes Care 2018; 41: 620–22). Nevertheless, GLP-1 RAs should not be used in patients with a personal or familiar history of medullary thyroid carcinoma.

Injection Site Reactions

It is difficult to compare injection site reactions across all studies because of differences in methods of reporting outcomes. Overall, once-weekly GLP-1 RAs appear to be associated with higher incidences of injection site reaction than exenatide twice daily (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016, 2009) or liraglutide once daily (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016, 2009).

The exception appears to be dulaglutide once weekly, which in AWARD-6 was associated with low rates (<1%) of injection site reactions, comparable to those observed with liraglutide (Dungan et al. 2014). In SUSTAIN 7 injection site reaction did not differ between semaglutide and dulaglutide (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31, Epub ahead of print).

Immunogenicity

As GLP-1 RAs are peptides, antibody formation could potentially occur, which might result in injection site reactions, loss of efficacy, and anaphylaxis. Antibody formation has been reported in several head-to-head trials, as discussed in relation to the individual GLP-1 RAs (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016), but has not resulted in major immune reactions.

Cardiovascular Effects and Endpoint Studies with GLP-1 RAs

Endothelial Function

Multiple studies have demonstrated a role for GLP-1 to regulate endothelial function, but it remains unclear whether direct or indirect activation of GLP-1 receptors in blood vessels is involved in the regulation of blood flow (Drucker 2016; Pujadas and Drucker 2016). It also remains uncertain whether endothelial cells within blood vessels express the GLP-1 receptor.

Blood Pressure and Heart Rate

Improvements in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) preceding weight loss have been reported in clinical trials of GLP-1 RAs (Gallwitz et al. 2010). Indeed, a meta-analysis of trials involving exenatide OW, exenatide BID, or liraglutide found that these treatments all significantly decreased SBP by −1.79 and −2.39 mmHg compared with placebo and active controls, respectively (Robinson et al. 2013). There was also a trend toward decreased DBP with GLP-1 RAs, but the reductions did not reach statistical significance. In the clinical studies, office blood pressure measurements have been used. However, in four studies using 24 h ambulatory blood glucose monitoring in subjects with type 2 diabetes and in one study in type 1 diabetic patients, treatment with liraglutide did not show any significant blood pressure-lowering effect (Dejgaard et al. 2017; Kumarathurai et al. 2017b). The mechanisms linking GLP-1 RA treatment to reduction in blood pressure are poorly understood, but potential mechanisms include vasodilation and natriuresis or unknown neurohormonal mechanisms.

Increases in resting heart rate and cardiac output have been reported with GLP-1 RAs (Drucker 2016; Pujadas and Drucker 2016). Although the underlying

physiological mechanisms have not yet been defined, activation of the GLP-1 receptors in the sinoatrial node and changes in the activity of the autonomic nervous system by enhancing sympathetic and reducing parasympathetic nervous system activity have been proposed to be responsible for the changes (Pyke et al. 2014; Smits et al. 2016). Another potential explanation for the increased heart rate could include a reflex mechanism compensating for vasodilation and lowering of BP (Asmar et al. 2015). A meta-analysis of studies involving exenatide OW, exenatide BID, or liraglutide found that these treatments increased heart rate by 1.86 beats/min (bpm) versus placebo and by 1.90 bpm versus active comparators (Robinson et al. 2013). However, in studies involving 24-h heart rate registration much greater increases may be seen (Kumarathurai et al. 2017a). The acute effect of GLP-1 on BP is an increase, consistent with the increase in heart rate and a consequent increase in cardiac output (Asmar et al. 2015). Postmarketing reports have not demonstrated any prolongation of QT interval during treatment with a GLP-1 RA, but the GLP-1 RA liraglutide has been shown to reduce heart rate variability in conjunction with a decrease in parasympathetic activity suggesting that liraglutide may affect sympatho-vagal balance (Kumarathurai et al. 2017a).

Lipids and Cardiovascular Risk Markers

Effects on lipids have in most trials, including the large outcome trial with liraglutide, been minimal (Marso et al. 2016a, b; Pfeffer et al. 2015; Holman et al. 2017; Pujadas and Drucker 2016), but an interesting study with liraglutide 1.8 mg suggested that liraglutide treatment in patients with T2DM significantly and markedly reduces postprandial excursions of triglyceride and apolipoprotein B48 after a fat-rich meal, independently of gastric emptying (Hermansen et al. 2013). Cardiovascular risk markers as PAI-1, B-type natriuretic peptide, ICAM-1, monocyte chemoattractant protein-1 (MCP-1), and CRP levels were reduced during treatment with GLP-1 RAs (Pujadas and Drucker 2016). Whether GLP-1 RAs exert clinically relevant effects on platelets and coagulation is not yet known (Drucker 2016; Pujadas and Drucker 2016).

Cardioprotection

Animal studies have demonstrated cardioprotection in experimental models of myocardial infarction, reviewed in Drucker (2016) and Pujadas and Drucker (2016). Administration of a GLP-1 RA reduced infarct size, improved survival, and preserved left ventricular function in mice (Drucker 2016; Pujadas and Drucker 2016). However, the precise mechanisms explaining the results remain unclear, especially since it has been a challenge to find GLP-1 receptors on the myocytes and endothelial cells in the heart (Drucker 2016; Pujadas and Drucker 2016). Studies in mice suggested that the primary metabolite of GLP-1 ($9\text{-}36NH_2$) may mediate some of the effects via hypothetical non-GLP-1 receptor-mediated cardioprotective

actions (Ban et al. 2008). Support for a similar mechanism to operate in humans is lacking. In a pilot study, 72 h infusion of native GLP-1 in human with acute myocardial infarction and impaired ejection fraction (< 40%) improved ventricular function (Nikolaidis et al. 2004). In an acute study, intravenous infusions of exenatide were demonstrated to be cardioprotective as an adjunct to primary percutaneous coronary intervention in patients with ST-segment-elevation myocardial infarction (STEMI) (Lonborg et al. 2012). The infusion was commenced 15 min before intervention and maintained for 6 h after the procedure. The exenatide treatment was associated with a 30% decrease in final infarct size, if treatment could be instituted within 130 min after the attack, whereas there was no cardioprotective effect in patients with longer system delay (Lonborg et al. 2012). In another study in patients with STEMI, liraglutide administered 30 min before PCI and continued for 7 days lowered level of troponin T and improved ventricular function (Chen et al. 2015).

Heart Failure

However, 48 h of native GLP-1 infusion in patients with NYHA class II-III failed to show any benefit (Halbirk et al. 2010). Albiglutide versus placebo over 12 weeks did not improve ventricular function in patients with EF < 40% (Lepore et al. 2016). In a double-blind, placebo-controlled, randomized clinical trial, patients with established heart failure and reduced LVEF (median LVEF of 25%) were randomized to liraglutide 1.8 mg (n = 154) or placebo (n = 146) for 180 days (Margulies et al. 2016). Compared with placebo, liraglutide had no significant effect on the number of deaths or rehospitalizations for heart failure (Margulies et al. 2016). In two other studies, no effect of liraglutide treatment for 12–24 weeks on left ventricular function was reported in patients with or without type 2 diabetes and stable heart failure (Jorsal et al. 2017; Kumarathurai et al. 2016). On the contrary a tendency to an increased frequency of adverse cardiovascular events was reported in Jorsal et al. (2017). These findings do not support the use of liraglutide for the treatment of heart failure. Notably, in the ELIXA, LEADER, SUSTAIN-6, and EXSCEL studies, there was no increased risk of hospitalization because of heart failure in the treated groups (Marso et al. 2016a, b; Pfeffer et al. 2015; Holman et al. 2017).

Cardiovascular Endpoint Studies

In 2008 the FDA recommended that all drugs investigated for diabetes should be evaluated for cardiovascular effects in large and long-term trials. The short-acting GLP-1 RA, lixisenatide, was assessed with respect to cardiovascular outcome versus placebo (the ELIXA trial) in 6068 patients with type 2 diabetes, who had had a recent acute coronary event (Pfeffer et al. 2015). The primary endpoint of cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina did not differ between the lixisenatide and placebo groups after a median of 25 months

follow-up (Pfeffer et al. 2015). There was no difference in heart failure or death. Lixisenatide treatment was not associated with a higher risk of hypoglycemia, pancreatitis, or pancreatic neoplasm (Pfeffer et al. 2015).

The safety of liraglutide was evaluated in the LEADER trial, a double-blinded trial, including 9340 type 2 patients with a mean follow-up of 3.8 years (Marso et al. 2016a). Patients included had cardiovascular or kidney disease or were at high risk for developing cardiovascular disease. The primary endpoint: death, nonfatal myocardial infarction, and nonfatal stroke, was reduced by 13% ($p < 0.001$), and mortality from cardiovascular disease was reduced by 22% ($p = 0.007$) and death of any course by 15%, ($p = 0.002$) (Marso et al. 2016a). The incidence of pancreatitis was nonsignificantly *lower* in the liraglutide group. There was a significant reduction in severe hypoglycemic episodes in the liraglutide group. Subgroup analysis showed benefit with liraglutide in patients with eGFR less than 60 ml/min/1.72 m^2 compared with those with higher eGFRs; the benefit also appeared greater in patients with established CVD compared to patients with risk factors for CVD (Marso et al. 2016a). In total, 66 patients had to be treated for 3 years to prevent 1 primary endpoint and 98 patients to prevent 1 death from any cause (Marso et al. 2016a). A secondary analysis shows that liraglutide resulted in lower rates of development and progression of diabetic kidney disease than placebo (Mann et al. 2017). This result was driven primarily by the new onset of persistent macroalbuminuria, which occurred in fewer participants in the liraglutide group than in the placebo group (HR, 0.74).

In SUSTAIN-6, semaglutide for once-weekly administration was evaluated in 2 doses (0.5 mg or 1.0 mg) versus placebo in 3297 type 2 diabetic patients (Marso et al. 2016b). At baseline 83% had established cardiovascular disease, chronic kidney disease, or both. The primary outcome: cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, was after 104 weeks follow-up reduced by 26% ($p < 0.001$), nonfatal myocardial infarction by 26% ($p = 0.12$), and nonfatal stroke by 39% ($p = 0.04$) (Marso et al. 2016b). Rates of death, including cardiovascular death, were similar in the two groups. In total 45 patients would need to be treated for 2 years to prevent 1 primary endpoint. Revascularization surgery rates were also greatly reduced by semaglutide compared with placebo. Semaglutide is in late phase 3 development and will probably enter the market within the next few years.

In the EXSCEL trial, 14,752 patients (of whom 10,782 (73%) had previous cardiovascular disease) were randomized to treatment with exenatide once weekly or placebo as add-on to usual therapy and followed for a median of 3.2 years (Holman et al. 2017). The primary composite endpoint: death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke, occurred in 839 versus 905 participants in the exenatide and placebo groups (HR, 0.91, $P = 0.06$ for superiority). Once-weekly exenatide did not increase risk of hospitalization for heart failure. Cardiovascular death did not differ between exenatide and placebo groups, but exenatide reduced total mortality by 14%, which was statistically significant (Holman et al. 2017). The incidence of acute pancreatitis, pancreas cancer, and thyroid carcinoma did not differ between the groups.

Taken together, the short-acting lixisenatide had a neutral effect on cardiovascular risk, whereas liraglutide and semaglutide showed a benefit. Liraglutide reduced

cardiovascular and total mortality. Nonfatal stroke was reduced with semaglutide but not with liraglutide. Exenatide OW also reduced cardiovascular risk and total mortality significantly. The GLP-1 receptor agonist had no effect on heart failure in the four endpoint trials.

The mechanism of the cardiovascular benefits is unknown. One suggestion is that GLP-1 RAs have some beneficial effects on the progression of atherosclerosis by reducing the plaque burden or increasing plaque stability. The GLP-1 RAs also have beneficial effects on blood pressure, weight and postprandial lipids, low-grade inflammation, and on the myocardium, but these effects do not readily explain the findings. Therefore, the mechanisms of action of GLP-1 RAs have yet to be elucidated. It is also debated how the four randomized studies with lixisenatide, liraglutide, semaglutide, and exenatide could generate so different results (Marso et al. 2016a, b; Pfeffer et al. 2015; Holman et al. 2017)? First, the patients in ELIXA appeared to be at higher risk for further cardiovascular disease progression than the patients in LEADER, SUSTAIN-6, and EXSCEL, meaning that even a significant beneficial effect of lixisenatide might not be able to influence the very high event rate in this group of patients. In addition, lixisenatide has a short half-life and covers only about 8 h of the day, while liraglutide, semaglutide, and exenatide QW cover all 24 h. In addition, the duration of the trials differ significantly. Moreover, the molecules are quite different and differ in their receptor signalling capacity and biological effects (see Table 1). It is an ongoing discussion, whether the CV benefit of the long-acting human GLP-1 RAs in LEADER and SUSTAIN-6 versus EXSCEL trials can be considered a class effect or might be specific for the liraglutide/semaglutide and exenatide molecules. Additional cardiovascular endpoint studies will be published in the future with FREEDOM-CVO (ITCA 659) and REWIND (dulaglutide). At any rate liraglutide, semaglutide, and exenatide QW have demonstrated beneficial effects on cardiovascular events and mortality in type 2 patients with cardiovascular disease or at high risk for a future cardiovascular events, which is important for the treatment of these patients.

Head-To-Head Comparisons of GLP-1 RAs

Currently, six glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are approved for treating type 2 diabetes (Madsbad 2016), and ten published phase 3 head-to-head trials of 24–30 weeks duration have compared the efficacy and safety of these six GLP-1 RAs and taspoglutide (Buse et al. 2009, 2013; Drucker et al. 2008; Rosenstock et al. 2013a, b; Nauck et al. 2016b; Pratley et al. 2014; Dungan et al. 2014; Blevins et al. 2011; Ji et al. 2013). Exenatide BID and liraglutide were the most common comparators (Figs. 4 and 5).

In general, baseline characteristics were similar across trial populations and between treatment groups (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016). The mean age of participants ranged from 55 to 61 years across the studies, with mean duration of diabetes ranging from 6 to 9 years (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016). Mean baseline HbA1c levels were in the range

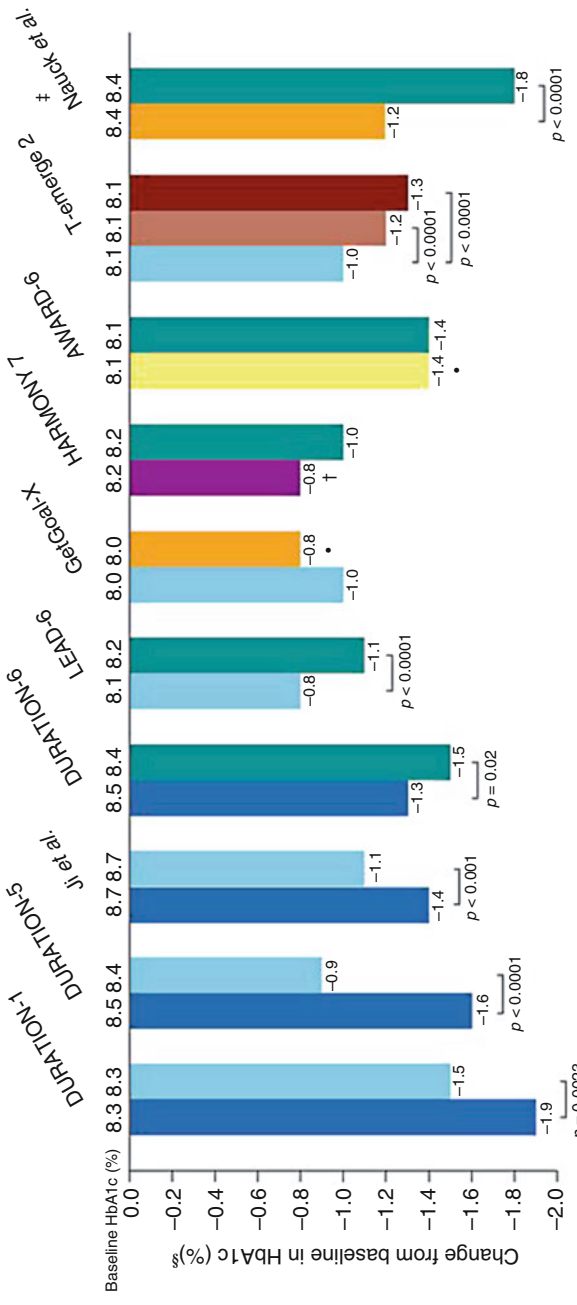


Fig. 4 Reduction in HbA1c in published phase III randomized head-to-head studies of glucagon-like peptide-1 receptor agonists in type 2 diabetes. Duration of studies 24–30 weeks. (Has been modified from reference Lau et al. 2015)

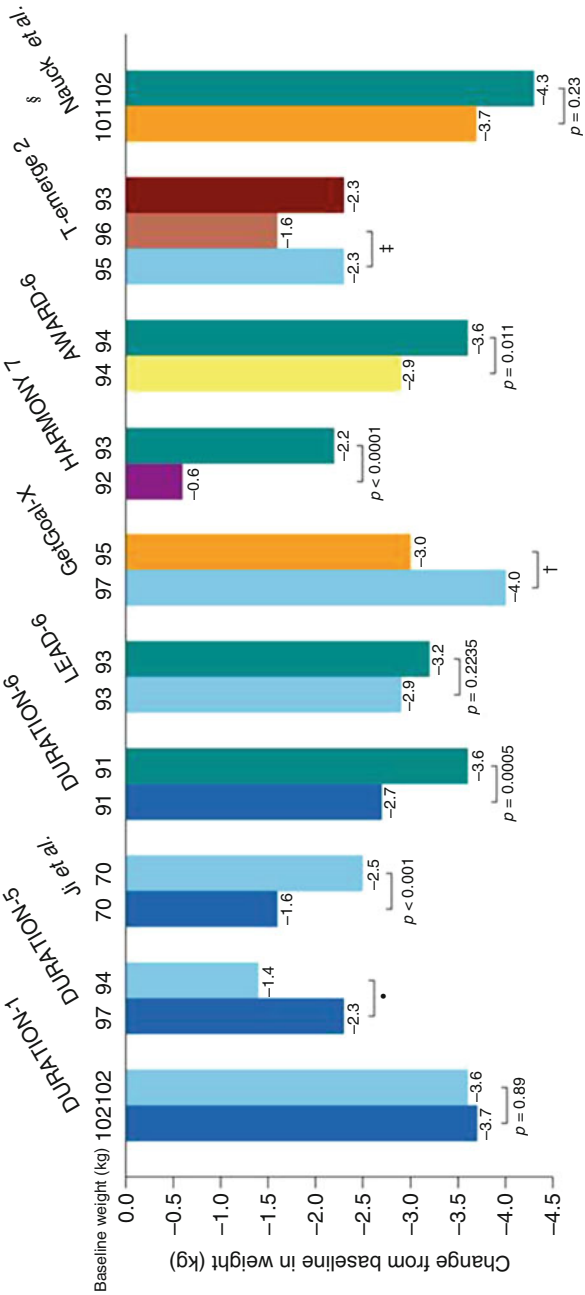


Fig. 5 Reduction in weight in published phase III randomized head-to-head studies of glucagon-like peptide-1 receptor agonists in type 2 diabetes. Duration of studies 24–30 weeks. (Has been modified from reference Lau et al. 2015)

of 8.0 (64 mmol/mol) to 8.7% (72 mmol/mol) across the studies (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016). Fasting glucose concentrations ranged from 9.1 to 9.9 mmol/l, and mean baseline body weight was consistently in the range 91–102 kg (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016).

Effect on Glycemic Control

All of the phase 3 trials examined changes in HbA1c as the primary endpoint (Ostergaard et al. 2016; Madsbad et al. 2011; Madsbad 2016). All trials were associated with notable reductions in HbA1c, although liraglutide led to greater decreases than exenatide formulations, lixisenatide, and albiglutide (Fig. 4; Ostergaard et al. 2016; Madsbad 2016). HbA1c reductions did not differ between liraglutide and dulaglutide after 26 weeks (Fig. 4; Dungan et al. 2014). Exenatide once weekly produced more consistent and significantly greater reductions in HbA1c than exenatide twice daily (Drucker et al. 2008; Blevins et al. 2011; Ji et al. 2013). In the T-emerge 2 study, taspoglutide at 10 and 20 mg led to greater reductions in HbA1c than exenatide 10 μg BID (Rosenstock et al. 2013b). In the SUSTAIN-7 trial, semaglutide and dulaglutide as add-on to metformin during 40 weeks were compared (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print). Patients in the 0.5 mg semaglutide group had a reduction in HbA1c of 1.5% against a 1.1% reduction in the 0.75 mg dulaglutide group. Additionally, 1.0 mg of semaglutide reduced HbA1c with 1.8% compared with a decrease of 1.4% among patients treated with 1.5 mg dulaglutide. The reductions in HbA1c thus ranged from 0.3 to 1.9% (Fig. 4; Buse et al. 2009, 2013; Drucker et al. 2008; Rosenstock et al. 2013a, b; Nauck et al. 2016b; Pratley et al. 2014; Dungan et al. 2014; Blevins et al. 2011; Ji et al. 2013).

The postprandial glucose excursions and fasting plasma glucose were also assessed in many of these trials. As expected, based on the delayed gastric emptying seen with the short-acting GLP-1 RAs, exenatide BID and lixisenatide demonstrated greater effects on postprandial glucose excursions than the longer-acting GLP-1 RAs, but this improvement was seen mainly after the meal following the injection, whereas the longer-acting compounds reduced plasma glucose throughout the 24-h period studied (Fig. 2; Ostergaard et al. 2016; Madsbad 2016; Nauck et al. 2016b). Hence the longer acting resulted in greater improvements in HbA1c compared with the short-acting GLP-1 RAs (Ostergaard et al. 2016; Madsbad 2016; Dungan et al. 2014).

Effect on Weight

Liraglutide was associated with weight reductions similar to those with exenatide BID (3.2 and 2.9 kg, respectively) but greater than those with exenatide OW, albiglutide, and dulaglutide (Fig. 5; Buse et al. 2009, 2013; Drucker et al. 2008; Rosenstock et al. 2013a, b; Nauck et al. 2016b; Pratley et al. 2014; Dungan et al. 2014; Blevins et al. 2011; Ji et al. 2013). Compared to lixisenatide, the weight loss

tended to be greater with liraglutide (4.3 kg vs. 3.7 kg) (Nauck et al. 2016b). Weight loss was not significantly different between the two exenatide formulations. In the T-emerge 2 study, exenatide BID showed a greater (nonsignificant) reduction in weight than taspoglutide 10 mg OW but showed no difference in weight loss compared with taspoglutide 20 mg OW (Rosenstock et al. 2013b). Exenatide BID was associated with greater (nonsignificant) weight loss than lixisenatide. In SUSTAIN-7 those on 0.5 mg semaglutide lost on average 4.6 kg of body weight compared to 2.3 kg with 0.75 mg dulaglutide. The higher doses led to losses of 6.5 kg and 3.0 kg, respectively (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print). Taken together, all GLP-1 receptor agonists have weight-reduction effect (Fig. 5), and the head-to-head studies revealed significantly greater reduction in weight with liraglutide than the once-weekly GLP-1 RAs (except for semaglutide). The explanation for the different magnitude of weight loss is a matter of contention. It is unclear whether the large molecules albiglutide and dulaglutide hinder transport across the blood-brain barrier or through fenestrated capillaries around hypothalamus (Secher et al. 2014). Alternatively, suboptimal dosing of the once-weekly GLP-1 RAs may play a role; this may also explain the differences in reduction in HbA1c level of the GLP-1 RAs.

Effect on Blood Pressure

Head-to-head trials have not revealed significant differences in effects on blood pressure (BP) among different GLP-1 RAs (Madsbad 2016). However, in the extension phases of DURATION-1 and LEAD-6, which continued to 52 weeks, participants switching from exenatide BID to either exenatide OW or liraglutide experienced further reductions in SBP (–3.8 mmHg in both studies) (Ostergaard et al. 2016; Buse et al. 2010a, b). In a 26-week study, changes in blood pressure did not differ between lixisenatide and liraglutide (Nauck et al. 2016b). Effect on blood pressure did not differ between semaglutide and dulaglutide in SUSTAIN 7 trial (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print).

Heart Rate

Head-to-head trials have suggested that heart rate increases may be smaller with exenatide twice daily than exenatide once weekly or liraglutide (Buse et al. 2009; Drucker et al. 2008). Dulaglutide is also associated with an increase in heart rate, of similar magnitude, to that with liraglutide (Dungan et al. 2014). Albiglutide did not appear to be associated with clinically relevant increases in heart rate (Pratley et al. 2014).

Since heart rate was mostly estimated during daytime, 24-h monitoring was needed to understand the different effects of the short- and long-acting GLP-1 RAs on heart rate. In a 8-week study, liraglutide doses increased the mean ± SE 24-h heart rate from baseline by 9 ± 1 bpm versus 3 ± 1 bpm with lixisenatide

($P < 0.001$) (Meier et al. 2015). Greater heart rate increases at week 8 with liraglutide were observed at nighttime, while heart rate increases with lixisenatide were greatest during the day (Meier et al. 2015). In another comparison between lixisenatide and liraglutide, the increase in pulse was 2.5 bpm with liraglutide, while a decrease by 1.1 bpm was reported with lixisenatide after 26 weeks (Seino et al. 2012). Note that heart rate primarily has been estimated during daytime in an ambulatory consultation; 24 h monitoring is needed to understand the different effects of short- and long-acting GLP-1 receptor agonists on heart rate. In one study, liraglutide increased 24-h heart rate from baseline by 9 bpm versus 3 bpm with lixisenatide (Meier et al. 2015). Greater heart rate increases with liraglutide were observed at nighttime (Meier et al. 2015). Increase in heart rate was greater with semaglutide 1.0 mg compared with dulaglutide 1.5 mg (4.0 vs 2.4 bpm), while the increase did not differ with the lowest doses (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print).

Gastrointestinal Adverse Effects

The most frequently observed AEs with GLP-1 RAs were gastrointestinal disorders, particularly nausea, vomiting, and diarrhea; nausea, however, occurred less frequently with exenatide OW and albiglutide than exenatide BID and liraglutide (Buse et al. 2009, 2013; Drucker et al. 2008; Rosenstock et al. 2013a, b; Nauck et al. 2016b; Pratley et al. 2014; Dungan et al. 2014; Blevins et al. 2011; Ji et al. 2013). However, by far the highest rates of nausea were observed with taspoglutide: 53% and 59% with 10 and 20 mg OW, respectively, compared with 35% among participants treated with exenatide BID (Rosenstock et al. 2013b). In a meta-analysis of 32 phase 3 clinical trials with GLP-1 RAs, it was concluded that presence of a background treatment with metformin was associated with more nausea and vomiting (Bettge et al. 2017). Compared to exenatide BID, there was less nausea and diarrhea with lixisenatide (Bettge et al. 2017). Compared to liraglutide, there was a similar risk associated with dulaglutide and less with exenatide QW and albiglutide (Bettge et al. 2017). Long-acting GLP-1 RAs were associated with less nausea and vomiting but with more diarrhea than short-acting agents (Bettge et al. 2017). More premature discontinuation, mostly due to gastrointestinal adverse events was observed with semaglutide compared with dulaglutide in SUSTAIN 7 (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print).

Injection Site Reactions

Both exenatide formulations and albiglutide may be associated with higher incidences of injection site reactions than liraglutide and dulaglutide (Buse et al. 2009, 2013; Drucker et al. 2008; Rosenstock et al. 2013a, b; Nauck et al. 2016b; Pratley et al. 2014; Dungan et al. 2014; Blevins et al. 2011; Ji et al. 2013), but the once-weekly GLP-1 RAs appear to be associated with higher incidences of injection site reaction

than exenatide BID or liraglutide OD. The exception appears to be dulaglutide OW, in AWARD-6, which was associated with low rates (<1%) of injection site reactions, comparable to those observed with liraglutide (Dungan et al. 2014). Injection site reaction did not differ between semaglutide and dulaglutide in SUSTAIN 7 (Pratley RE et al. Lancet Diabetes Endocrinol 2018, Jan 31 Epub ahead of print).

Antibodies

In head-to-head studies, anti-exenatide antibodies were more common – and titers were higher – with exenatide OW than with exenatide BID (Drucker et al. 2008; Blevins et al. 2011; Ji et al. 2013). However, reductions in HbA1c were still significant in participants with or without antibodies, and the presence of antibodies did not correlate with reported rates of AEs (Drucker et al. 2008; Blevins et al. 2011; Ji et al. 2013).

Antibody formation has also been reported in liraglutide clinical trials, although a meta-analysis of the LEAD studies found lower immunogenicity with liraglutide than with exenatide BID and no effect of antibodies on glycemic efficacy with liraglutide (Buse et al. 2011). Development of antibodies was reported in 56–60% of participants (undergoing different treatment regimens) treated with 20 μg lixisenatide OD (Rosenstock et al. 2013a; Nauck et al. 2016b). In another study, antibodies were found in 43% and 71% of participants treated with 10 μg lixisenatide once daily and 20 μg twice daily, respectively (Ratner et al. 2010; Fonseca et al. 2012). No notable differences were reported in terms of safety and efficacy between antibody-positive and negative participants (Ratner et al. 2010; Fonseca et al. 2012).

Antibody formation occurred relatively rarely in phase 3 trials of dulaglutide and albiglutide, but no comparison could be made with liraglutide in these studies, as anti-liraglutide antibodies were not assessed (Pratley et al. 2014; Dungan et al. 2014).

Finally, in the T-emerge 2 study, anti-taspoglutide antibodies were detected in 49% of participants. In this trial, levels of systemic allergic reactions were also considered to be unacceptably high (6% of participants in each of the taspoglutide groups) (Rosenstock et al. 2013b).

The immunogenicity reported in the trials of exenatide, lixisenatide, and liraglutide appeared to have little impact on the efficacy and safety of these GLP-1 RAs.

Fixed-Ratio Combination Therapy with a GLP-1 Receptor Agonist and Basal Insulin

The complex pathophysiology of type 2 diabetes (T2D) is associated with insulin resistance, obesity, and declining beta-cell function (DeFronzo 2009). It also includes defects in glucagon secretion and a severely impaired incretin effect of glucagon-like peptide-1 (GLP-1) and glucose-independent polypeptide (GIP) in response to a meal (Holst et al. 2011). Consequently, combination therapies,

addressing several of the underlying abnormalities and effectively reducing glycated hemoglobin A1c (HbA1c), mitigating weight gain or inducing weight loss, combined with an impact on the comorbidities associated with T2D are of high interest (Balena et al. 2013; Eng et al. 2014). On this background, in the real world, combination therapy with basal insulin and a GLP-1 RA has turned out to be very popular, and a recent meta-analysis suggests that basal insulin in combination with a GLP-1 RA results in superior glycemic control with no increase in hypoglycemic episodes or weight gain, as compared with basal insulin alone (Eng et al. 2014).

IDegLira

A fixed-ratio combination of the basal insulin degludec and the GLP-1 RA, liraglutide (IDegLira, 50 units degludec/1.8 mg liraglutide), has been approved under the brand name Xultophy 100/3.6 as a once-daily injection for the treatment of type 2 diabetes (T2D). Insulin degludec is an ultra-long-acting basal insulin analogue with a half-life of approximately 25 h and a duration of action of about 41 h compared with about 12 h for insulin glargine (Haahr and Heise 2014). Steady state is obtained within 2–3 days of treatment (Haahr and Heise 2014). Insulin degludec has demonstrated lower intraindividual glycemic variability and lower risk of hypoglycemia as compared to the shorter-acting insulin glargine (Haahr and Heise 2014; Vora et al. 2014). IDegLira has been approved by FDA to improve glycemic control in patients inadequately controlled on basal insulin in doses of up to 50 units/day or a GLP-1 RA. IDegLira has also been approved for use in Europe for the treatment of type 2 diabetes in combination with oral glucose-lowering agents alone or combined with basal insulin.

IDegLira is available in prefilled pen injectors which contain 3 ml, equivalent to 300 units of insulin degludec and 10.8 mg of liraglutide. Each dose step is 1 unit of insulin degludec and 0.036 mg of liraglutide. Administration is by once-daily injection, independent of meal intake or time of day (although it should ideally be injected at the same time each day). The maximal dose is 50 steps corresponding to 50 units of insulin degludec and 1.8 mg of liraglutide. The recommended starting dose in patients treated with OADs alone is 10 dose steps (10 units/0.36 mg), whereas the starting dose is 16 dose steps (16 units/0.6 mg) in patients that were already treated with a GLP-1 RA or insulin.

IDegLira has been investigated in eight 26-week randomized trials (the DUAL™ program) (Buse et al. 2014; Gough et al. 2014; Lingvay et al. 2016; Linjawi et al. 2017; Rodbard et al. 2017). IDegLira reduces HbA1c more than monotherapy with a GLP-1 RA (liraglutide) or insulin (degludec or glargine) despite the fact that IDegLira and insulin degludec or insulin glargine were titrated to similar FPG levels, indicating that the further improvement also includes better PPG control effected by the liraglutide component of the combination therapy (Buse et al. 2014; Gough et al. 2014, 2015; Lingvay et al. 2016; Linjawi et al. 2017; Rodbard et al. 2017). Furthermore, combination therapy leads to weight loss, or a stable body weight, with no increase in hypoglycemia despite the lower HbA1c in the IDegLira group

(Buse et al. 2014; Gough et al. 2014, 2015; Lingvay et al. 2016; Linjawi et al. 2017; Rodbard et al. 2017). These results were found in both insulin-naïve and insulin-treated patients with T2D, independent of diabetes duration and baseline HbA1c (Buse et al. 2014; Gough et al. 2014, 2015; Lingvay et al. 2016; Linjawi et al. 2017; Rodbard et al. 2017). In DUAL VII, IDegLira was compared with basal-bolus insulin therapy (glargine plus insulin aspart up to four times daily) in patients uncontrolled on metformin and insulin glargine. After 26 weeks the HbA1c did not differ between groups (6.7%), but body weight decreased with IDegLira (−0.9 kg) and increased with basal-bolus therapy (+2.6 kg); the rate of hypoglycemia was eightfold lower with IDegLira (Billings LK et al. Diabetes Care 2018; Feb, Epub ahead of print). Daily dose of insulin was 40 units in the IDegLira group compared with 84 units (basal 52 units and bolus 32 units) in the patients treated with basal-bolus. Notably, these results were obtained by one injection and one fasting blood glucose measurement in the IDegLira group compared with multiple injections and multiple blood glucose measurements in the basal-bolus group.

In the DUAL studies, rates of adverse events did not differ between treatment groups; however, gastrointestinal side effects were fewer with IDegLira compared with liraglutide treatment alone, which was titrated using the recommended dose escalation of 0.6 mg per week until a dose of 1.8 mg (Gough et al. 2014, 2015) (although, because of insulin titration, only a maximum dose of 1.4 mg was actually achieved in the large DUAL 1 study). IDegLira may be of more limited value in patient populations that are challenging to manage, e.g., patients with HbA1c values >10%, BMI >40 kg/m^2, or patients receiving insulin doses in excess of 50 U/day. This has to be taken into consideration when switching people treated with large doses of insulin; potentially this may lead to a transient worsening of glycemic control.

iGlarLixi

The combination of once-daily insulin glargine and the short-acting GLP-1 RA lixisenatide (iGlarLixi, formerly known as LixiLan) is recommended to be injected about 1 h before the largest meal (Aroda et al. 2016; Rosenstock et al. 2016a, b). iGlarLixi has been approved by FDA to improve glycemic control in patients inadequately controlled on basal insulin up to 60 units/day or a GLP-1 RA alone. iGlarLixi has also been approved for use in Europe for the treatment of type 2 diabetes in combination with oral glucose-lowering agents alone or combined with basal insulin. iGlarLixi will be available as prefilled pens for dosing of 10–40 units of glargine with 5–20 mcg of lixisenatide or 30–60 units of glargine with 10–20 mcg of lixisenatide. Each dose step contains 1 unit of glargine and 0.33 mcg of lixisenatide.

In the phase 3 program, iGlarLixi demonstrated better HbA1c reduction versus insulin glargine in patients treated with metformin and reduced weight by approximately 1 kg versus an increase of 0.5 kg for those who received glargine (Rosenstock et al. 2016a). Final dose of insulin was 36 versus 39 units and risk of

hypoglycemia did not differ between groups. In the second trial including patients inadequately controlled on basal insulin and with up to 2 oral glucose-lowering agents, iGlarLixi compared to glargine demonstrated better reduction in HbA1c over 30 weeks and a greater proportion of patients (55% vs. 30%) achieving target of <7%. Body weight was reduced with 0.7 kg versus + 0.7 kg, respectively, while final dose of insulin (47 units) and risk of hypoglycemia did not differ between groups (Aroda et al. 2016). In a third study including patients on metformin with or without a second oral glucose-lowering agents, iGlarLixi reduced HbA1c significantly more than with either glargine or lixisenatide alone (−1.6%, −1.3%, and −0.9%, respectively) without increased risk of hypoglycemia with iGlarLixi compared with glargine alone (Rosenstock et al. 2016b). Insulin dose was 39.8 units in the iGlarLixi group and 40.3 units in the glargine group. Changes in body weight were −0.3 kg, +1.1 kg, and −2.3 kg, respectively.

Lixisenatide has a more pronounced effect on PPG excursions in relation to the meal following the injection when compared with liraglutide. Thus, addition of a short-acting GLP-1 RA may be a more convenient intensification strategy compared to adding mealtime rapid-acting insulin, because the fixed dosing does not require adjustments for meal size and carbohydrate content. A limitation with iGlarLixi may be the short duration of lixisenatide and the once-daily administration given 30–60 min before one of the main meals, while IDegLira can be taken independent of meals. A head-to-head comparison with LixiLan and IDegLira will be of interest.

A possible drawback of the combination therapies is the fixed-dose principle, which reduces the flexibility to adjust insulin and GLP-1 RA treatment in an individualized manner. In patients where weight loss is a major aim, a more optimal treatment may be to titrate liraglutide to the maximal dose of 1.8 mg and then add basal insulin (Balena et al. 2013). Thereby less insulin is probably also needed. Nevertheless, the fixed-ratio combinations have been shown to be very effective at lowering glycemia while being associated with lower rate of hypoglycemia and weight gain compared to basal insulin alone and lower gastrointestinal side effects than liraglutide and lixisenatide alone.

GLP-1 RA: Place in Therapy of Type 2 Diabetes

Metformin is considered the first-line therapy in the treatment of type 2 diabetes, but ADA, EASD, and AACE recommend GLP-1 receptor agonists as potential add-on therapy for patients with uncontrolled type 2 diabetes (Garber et al. 2016; Inzucchi et al. 2015). They also may be considered as monotherapy for patients with metformin intolerance. GLP-1 RAs are becoming increasingly popular for the treatment of T2DM because of their excellent HbA1c lowering, positive effects on weight loss, low risk of hypoglycemia, and influence on cardiovascular risk factors (Ostergaard et al. 2016). Their superiority to OADs has been demonstrated in most studies, with greater reductions in both HbA1c and weight (Ostergaard et al. 2016). The fear of injections will, in some patients, remain a barrier for the use of GLP-1 RAs, but this problem can be reduced by using the long-acting agonists for once-weekly injection

or ITCA 650 infusion pump. Compared with insulin, GLP-1 RAs are much easier to initiate, with less need for dose titration and blood glucose monitoring (Ostergaard et al. 2016). Furthermore, in patients in whom weight loss is advisable, GLP-1 RA treatment could be an option instead of insulin, which for many patients is associated with weight gain (Inzucchi et al. 2015). The addition of a GLP-1 RA to insulin treatment has been demonstrated to improve glycemic control, help patients lose weight, and lower the need for insulin (Eng et al. 2014). The results from the degludec/liraglutide and the glargine/lixisenatide fixed combination studies support the concept that initiation of insulin therapy is best carried out as an insulin/GLP-1 combination rather than insulin alone. None of the GLP-1 RAs are marketed for use with basal-bolus regimens.

Recently the ADA and some other national guidelines have suggested that in patients with type 2 diabetes and established CVD treatment should begin with lifestyle management and metformin and subsequently in patients not achieving glycemic goal an agent proven to reduce major cardiovascular events and cardiovascular mortality (liraglutide and empagliflozin) is recommended (ADA Position Statement. Diabetes Care 2018; 41 (Suppl 1): S73–S85).

GLP-1 RA use in clinical practice should be customized for individual patients, based on the clinical profile and patient preferences. Survey data on patient preferences have revealed that efficacy (lowering of HbA1c) is the most important attribute influencing patient preference, followed by absence of nausea and hypoglycemia and simplicity of dosing schedule (Polster et al. 2010). In a survey more patients were likely to prefer once-weekly injection because of greater convenience (Polonsky et al. 2011).

The GLP-1 RAs are generally well-tolerated. The main side effects are gastrointestinal, i.e., nausea and vomiting, which often are transient and can be partly avoided by slowly up-titrating the dose (Ostergaard et al. 2016; Gough et al. 2015). The GLP-1 RAs are not recommended for people with impaired kidney function (estimated glomerular filtration rate (eGFR) <30 ml/min) or for elderly people with reduced appetite and food intake. At present, no clear evidence of a causal relationship between GLP-1 RAs and pancreatitis and pancreatic cancer exists (Ostergaard et al. 2016; Marso et al. 2016a, b; Pfeffer et al. 2015).

The major drawback of GLP-1 RAs is the higher cost compared with that of other antidiabetic agents.

Treatment of Type 1 Diabetic Patients with GLP-1 Receptor Agonists

Type 1 diabetes (T1D) is characterized by severely impaired or absent or minimal insulin secretion (Madsbad 1983). Even the most rapid-acting insulin analogues peak too late when given with meals to match the postprandial glucose absorption resulting in large postprandial glucose excursions. Intensive insulin treatment is associated with weight gain, and about 50% of persons with T1D are overweight in economically developed countries (Conway et al. 2010). In theory, treatment

regimens in T1D may be improved by combining a GLP-1 RA with insulin. Accordingly, acute infusions of native GLP-1 in C-peptide-negative patients with type 1 diabetes resulted in inhibition of gastric emptying, as well as reduction of glucagon levels, which seems to explain the glucose-regulating effect of GLP-1 during a meal, whereas in patients with residual beta-cell function, enhancement of the endogenous insulin secretion is probably also of importance (Kielgast et al. 2011).

Results from open-label short and small clinical trials indicate that GLP-1 RA treatment induces weight loss and reduces insulin requirements, with either improved or unaltered glycemic control, reviewed in Frandsen et al. (2016). In most of the trials, liraglutide has been used (Dejgaard et al. 2016a).

In the first placebo-controlled trial in normal weight type 1 patients with liraglutide 1.2 mg once daily, there was no effect on HbA1c or glycemic variation compared with placebo (Frandsen et al. 2015). Changes in body weight were −3.13 and +1.12 kg with liraglutide and placebo, respectively. The bolus insulin dose decreased in liraglutide-treated patients and did not change with placebo treatment (−4.0 ± 1.3 vs. 0.0 ± 1.0 IU), and systolic blood pressure decreased compared with placebo (between-group difference 3.21 mmHg) (Frandsen et al. 2015). The incidence of hypoglycemia did not differ between groups. Liraglutide does not compromise glycemic recovery, gastric emptying rate, or counterregulatory hormone responses in T1D during hypoglycemia (Frandsen et al. 2017).

In the second trial with obese type 1 patients, HbA1c and glycemic variability did not differ between liraglutide 1.8 mg and placebo after 24 weeks of treatment, but the number of hypoglycemic events was reduced with liraglutide (Dejgaard et al. 2016b). Both bolus insulin (difference −5·8 IU) and body weight (difference −6·8 kg) decreased with liraglutide treatment compared with placebo. Heart rate increased with liraglutide, with a difference between groups of 7·5 bpm (Dejgaard et al. 2016b). Daytime heart rate increased by 3.7 and nighttime heart rate by 7.5 pbm (Dejgaard et al. 2017).

In the randomized, double-blind, placebo-controlled trial ADJUNCT ONE™, liraglutide 0·6 mg, 1·2 mg, 1·8 mg, and placebo as adjunct to insulin treatment were investigated in 1,398 persons with T1D for 52 weeks (Mathieu et al. 2016). From a mean baseline HbA1c of around 8·2%, those treated with 1·2 mg and 1·8 mg showed a numerically greater improvement in HbA1c of around 0·5% compared with 0·3% for placebo (Mathieu et al. 2016). From a baseline body weight of 86 kg, persons treated with 1·2 mg and 1·8 mg achieved a statistically significantly greater weight loss between 3 kg and 4 kg, whereas the placebo groups experienced a weight gain of around 1 kg (Mathieu et al. 2016). The rates of severe hypoglycemia appeared numerically, but not statistically, lower for all doses of liraglutide compared with placebo. A statistically higher rate of confirmed symptomatic hypoglycemia was observed among persons treated with liraglutide 1·2 mg and 1·8 mg compared with those treated with placebo (Mathieu et al. 2016).

In the ADJUNCT TWO™ trial, 835 participants were enrolled in a 26-week, double-blind, placebo-controlled trial and assigned to liraglutide 0·6 mg, 1·2 mg, 1·8 mg, and placebo (Ahren et al. 2016). Maximum insulin dose was fixed for all

treatment arms. From a baseline HbA1c of about 8·1%, the groups treated with liraglutide showed statistically significantly improvements of HbA1c by 0·2% and 0·3% compared with unaltered glycemic control in the placebo-treated group (Ahren et al. 2016). Additionally, the total insulin dose was reduced with liraglutide compared with placebo after 26 weeks. From a baseline body weight of 84 kg, the weight loss in the liraglutide groups was 1–5 kg, whereas the weight was stable in placebo-treated patients (Ahren et al. 2016). A higher rate of symptomatic hypoglycemia was observed among persons treated with liraglutide 1·2 mg (but not with the higher dose) compared with placebo treatment. The incidence of severe hypoglycemia and nocturnal hypoglycemia did not differ between groups (Ahren et al. 2016). Notable, in C-peptide positive patients, liraglutide reduced HbA1c by 0.77% and 0.69% for the 1.8 mg and 1.2 mg doses, respectively (Ahren et al. 2016).

Lastly, efficacy of liraglutide 1.8 mg has also been evaluated in inadequately controlled (HbA1c 8.2%) insulin pump-treated type 1 patients (ADA 2017 abstract OR 71). After 26 weeks the reduction in HbA1c was −0.6% in the liraglutide group, while an increase of 0.2% was observed in the placebo group (between groups, $p < 0.001$), without increased risk of hypoglycemia. Doses of insulin were unchanged in both groups. Body weight was reduced with −7.3 kg in the groups treated with liraglutide and −0.6 kg in the placebo group.

Thus GLP-1 RAs (at least liraglutide) reduce body weight and insulin dose with improved or unaltered glycemic control, without increased risk of hypoglycemia (Frandsen et al. 2016). The effects on HbA1c are conflicting with small, uncontrolled studies showing the most positive findings (Frandsen et al. 2016). In the randomized, placebo-controlled studies, no effect on HbA1c and glucose variability was reported compared with placebo treatment (Frandsen et al. 2016). One area of interest is treatment with a GLP-1 RA from time of diagnosis with the aim to improve and prolong the remission phase, the first years after diagnosis. From animal and in vitro human models, there is evidence that GLP-1 RAs preserve beta cells from destruction as reviewed in Kielgast et al. (2009), which has initiated ongoing trials in new-onset T1D. Whether treatment with a GLP-1 RA has a future in C-peptide-negative T1D is questionable if the primary indication is to improve glycemic control, especially when taking cost into account.

GLP-1 RAs a New Option for Treatment of Obesity

The exact mechanism by which GLP-1 exerts its anorectic effects is a matter of controversy, but both peripheral and brain GLP-1 receptors seem to be involved (Secher et al. 2014; Madsbad 2014). Since albumin-conjugated GLP-1 which presumably does not cross the blood-brain barrier still reduces food intake, one would assume that a peripheral action on vagal afferent neurons could be involved (Madsbad 2016). On the other hand, it is possible that the reduced weight loss obtained with the large molecules, albiglutide and dulaglutide, compared with liraglutide, can be explained by less direct activation of the GLP-1 receptors in the hypothalamic areas and brain stem. Indeed, compared with liraglutide, the larger

molecular sizes of albiglutide and dulaglutide may hinder transport across the blood-brain barrier or through fenestrated capillaries at the area of hypothalamus (Secher et al. 2014; Madsbad 2014). Liraglutide has been reported to directly stimulate pro-opiomelanocortin (POMC) neurons and inhibit neuropeptide-Y and Agouti-related peptide neurons in the hypothalamus resulting in appetite suppression (Secher et al. 2014). The weight-reducing effect may also be explained by attenuation of the decrease in the levels of the anorexigenic hormone, leptin, which accompanies weight loss (Iepsen et al. 2015). GLP-1 RAs do not influence energy expenditure in humans (Harder et al. 2004). Alternatively, however, it is possible that the once-weekly GLP-1 RAs have been suboptimally dosed (with respect to weight loss); in fact, this may also explain the differences with respect to reduction of HbA1c (Madsbad et al. 2011; Madsbad 2016).

Obesity is known as a risk factor for several common diseases including cardiovascular disease, type 2 diabetes, cancers, and osteoarthritis, and obesity is associated with reduced quality of life (Guh et al. 2009). Obesity guidelines mention the use of pharmacological therapy as a possible adjunctive therapy to diet, exercise, and behavior modification in certain patients (Jensen et al. 2014). Weight loss medications can be consider in adults, who have a BMI of 30 kg/m2 or higher or in patients with a BMI of 27 kg/m2 and having at least one overweight-related comorbid condition, e.g., hypertension, dyslipidemia, and type 2 diabetes (Jensen et al. 2014). The response should be evaluated after 3 months treatment, and if weight loss is less than 5%, the treatment should be stopped.

Liraglutide 3.0 mg has been developed for treatment of obesity and was approved in the USA in 2014 and in Europe in 2015. In a dose-finding study of 1.2, 1.8, 2.4, and 3.0 mg doses, it became clear that 3.0 mg was the most effective dose for inducing weight loss (4.8, 5.5, 6.3, and 7.2 kg, respectively) (Astrup et al. 2009).

In the SCALE-Obesity and Prediabetes study with a duration of 56 weeks, 3731 subjects were included, 2285 of whom had prediabetes, a baseline weight of about 106 kg, and BMI about 38 kg/m2 (Pi-Sunyer et al. 2015). The prediabetes group was followed for 160 weeks to assess the ability of liraglutide to delay the onset of progression to type 2 diabetes. After 56 weeks the weight loss was 8 kg in the liraglutide group compared with 2.6 kg in the placebo group. In total 63.2% versus 27.1% and 33.1% versus 10.6% lost more than 5% or 10% of body weight in the liraglutide and placebo group, respectively (Pi-Sunyer et al. 2015). In total 9.9% and 3.8% withdrew due to adverse events in the liraglutide and placebo groups (Pi-Sunyer et al. 2015). Liraglutide was associated with a reduced progression to prediabetes (7.2% vs. 20.7%) and increased reversal of prediabetes (69.2% vs. 32.7%).

In a follow-up after 160 weeks, 2254 participants with prediabetes 1128 had completed the study (Roux et al. 2017). At week 160 2% in the liraglutide group compared with 6% in the placebo group were diagnosed with diabetes while on treatment. The time to onset of diabetes was 2.7 times longer with liraglutide than with placebo, corresponding to a hazard ratio of 0.21 (Roux et al. 2017). Weight loss was greater with liraglutide (−6.1% vs. −1.9%) than with placebo. In a post hoc analysis for individuals who lose >5% body weight after 16 weeks of treatment, the

weight loss was 12% after 1 year and 8.6% at week 160, where 37% and 19% have a weight loss of 10% and 15%, respectively. Thus, early responders achieved greater long-term weight loss than non-responders, and fewer early responders developed type 2 diabetes, and more regressed to normoglycemia while on treatment.

In the SCALE-Diabetes study, 846 patients with type 2 diabetes and a BMI of 37 kg/m2 (106 kg) were followed for 56 weeks and randomized to liraglutide 3.0 mg, 1.8 mg, or placebo (Davies et al. 2015). The weight loss was 6.4 kg, 5.0 kg, and 2.0 kg, corresponding to 54.3%, 40.4%, and 21.4% obtaining a weight loss of at least 5% and 25.2%, 15.9%, and 6.7% obtaining a weight loss of more than 10%. The reduction in HbA1c was −1.3%, −1.1%, and −0.3%, respectively (Davies et al. 2015).

In the SCALE Maintenance trial, overweight subjects (BMI 38 kg/m2, 106 kg) undertook a 1200–1400 kcal/day diet (Wadden et al. 2015) and entered into the trial if they managed to lose >5% in body weight after 12 weeks. The mean weight loss at randomization was 6%. After 1 year, 6.2% more weight loss was obtained with liraglutide than those on placebo (−0.2%). An extra weight loss of >10% was obtained by 26.1% versus 6.3%, respectively (Wadden et al. 2015).

In a randomized study, liraglutide induced a significant reduction in obstructive sleep apnea compared with placebo (Blackman et al. 2016). Recently a 5-week trial that assesses safety and tolerability of 3.0 mg liraglutide in obese adolescents aged 12–17 years concluded that dosing regimen for adults may be appropriate for use in adolescents (Danne et al. 2017).

In the SCALE studies, small reductions in LDL, VLDL, triglycerides, and systolic and diastolic blood pressure were reported.

The adverse events to liraglutide 3.0 mg were the usual gastrointestinal events including nausea, diarrhea, constipation, and vomiting but also gallbladder disease. Nausea peaked after 4 weeks of treatment and subsided thereafter. Liraglutide was associated with a small increase in heart rate, between 2 and 4 beats/min. Gallbladder-related complications including cholelithiasis and cholecystitis were more common in the liraglutide arms. No incidence of medullary thyroid cancer was reported. Hypoglycemia was not a problem during the studies (Pi-Sunyer et al. 2015; Roux et al. 2017; Davies et al. 2015).

Liraglutide 3.0 mg has demonstrated superior weight loss compared with orlistat, but has not been compared with other weight loss medications (Astrup et al. 2012). Tolerability may be problematic for some patients, although it partly can be avoided by a slower up-titration than 0.6 mg per week. Liraglutide 3.0 mg is expensive, and it is currently priced much higher than other pharmacological agents for the treatment of obesity. Patients who may particularly benefit from liraglutide 3.0 mg are those who have prediabetes due to its glucose-lowering effect and potential to delay the progression from prediabetes to diabetes (Pi-Sunyer et al. 2015; Roux et al. 2017). The appropriate duration of treatment is not established, but obesity is a chronic disease, and the weight loss effects are only sustained as long as liraglutide is taken.

In a phase 2 study obese patients treated with semaglutide 0.4 mg daily lost up to 13.8% of body weight after 52 weeks compared with 2.3% in the placebo group. In the semaglutide group 65% lost more than 10% of their body weight (O'Neil PM et

al. Presented at ENDO March 2018 (OR12)). Semaglutide is at present in phase 3 development as an obesity drug.

Future Perspective of GLP-1 RAs

In light of the relatively narrow therapeutic window defined by the balance between efficacy and gastrointestinal side effects, future subcutaneously administered long-acting GLP-1 RAs will probably not provide much better efficacy than observed with, for instance, semaglutide. In the future, the oral administration of GLP-1 or GLP-1 enhancers may be of interest to increase the treatment compliance (Meier and Nauck 2015). Oral GLP-1 for once-daily administration is in phase 3 development, and in a dose-finding study, 40 mg of oral semaglutide reduced HbA1c with 1.9% from a baseline of 7.9%. Reduction in weight was 6.9 kg (Davies M et al. JAMA 2017; 318: 1460–70).

In rodents, co-agonism at the GLP-1 and glucagon receptors has been investigated to achieve weight loss. Rats treated with co-agonism achieved superior weight loss without induction of hyperglycemia compared to rats treated with GLP-1 receptor-selective agonists (Day et al. 2012).

Peptide YY (PYY) is secreted from intestinal L cells and reduces appetite; co-agonism stimulating both the GLP-1 and PYY receptor pathways reduced food intake in humans in experimental studies (De et al. 2011; Tan et al. 2014). Similarly co-agonism with several hormones may open up new treatments for T2DM and obesity. Recently, the first 12 weeks clinical study in patients with type 2 diabetes was published showing sustained effects of a dual GIP/GLP-1 receptor agonist (Frias et al. 2017). The agonist significantly improved glycemic control and reduced body weight, total cholesterol, and leptin compared with placebo.

Reference

Agerso H, Jensen LB, Elbrond B, Rolan P, Zdravkovic M. The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. Diabetologia. 2002;45:195–202.

Ahren B, Hirsch IB, Pieber TR, et al. Efficacy and safety of Liraglutide added to capped insulin treatment in subjects with type 1 diabetes: the ADJUNCT TWO randomized trial. Diabetes Care. 2016;39:1693–701.

Ahren B, Masmiquel L, Kumar H, et al. Efficacy and safety of once-weekly semaglutide versus once-daily sitagliptin as an add-on to metformin, thiazolidinediones, or both, in patients with type 2 diabetes (SUSTAIN 2): a 56-week, double-blind, phase 3a, randomised trial. Lancet Diabetes Endocrinol. 2017;5:341–54.

Aroda VR, Rosenstock J, Wysham C, et al. Efficacy and safety of LixiLan, a titratable fixed-ratio combination of insulin glargine plus Lixisenatide in type 2 diabetes inadequately controlled on basal insulin and metformin: the LixiLan-L randomized trial. Diabetes Care. 2016;39:1972–80.

Aroda VR, Bain SC, Cariou B, et al. Efficacy and safety of once-weekly semaglutide versus once-daily insulin glargine as add-on to metformin (with or without sulfonylureas) in insulin-naive

patients with type 2 diabetes (SUSTAIN 4): a randomised, open-label, parallel-group, multicentre, multinational, phase 3a trial. Lancet Diabetes Endocrinol. 2017;5:355–66.
Asmar A, Simonsen L, Asmar M, et al. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. Am J Physiol Endocrinol Metab. 2015;308:E641–9.
Astrup A, Rossner S, Van GL, et al. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. Lancet. 2009;374:1606–16.
Astrup A, Carraro R, Finer N, et al. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. Int J Obes (Lond). 2012;36:843–54.
Athauda D, Maclagan K, Skene SS, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet. 2017;390:1664.
Balena R, Hensley IE, Miller S, Barnett AH. Combination therapy with GLP-1 receptor agonists and basal insulin: a systematic review of the literature. Diabetes Obes Metab. 2013;15:485–502.
Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation. 2008;117:2340–50.
Barrington P, Chien JY, Showalter HD, et al. A 5-week study of the pharmacokinetics and pharmacodynamics of LY2189265, a novel, long-acting glucagon-like peptide-1 analogue, in patients with type 2 diabetes. Diabetes Obes Metab. 2011;13:426–33.
Bettge K, Kahle M, Abd El Aziz MS, Meier JJ, Nauck MA. Occurrence of nausea, vomiting and diarrhoea reported as adverse events in clinical trials studying glucagon-like peptide-1 receptor agonists: a systematic analysis of published clinical trials. Diabetes Obes Metab. 2017;19:336–47.
Bjerre KL, Madsen LW, Andersen S, et al. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology. 2010;151:1473–86.
Blackman A, Foster GD, Zammit G, et al. Effect of liraglutide 3.0 mg in individuals with obesity and moderate or severe obstructive sleep apnea: the SCALE Sleep Apnea randomized clinical trial. Int J Obes (Lond). 2016;40:1310–9.
Blair HA, Keating GM. Albiglutide: a review of its use in patients with type 2 diabetes mellitus. Drugs. 2015;75:651–63.
Blevins T, Pullman J, Malloy J, et al. DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. J Clin Endocrinol Metab. 2011;96:1301–10.
Bolli GB, Munteanu M, Dotsenko S, et al. Efficacy and safety of lixisenatide once daily vs. placebo in people with type 2 diabetes insufficiently controlled on metformin (GetGoal-F1). Diabet Med. 2014;31:176–84.
Brunton S, Davidson JA. Exenatide once weekly: a review of pharmacology and treatment considerations in type 2 diabetes. Clin Ther. 2016;38:582–94.
Bunck MC, Corner A, Eliasson B, et al. Effects of exenatide on measures of beta-cell function after 3 years in metformin-treated patients with type 2 diabetes. Diabetes Care. 2011;34:2041–7.
Buse JB, Rosenstock J, Sesti G, et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet. 2009;374:39–47.
Buse JB, Drucker DJ, Taylor KL, et al. DURATION-1: exenatide once weekly produces sustained glycemic control and weight loss over 52 weeks. Diabetes Care. 2010a;33:1255–61.
Buse JB, Sesti G, Schmidt WE, et al. Switching to once-daily liraglutide from twice-daily exenatide further improves glycemic control in patients with type 2 diabetes using oral agents. Diabetes Care. 2010b;33:1300–3.
Buse JB, Garber A, Rosenstock J, et al. Liraglutide treatment is associated with a low frequency and magnitude of antibody formation with no apparent impact on glycemic response or increased frequency of adverse events: results from the Liraglutide Effect and Action in Diabetes (LEAD) trials. J Clin Endocrinol Metab. 2011;96:1695–702.

Buse JB, Nauck M, Forst T, et al. Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. Lancet. 2013;381:117–24.

Buse JB, Vilsboll T, Thurman J, et al. Contribution of liraglutide in the fixed-ratio combination of insulin degludec and liraglutide (IDegLira). Diabetes Care. 2014;37:2926–33.

Calsolaro V, Edison P. Novel GLP-1 (Glucagon-like Peptide-1) analogues and insulin in the treatment for Alzheimer's disease and other neurodegenerative diseases. CNS Drugs. 2015;29:1023–39.

Cefalu WT, Buse JB, Del PS, et al. Beyond metformin: safety considerations in the decision-making process for selecting a second medication for type 2 diabetes management: reflections from a diabetes care editors' expert forum. Diabetes Care. 2014;37:2647–59.

Cervera A, Wajcberg E, Sriwijitkamol A, et al. Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab. 2008;294: E846–52.

Chen WR, Hu SY, Chen YD, et al. Effects of liraglutide on left ventricular function in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Am Heart J. 2015;170:845–54.

Conway B, Miller RG, Costacou T, et al. Temporal patterns in overweight and obesity in type 1 diabetes. Diabet Med. 2010;27:398–404.

Danne T, Biester T, Kapitzke K, et al. Liraglutide in an adolescent population with obesity: a randomized, double-blind, placebo-controlled 5-week trial to assess safety, tolerability, and pharmacokinetics of Liraglutide in adolescents aged 12–17 years. J Pediatr. 2017;181:146–53.

Davies MJ, Bergenstal R, Bode B, et al. Efficacy of Liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. JAMA. 2015;314:687–99.

Day JW, Gelfanov V, Smiley D, et al. Optimization of co-agonism at GLP-1 and glucagon receptors to safely maximize weight reduction in DIO-rodents. Biopolymers. 2012;98:443–50.

De SA, Salem V, Long CJ, et al. The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. Cell Metab. 2011;14:700–6.

Deacon CF, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ. Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. Diabetologia. 1998;41:271–8.

DeFronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58:773–95.

Dejgaard TF, Frandsen CS, Holst JJ, Madsbad S. Liraglutide for treating type 1 diabetes. Expert Opin Biol Ther. 2016a;16:579–90.

Dejgaard TF, Frandsen CS, Hansen TS, et al. Efficacy and safety of liraglutide for overweight adult patients with type 1 diabetes and insufficient glycaemic control (Lira-1): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2016b;4:221–32.

Dejgaard TF, Johansen NB, Frandsen CS, et al. Effects of liraglutide on cardiovascular risk factors in patients with type 1 diabetes. Diabetes Obes Metab. 2017;19:734–8.

Diamant M, Van GL, Guerci B, et al. Exenatide once weekly versus insulin glargine for type 2 diabetes (DURATION-3): 3-year results of an open-label randomised trial. Lancet Diabetes Endocrinol. 2014;2:464–73.

Dong JZ, Shen Y, Zhang J, Tsomaia N, Mierke DF, Taylor JE. Discovery and characterization of taspoglutide, a novel analogue of human glucagon-like peptide-1, engineered for sustained therapeutic activity in type 2 diabetes. Diabetes Obes Metab. 2011;13:19–25.

Drucker DJ. The cardiovascular biology of glucagon-like Peptide-1. Cell Metab. 2016;24:15–30.

Drucker DJ, Buse JB, Taylor K, et al. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. Lancet. 2008;372: 1240–50.

Dungan KM, Povedano ST, Forst T, et al. Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. Lancet. 2014;384:1349–57.

Egan AG, Blind E, Dunder K, et al. Pancreatic safety of incretin-based drugs – FDA and EMA assessment. N Engl J Med. 2014;370:794–7.

Eng C, Kramer CK, Zinman B, Retnakaran R. Glucagon-like peptide-1 receptor agonist and basal insulin combination treatment for the management of type 2 diabetes: a systematic review and meta-analysis. Lancet. 2014;384:2228–34.

Fonseca VA, Alvarado-Ruiz R, Raccah D, Boka G, Miossec P, Gerich JE. Efficacy and safety of the once-daily GLP-1 receptor agonist lixisenatide in monotherapy: a randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes (GetGoal-Mono). Diabetes Care. 2012;35:1225–31.

Frandsen CS, Dejgaard TF, Holst JJ, Andersen HU, Thorsteinsson B, Madsbad S. Twelve-week treatment with Liraglutide as add-on to insulin in normal-weight patients with poorly controlled type 1 diabetes: a randomized, placebo-controlled, double-blind parallel study. Diabetes Care. 2015;38:2250–7.

Frandsen CS, Dejgaard TF, Madsbad S. Non-insulin drugs to treat hyperglycaemia in type 1 diabetes mellitus. Lancet Diabetes Endocrinol. 2016;4:766–80.

Frandsen CS, Dejgaard TF, Andersen HU, et al. Liraglutide as adjunct to insulin treatment in type 1 diabetes does not interfere with glycaemic recovery or gastric emptying rate during hypoglycaemia: a randomized, placebo-controlled, double-blind, parallel-group study. Diabetes Obes Metab. 2017;19:773–82.

Frias JP, Guja C, Hardy E, et al. Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy (DURATION-8): a 28 week, multicentre, double-blind, phase 3, randomised controlled trial. Lancet Diabetes Endocrinol. 2016;4:1004–16.

Frias JP, Bastyr EJ III, Vignati L, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. Cell Metab. 2017;26:343–52.

Gallwitz B, Ropeter T, Morys-Wortmann C, Mentlein R, Siegel EG, Schmidt WE. GLP-1-analogues resistant to degradation by dipeptidyl-peptidase IV in vitro. Regul Pept. 2000;86:103–11.

Gallwitz B, Vaag A, Falahati A, Madsbad S. Adding liraglutide to oral antidiabetic drug therapy: onset of treatment effects over time. Int J Clin Pract. 2010;64:267–76.

Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus statement by the american association of clinical endocrinologists and american college of endocrinology on the comprehensive type 2 diabetes management algorithm – 2016 executive summary. Endocr Pract. 2016;22:84–113.

Glaesner W, Vick AM, Millican R, et al. Engineering and characterization of the long-acting glucagon-like peptide-1 analogue LY2189265, an Fc fusion protein. Diabetes Metab Res Rev. 2010;26:287–96.

Gough SC, Bode B, Woo V, et al. Efficacy and safety of a fixed-ratio combination of insulin degludec and liraglutide (IDegLira) compared with its components given alone: results of a phase 3, open-label, randomised, 26-week, treat-to-target trial in insulin-naive patients with type 2 diabetes. Lancet Diabetes Endocrinol. 2014;2:885–93.

Gough SC, Bode BW, Woo VC, et al. One-year efficacy and safety of a fixed combination of insulin degludec and liraglutide in patients with type 2 diabetes: results of a 26-week extension to a 26-week main trial. Diabetes Obes Metab. 2015;17:965–73.

Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health. 2009;9:88.

Haahr H, Heise T. A review of the pharmacological properties of insulin degludec and their clinical relevance. Clin Pharmacokinet. 2014;53:787–800.

Halbirk M, Norrelund H, Moller N, et al. Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in compensated chronic patients with heart failure. Am J Physiol Heart Circ Physiol. 2010;298:H1096–102.

Harder H, Nielsen L, Tu DT, Astrup A. The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. Diabetes Care. 2004;27:1915–21.

Harkavyi A, Abuirmeileh A, Lever R, Kingsbury AE, Biggs CS, Whitton PS. Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. J Neuroinflammation. 2008;5:19.

Hegedus L, Moses AC, Zdravkovic M, Le TT, Daniels GH. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. J Clin Endocrinol Metab. 2011;96:853–60.

Henry RR, Rosenstock J, Logan DK, Alessi TR, Luskey K, Baron MA. Randomized trial of continuous subcutaneous delivery of exenatide by ITCA 650 versus twice-daily exenatide injections in metformin-treated type 2 diabetes. Diabetes Care. 2013a;36:2559–65.

Henry RR, Logan D, Alessi T, Baron MA. A randomized, open-label, multicenter, 4-week study to evaluate the tolerability and pharmacokinetics of ITCA 650 in patients with type 2 diabetes. Clin Ther. 2013b;35:634–45.

Henry RR, Rosenstock J, Logan D, Alessi T, Luskey K, Baron MA. Continuous subcutaneous delivery of exenatide via ITCA 650 leads to sustained glycemic control and weight loss for 48 weeks in metformin-treated subjects with type 2 diabetes. J Diabetes Complicat. 2014;28:393–8.

Hermansen K, Baekdal TA, During M, et al. Liraglutide suppresses postprandial triglyceride and apolipoprotein B48 elevations after a fat-rich meal in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, cross-over trial. Diabetes Obes Metab. 2013;15:1040–8.

Holman RR, Bethel MA, Mentz RJ, et al. Effects of once-weekly Exenatide on cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2017;377:1228–39.

Holst JJ, Knop FK, Vilsboll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. Diabetes Care. 2011;34(Suppl 2):S251–7.

Iepsen EW, Lundgren J, Dirksen C, et al. Treatment with a GLP-1 receptor agonist diminishes the decrease in free plasma leptin during maintenance of weight loss. Int J Obes (Lond). 2015;39:834–41.

Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia. 2015;58:429–42.

Jelsing J, Vrang N, Hansen G, Raun K, Tang-Christensen M, Knudsen LB. Liraglutide: short-lived effect on gastric emptying – long lasting effects on body weight. Diabetes Obes Metab. 2012;14:531–8.

Jendle J, Grunberger G, Blevins T, Giorgino F, Hietpas RT, Botros FT. Efficacy and safety of dulaglutide in the treatment of type 2 diabetes: a comprehensive review of the dulaglutide clinical data focusing on the AWARD phase 3 clinical trial program. Diabetes Metab Res Rev. 2016;32:776–90.

Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. J Am Coll Cardiol. 2014;63:2985–3023.

Ji L, Onishi Y, Ahn CW, et al. Efficacy and safety of exenatide once-weekly vs exenatide twice-daily in Asian patients with type 2 diabetes mellitus. J Diabetes Investig. 2013;4:53–61.

Jorsal A, Kistorp C, Holmager P, et al. Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE)-a multicentre, double-blind, randomised, placebo-controlled trial. Eur J Heart Fail. 2017;19:69–77.

Kapitza C, Forst T, Coester HV, Poitiers F, Ruus P, Hincelin-Mery A. Pharmacodynamic characteristics of lixisenatide once daily versus liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. Diabetes Obes Metab. 2013;15:642–9.

Kapitza C, Nosek L, Jensen L, Hartvig H, Jensen CB, Flint A. Semaglutide, a once-weekly human GLP-1 analog, does not reduce the bioavailability of the combined oral contraceptive, ethinylestradiol/levonorgestrel. J Clin Pharmacol. 2015;55:497–504.

Kielgast U, Holst JJ, Madsbad S. Treatment of type 1 diabetic patients with glucagon-like peptide-1 (GLP-1) and GLP-1R agonists. Curr Diabetes Rev. 2009;5:266–75.

Kielgast U, Holst JJ, Madsbad S. Antidiabetic actions of endogenous and exogenous GLP-1 in type 1 diabetic patients with and without residual beta-cell function. Diabetes. 2011;60:1599–607.

Knudsen LB, Nielsen PF, Huusfeldt PO, et al. Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. J Med Chem. 2000;43: 1664–9.

Kolterman OG, Kim DD, Shen L, et al. Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. Am J Health Syst Pharm. 2005;62:173–81.

Kumarathurai P, Anholm C, Nielsen OW, et al. Effects of the glucagon-like peptide-1 receptor agonist liraglutide on systolic function in patients with coronary artery disease and type 2 diabetes: a randomized double-blind placebo-controlled crossover study. Cardiovasc Diabetol. 2016;15:105.

Kumarathurai P, Anholm C, Larsen BS, et al. Effects of Liraglutide on heart rate and heart rate variability: a randomized, double-blind, placebo-controlled crossover study. Diabetes Care. 2017a;40:117–24.

Kumarathurai P, Anholm C, Fabricius-Bjerre A, et al. Effects of the glucagon-like peptide-1 receptor agonist liraglutide on 24-h ambulatory blood pressure in patients with type 2 diabetes and stable coronary artery disease: a randomized, double-blind, placebo-controlled, crossover study. J Hypertens. 2017b;35:1070–8.

Lau J, Bloch P, Schaffer L, et al. Discovery of the once-weekly glucagon-like Peptide-1 (GLP-1) analogue Semaglutide. J Med Chem. 2015;58:7370–80.

le Roux CW, Astrup A, Fujioka K, et al. 3 years of liraglutide versus placebo for type 2 diabetes risk reduction and weight management in individuals with prediabetes: a randomised, double-blind trial. Lancet. 2017;389:1399–409.

Lepore JJ, Olson E, Demopoulos L, et al. Effects of the novel long-acting GLP-1 agonist, Albiglutide, on cardiac function, cardiac metabolism, and exercise capacity in patients with chronic heart failure and reduced ejection fraction. JACC Heart Fail. 2016;4:559–66.

Lingvay I, Perez MF, Garcia-Hernandez P, et al. Effect of insulin glargine up-titration vs insulin Degludec/Liraglutide on glycated hemoglobin levels in patients with uncontrolled type 2 diabetes: the DUAL V randomized clinical trial. JAMA. 2016;315:898–907.

Linjawi S, Bode BW, Chaykin LB, et al. The efficacy of IDegLira (Insulin Degludec/Liraglutide combination) in adults with type 2 diabetes inadequately controlled with a GLP-1 receptor agonist and oral therapy: DUAL III randomized clinical trial. Diabetes Ther. 2017;8:101–7.

Lonborg J, Kelbaek H, Vejlstrup N, et al. Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. Circ Cardiovasc Interv. 2012;5:288–95.

Madsbad S. Prevalence of residual B cell function and its metabolic consequences in type 1 (insulin-dependent) diabetes. Diabetologia. 1983;24:141–7.

Madsbad S. Exenatide and liraglutide: different approaches to develop GLP-1 receptor agonists (incretin mimetics) – preclinical and clinical results. Best Pract Res Clin Endocrinol Metab. 2009;23:463–77.

Madsbad S. The role of glucagon-like peptide-1 impairment in obesity and potential therapeutic implications. Diabetes Obes Metab. 2014;16:9–21.

Madsbad S. Review of head-to-head comparisons of glucagon-like peptide-1 receptor agonists. Diabetes Obes Metab. 2016;18:317–32.

Madsbad S, Kielgast U, Asmar M, Deacon CF, Torekov SS, Holst JJ. An overview of once-weekly glucagon-like peptide-1 receptor agonists – available efficacy and safety data and perspectives for the future. Diabetes Obes Metab. 2011;13:394–407.

Mann KV, Raskin P. Exenatide extended-release: a once weekly treatment for patients with type 2 diabetes. Diabetes Metab Syndr Obes. 2014;7:229–39.

Mann JFE, Orsted DD, Brown-Frandsen K, et al. Liraglutide and renal outcomes in type 2 diabetes. N Engl J Med. 2017;377:839–48.

Margulies KB, Hernandez AF, Redfield MM, et al. Effects of Liraglutide on clinical stability among patients with advanced heart failure and reduced ejection fraction: a randomized clinical trial. JAMA. 2016;316:500–8.
Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016a;375:311–22.
Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med. 2016b;375:1834.
Mathieu C, Zinman B, Hemmingsson JU, et al. Efficacy and safety of Liraglutide added to insulin treatment in type 1 diabetes: the ADJUNCT ONE treat-to-target randomized trial. Diabetes Care. 2016;39:1702–10.
McClean PL, Parthsarathy V, Faivre E, Holscher C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. J Neurosci. 2011;31: 6587–94.
Meier JJ. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. Nat Rev Endocrinol. 2012;8:728–42.
Meier JJ, Nauck MA. Incretin-based therapies: where will we be 50 years from now? Diabetologia. 2015;58:1745–50.
Meier JJ, Gallwitz B, Salmen S, et al. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab. 2003;88:2719–25.
Meier JJ, Nauck MA, Kranz D, et al. Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. Diabetes. 2004;53:654–62.
Meier JJ, Rosenstock J, Hincelin-Mery A, et al. Contrasting effects of Lixisenatide and Liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. Diabetes Care. 2015;38:1263–73.
Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993;36:741–4.
Nauck MA, Petrie JR, Sesti G, et al. A phase 2, randomized, dose-finding study of the novel once-weekly human GLP-1 analog, Semaglutide, compared with placebo and open-label Liraglutide in patients with type 2 diabetes. Diabetes Care. 2016a;39:231–41.
Nauck M, Rizzo M, Johnson A, Bosch-Traberg H, Madsen J, Cariou B. Once-daily Liraglutide versus Lixisenatide as add-on to metformin in type 2 diabetes: a 26-week randomized controlled clinical trial. Diabetes Care. 2016b;39:1501–9.
Nikolaidis LA, Mankad S, Sokos GG, et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation. 2004;109:962–5.
Ostergaard L, Frandsen CS, Madsbad S. Treatment potential of the GLP-1 receptor agonists in type 2 diabetes mellitus: a review. Expert Rev Clin Pharmacol. 2016;9:241–65.
Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in patients with type 2 diabetes and acute coronary syndrome. N Engl J Med. 2015;373:2247–57.
Pinget M, Goldenberg R, Niemoeller E, Muehlen-Bartmer I, Guo H, Aronson R. Efficacy and safety of lixisenatide once daily versus placebo in type 2 diabetes insufficiently controlled on pioglitazone (GetGoal-P). Diabetes Obes Metab. 2013;15:1000–7.
Pi-Sunyer X, Astrup A, Fujioka K, et al. A randomized, controlled trial of 3.0 mg of Liraglutide in weight management. N Engl J Med. 2015;373:11–22.
Polonsky WH, Fisher L, Hessler D, Bruhn D, Best JH. Patient perspectives on once-weekly medications for diabetes. Diabetes Obes Metab. 2011;13:144–9.
Polster M, Zanutto E, McDonald S, Conner C, Hammer M. A comparison of preferences for two GLP-1 products – liraglutide and exenatide – for the treatment of type 2 diabetes. J Med Econ. 2010;13:655–61.

Pratley RE, Nauck MA, Barnett AH, et al. Once-weekly albiglutide versus once-daily liraglutide in patients with type 2 diabetes inadequately controlled on oral drugs (HARMONY 7): a randomised, open-label, multicentre, non-inferiority phase 3 study. Lancet Diabetes Endocrinol. 2014;2:289–97.

Pujadas G, Drucker DJ. Vascular biology of glucagon receptor superfamily peptides: mechanistic and clinical relevance. Endocr Rev. 2016;37:554–83.

Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology. 2014;155:1280–90.

Ratner RE, Rosenstock J, Boka G. Dose-dependent effects of the once-daily GLP-1 receptor agonist lixisenatide in patients with Type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled trial. Diabet Med. 2010;27:1024–32.

Riddle MC, Aronson R, Home P, et al. Adding once-daily lixisenatide for type 2 diabetes inadequately controlled by established basal insulin: a 24-week, randomized, placebo-controlled comparison (GetGoal-L). Diabetes Care. 2013;36:2489–96.

Robinson LE, Holt TA, Rees K, Randeva HS, O'Hare JP. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. BMJ Open. 2013;3:e001986.

Rodbard HW, Bode BW, Harris SB, et al. Safety and efficacy of insulin degludec/liraglutide (IDegLira) added to sulphonylurea alone or to sulphonylurea and metformin in insulin-naive people with Type 2 diabetes: the DUAL IV trial. Diabet Med. 2017;34:189–96.

Rosenstock J, Raccah D, Koranyi L, et al. Efficacy and safety of lixisenatide once daily versus exenatide twice daily in type 2 diabetes inadequately controlled on metformin: a 24-week, randomized, open-label, active-controlled study (GetGoal-X). Diabetes Care. 2013a;36:2945–51.

Rosenstock J, Balas B, Charbonnel B, et al. The fate of taspoglutide, a weekly GLP-1 receptor agonist, versus twice-daily exenatide for type 2 diabetes: the T-emerge 2 trial. Diabetes Care. 2013b;36:498–504.

Rosenstock J, Hanefeld M, Shamanna P, et al. Beneficial effects of once-daily lixisenatide on overall and postprandial glycemic levels without significant excess of hypoglycemia in type 2 diabetes inadequately controlled on a sulfonylurea with or without metformin (GetGoal-S). J Diabetes Complicat. 2014a;28:386–92.

Rosenstock J, Fonseca VA, Gross JL, et al. Advancing basal insulin replacement in type 2 diabetes inadequately controlled with insulin glargine plus oral agents: a comparison of adding albiglutide, a weekly GLP-1 receptor agonist, versus thrice-daily prandial insulin lispro. Diabetes Care. 2014b;37:2317–25.

Rosenstock J, Diamant M, Aroda VR, et al. Efficacy and safety of LixiLan, a titratable fixed-ratio combination of Lixisenatide and insulin glargine, versus insulin glargine in type 2 diabetes inadequately controlled on metformin monotherapy: the LixiLan proof-of-concept randomized trial. Diabetes Care. 2016a;39:1579–86.

Rosenstock J, Aronson R, Grunberger G, et al. Benefits of LixiLan, a titratable fixed-ratio combination of insulin glargine plus Lixisenatide, versus insulin glargine and Lixisenatide Monocomponents in Type 2 diabetes inadequately controlled on oral agents: the LixiLan-O randomized trial. Diabetes Care. 2016b;39:2026–35.

Secher A, Jelsing J, Baquero AF, et al. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. J Clin Invest. 2014;124:4473–88.

Seino Y, Min KW, Niemoeller E, Takami A. Randomized, double-blind, placebo-controlled trial of the once-daily GLP-1 receptor agonist lixisenatide in Asian patients with type 2 diabetes insufficiently controlled on basal insulin with or without a sulfonylurea (GetGoal-L-Asia). Diabetes Obes Metab. 2012;14:910–7.

Smits MM, Muskiet MH, Tonneijck L, et al. Exenatide acutely increases heart rate in parallel with augmented sympathetic nervous system activation in healthy overweight males. Br J Clin Pharmacol. 2016;81:613–20.

Sorli C, Harashima SI, Tsoukas GM, et al. Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in patients with type 2 diabetes (SUSTAIN 1): a double-blind, randomised, placebo-controlled, parallel-group, multinational, multicentre phase 3a trial. Lancet Diabetes Endocrinol. 2017;5:251–60.

Tan TM, Salem V, Troke RC, et al. Combination of peptide YY3-36 with GLP-1(7-36) amide causes an increase in first-phase insulin secretion after IV glucose. J Clin Endocrinol Metab. 2014;99:E2317–24.

Teramoto S, Miyamoto N, Yatomi K, et al. Exendin-4, a glucagon-like peptide-1 receptor agonist, provides neuroprotection in mice transient focal cerebral ischemia. J Cereb Blood Flow Metab. 2011;31:1696–705.

Vora J, Christensen T, Rana A, Bain SC. Insulin degludec versus insulin glargine in type 1 and type 2 diabetes mellitus: a meta-analysis of endpoints in phase 3a trials. Diabetes Ther. 2014;5:435–46.

Wadden TA, Hollander P, Klein S, et al. Weight maintenance and additional weight loss with liraglutide after low-calorie-diet-induced weight loss: the SCALE Maintenance randomized study. Int J Obes (Lond). 2015;39:187.

Weissman PN, Carr MC, Ye J, et al. HARMONY 4: randomised clinical trial comparing once-weekly albiglutide and insulin glargine in patients with type 2 diabetes inadequately controlled with metformin with or without sulfonylurea. Diabetologia. 2014;57:2475–84.

Young MA, Wald JA, Matthews JE, et al. Clinical pharmacology of albiglutide, a GLP-1 receptor agonist. Postgrad Med. 2014;126:84–97.

Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 2002;359:824–30.

Insulin Treatment

21

Agostino Consoli

Contents

Abstract

Impaired insulin secretion, although to different degrees, is present in all forms of diabetes mellitus. Thus insulin treatment is de facto an endocrine substitutive therapy in diabetes and it could be indicated in all forms of the disease. Of course insulin treatment is mandatory in type 1 diabetes, but it is also strongly advised in gestational diabetes, and it is still one of the most rationale options in type 2 diabetes. Since the discovery of insulin and its first clinical use in 1923, insulin therapy is greatly evolved, and the use of the most modern insulin analogues in combination allows today achievement of much more physiological glucose profiles with less and less hypoglycemia risk in subjects with diabetes. These advancements are reviewed in this chapter, together with risks, side effects, and

A. Consoli (✉)
Dipartimento di Medicina e Scienze dell'Invecchiamento e, Centro Scienze dell'Invecchiamento e Medicina Traslazionale, Università d'Annunzio di Chieti-Pescara, Chieti Scalo, Italy
e-mail: consoli@unich.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_21

limitations that still make insulin treatment a complex therapy. Insulin treatment needs to be coupled with appropriate patient education and empowerment. Self blood glucose monitoring must be carefully implemented and diet and lifestyle need to be optimized. However, the final goal of the joined efforts of patients and caregivers is to adapt treatment to life and not life to treatment. Progresses in insulin treatment are taking us very close to this goal.

Keywords
Insulin treatment · Type 1 diabetes · Type 2 diabetes · Gestational diabetes · Insulin analogues · Basal insulin · Prandial insulin

Introduction

Insulin therapy and its evolution in time have marked milestones in the history of modern medicine. Insulin has been the first hormone to be extracted for clinical use, the first hormone to be measured by radio immune assay, the first hormone to be synthesized by recombinant DNA technique, and the first hormone to be modified in its amino acid sequence to obtain more favorable pharmacokinetic and pharmacodynamics characteristics.

The introduction of insulin therapy for clinical use almost 100 years ago (January 1923) has radically changed the prognosis of what is now known as type 1 diabetes mellitus, which from a "fatal disease" became a "curable disease." It is therefore fair to say that, since then, insulin therapy has saved several millions of lives. Sadly, however, in some parts of the world, access to insulin therapy is still limited, and, in these areas, diabetes mellitus takes a much heavier toll on health and life.

It has become increasingly clear that in the pathogenesis of all forms of diabetes, an alteration of insulin secretion of some sort is involved (Del Guerra et al. 2005). Insulin therapy is therefore a key element for treatment of most forms of diabetes mellitus: it is mandatory in type 1 diabetes, it is strongly advised in gestational diabetes, and, although alternative therapies might be possible, it is often the best treatment option in LADA and in type 2 diabetes (Brunton et al. 2005).

Nowadays, insulin therapy (as diabetes therapy in general) has three main goals: (1) prevent acute, potentially fatal, diabetes complications (ketoacidosis, hyperglycemic hyperosmolar syndrome, etc.), (2) prevent the onset of diabetes vascular complications and/or delay their progression should they occur, and (3) allow a good quality of life. While the first of these goals is widely achieved, to reach the second requires a careful individualization of care and a skillful interaction between caregivers and patients. As to the third goal, this is where technologic innovation has been and will be in the future most helpful.

Indication to insulin therapy, treatment regimens, and targets to achieve and maintain vary greatly among different types of diabetes: in this chapter, we will first provide an overview of the different types of insulin available and will then offer a brief description of their use in type 1, type 2, and gestational diabetes. Another chapter in this publication deals with insulin therapy by insulin pumps.

Insulin Preparations

In routine outpatient settings, insulin is at present administered through subcutaneous injection. Alternative routes of insulin administration (pulmonary, nasal, oral, rectal) have been explored, and some are still under investigation, but, so far, none has known a prolonged and efficacious clinical use (Shah et al. 2016). Administration of exogenous insulin should result in plasma insulin concentration mimicking at best physiological insulin profiles, with an appropriate basal level of insulin throughout the day to which, following food ingestion, peaks proportional to meals' carbohydrate content are superimposed. Being endogenous insulin secretion delivered into the portal vein, and precisely driven by the glucose-sensing mechanisms in the beta cells, subcutaneous exogenous insulin administration will never be able to replicate physiological endogenous insulin profiles. However, an increasing number of insulin preparations, mostly based on insulin analogues, are becoming available. Such preparations, which differ in pharmacokinetics and pharmacodynamics, allow to better approximate physiology, and they make it less difficult to achieve optimal glycemic control without an excessive risk of hypoglycemia.

In the old times, other than in "type," different insulin preparations used to differ in concentration, species source, and purity. Nowadays insulin is obtained by recombinant DNA, so that highly purified human insulin or human insulin with specific amino acid substitutions apt to favorably modify its pharmacokinetic profile can be used. As to the concentration, this is no more such a relevant clinical issue, since 100 U/ml has become the standard concentration and insulin-delivering devices (syringes and pens) are labeled accordingly to deliver the proper amount of units. More concentrated preparations (200 U/ml) of insulin degludec and insulin lispro have recently become available for use in patients with very high insulin requirements. These preparations are isoequivalent, meaning that, unit per unit, no differences in pharmacokinetics and pharmacodynamics exist between the preparation at U100 or at U200. Different appears to be the case of glargine U300, where the increase in concentration seems to affect the absorption kinetics as we will describe later on.

As to the insulin types, different insulins generally differ in onset of action, height, and time of the peak of activity and duration of action. All of these are mainly dependent upon the rate of absorption from the subcutaneous tissue. According to the onset and duration of action, insulin preparations could be classified as rapid, short, intermediate, and long acting. From a clinical point of view, however, it might be more practical to group them in fast-acting insulins and protracted action insulins. Table 1 summarizes clinical characteristics of the more common insulin preparations on the market.

Until 1996, regular human insulin (developed in the early 1980s by recombinant DNA technique and used since then in lieu of porcine insulin) has been the only fast-acting insulin used to cover meal-related insulin requirements. With the advent of rapid-acting insulin analogues, however, it is now rarely used. Rapid-acting insulin analogues were developed to overcome the delay between subcutaneous administration and plasma peak concentrations occurring with regular human insulin.

Table 1 Pharmacokinetics of most commonly used insulin types

Type	Time of onset	Peak	Duration (hours)	Pregnancy class
Rapid acting				
Lispro U100–U200	5–15 min	30–90 min	3–5	B
Aspart	5–15 min	30–90 min	3–5	B
Glulisine	5–15 min	30–90 min	3–5	C
Short acting				
Regular	30–60 min	2–3 h	6–8	B
Intermediate acting				
NPH	60–120 min	5–7 h	13–16	B
Long acting				
Detemir	60–90 min	5–7 h	12–16	B
Glargine U100 (originator and biosimilar)	90–120 min	Slight at 12 h	20–22	C
Glargine U300	90–120 min	Virtually none	20–22	C
Degludec U100		None	Up to 42	C
Degludec U200		None	Up to 42	C

Regular human insulin molecules form hexamers in solution: regular insulin is thus injected into the subcutaneous tissue in hexameric form, while only insulin monomers can be absorbed into the circulation. Thus, after injection, a certain time is necessary for regular insulin to dissociate into monomers and being absorbed (Blundell et al. 1972). Insulin analogues have then been developed to overcome this problem. Insulin analogues are molecules very similar to regular insulin and with receptor affinity comparable to that of the natural hormone, presenting slight modifications (usually in the amino acid sequence) preventing the tendency to aggregate into hexamers (Hirsch 2005). This is why they are referred to as "monomeric" insulins. The first monomeric insulin to be introduced on the market was insulin lispro, differing from regular insulin because of the inversion of the amino acids lysine and proline at positions 28 and 29 in the C-terminus of the B chain (Jacobs et al. 1997). Soon after, insulin aspart was developed, differing from regular insulin in that proline at position 28 in the C-terminus of the B chain is replaced by the negatively charged aspartic acid (Raskin et al. 2000). Finally, insulin glulisine became available for clinical use, where asparagine in position B3 is replaced by lysine and lysine in position B29 is replaced by glutamic acid (Becker et al. 2007). Given their monomeric structure, as a result of the described amino acid modifications, lispro, aspart, and glulisine insulin are absorbed from the subcutaneous tissue twice as fast as regular insulin. This results in a much faster plasma concentration peak, with plasma concentration returning back to baseline more rapidly than with regular insulin. These pharmacokinetic characteristics allow a closer mimicking of physiological insulin excursions after a meal and provide greater convenience and flexibility for patients. Indeed, using rapid-acting analogue instead of regular insulin at mealtime, patients do not need to wait 20–30 min before starting eating after

injection and are much less worried about postprandial hypoglycemia or overlapping of insulin doses between meals (Cobry et al. 2010).

As to the protracted action insulins, the first to be used is the neutral protamine Hagedorn (NPH) insulin, devised by Hagedorn in 1936. The absorption of NPH insulin from the subcutaneous tissue is protracted due to the zinc-protamine in the preparation. Hagedorn applied this principle to bovine insulin, but this was then applied to porcine and human regular insulin and, lately, to lispro insulin. NPH insulin has an onset of action 1–2 h after injection, a distinct peak plasma concentration between 5 and 7 h after injection, and a duration of action of about 13 h. NPH lispro insulin pharmacokinetic and pharmacodynamics characteristics are indeed very similar to those of NPH insulin (Janssen et al. 1997). In the year 2000, the first insulin analogue close to be a basal insulin, insulin glargine, became available for clinical use. Glargine insulin differs from regular insulin in that asparagine in position 21 in the A chain is substituted by glycine and two arginine molecules are added to the B chain in position 30a and 30b. These modifications change the molecule isoelectric point, so that it becomes soluble in an acidic environment. Glargine insulin, solubilized in an acidic solution, precipitates forming microcrystals when injected into the subcutaneous tissue, due to the neutral environment (Bolli and Owens 2000). From these microcrystals, monomeric insulin molecules are than slowly and constantly released into the circulation. Glargine insulin has an onset of action about 2 h after injection, a barely appreciable peak plasma concentration about 12 h after injection, and a duration of action between 20 and 22 h (Lepore et al. 2000). Soon after insulin glargine, insulin detemir was marketed. Insulin detemir is characterized by deletion of the amino acid in position 30 in the B chain and by a link at this site with a molecule of myristic acid. This modification allows a reversible bonding of the molecule with albumin, which protracts its duration of action. As compared to NPH insulin, insulin detemir has a longer duration of action: however, its plasma concentrations still present a peak approximately 5 h after injection, and its duration of action does not extend past 12–16 h. Thus, as compared to glargine, insulin detemir appears to have shorter duration of action and a less flat concentration profile. This is why in subjects with very limited endogenous insulin secretion, it has generally to be administered twice a day. Nevertheless both insulin glargine and insulin detemir have a longer duration of action than insulin NPH and, more importantly, have more stable and consistent biologic activity resulting in more predictable glycemic levels and a lower risk of hypoglycemia (Meneghini et al. 2007; Caputo et al. 2013). Recently, a biosimilar of insulin glargine has been developed and marketed. Replicating the molecular structure of a complex molecule such as an insulin analogue is difficult. Therefore, the resulting product could never be identical to the originator and needs to undergo a set of registration trials aimed at proving that its efficacy and safety are not different from those of the originator. The biosimilar insulin glargine Basaglar now on the market meets these requirements and could be used in lieu of the originator molecule (Rosenstock et al. 2015a; Blevins et al. 2015).

More recently, two additional insulin analogue preparations, insulin degludec and insulin glargine U300, have demonstrated a protraction of biologic activity longer

than 24 h, and thus longer than glargine U100, considered the current technical standard for basal insulin replacement.

Insulin degludec is an ultra-long-acting basal insulin analogue with a half-life at steady state of greater than 25 h, twice as long as that of glargine U100 (Heise et al. 2012). The protraction mechanism is based on the formation of long strings of multihexamers, facilitated by a 16-carbon fatty acid chain linked via a glutamic acid spacer to the terminal end of the B chain of the insulin molecule (Lepore et al. 2000). As compared to glargine U100, insulin degludec also exhibited a flatter and more stable biologic activity and an impressively lower day-to-day intra-subject variability.

Insulin glargine U300 is essentially a threefold concentrated preparation of insulin glargine U100 that results in a two-third volume reduction and a one-half reduction in depot surface following subcutaneous administration (Owens 2016). The reduced depot surface area is presumed to account for much of the protracted absorption of glargine U300 from the subcutaneous tissues. Half-life of glargine U300 appears to be of about 19 h (5 h longer than glargine U100), and it also appears to have a flatter concentration profile after injection (Becker et al. 2015).

Insulin Treatment in Type 1 Diabetes

Without insulin therapy type 1 diabetes would still be a fatal disease: insulin therapy is thus mandatory in type 1 diabetes. The goal of insulin therapy in this condition is to provide insulin replacement in as physiologic fashion as possible. By doing this, one should aim at achieving a blood glucose profile as close as possible to that of a nondiabetic individual trying to avoid hypoglycemia. Thus, one must strive for a balance in each individual patient so to maximize glucose control and minimize hypoglycemia risk.

This, in type 1 diabetic patients who do not have endogenous insulin secretion, could only be approximated by a multiple daily insulin injections (MDI) regimen or by the use of a continuous subcutaneous insulin infusion (CSII) pump. Insulin regimens based on administration of admixture of NPH and regular insulin twice a day, while decreasing the number of injections, do not provide flexibility for the patient in terms of meal timing or time of insulin administration, are much less apt to obtain acceptable glucose profiles, and are marred by a higher risk of hypoglycemia. They should therefore no more be used in type 1 diabetic patient (Standards of Medical Care 2016).

Another chapter of this book covers insulin therapy by insulin pump in detail. As to MDI, to implement it in type 1 diabetic patients, it is paramount to provide them with adequate education and training on how to inject insulin, how to titrate insulin doses, how to perform self-monitoring of blood glucose (SMBG), and how to interpret its results. SMBG in this setting needs to be performed three to seven times a day, as evidence exists that an increased frequency in SMBG is associated with better overall glucose control.

The principle of MDI is that of providing a basal insulin level as constant as possible during the 24 h by injection of a long-acting insulin once or twice a day, and boluses of fast-acting insulin prior to each meal, to cover the blood glucose excursions caused by meal carbohydrate content. This offers to patients the most flexible lifestyle, since they can decide on when to have their meals and on what they want to eat, provided that they administer themselves their fast-acting insulin before each meal and are able to figure out its dose.

As to the type of insulins to use to obtain the basal insulin levels, as noticed above, long-acting insulin analogues have distinct advantages over human NPH insulin. In the treat-to-target trial, for the same level of HbA1c, insulin glargine was associated with a 42% reduction in the risk of hypoglycemia (Riddle et al. 2015). Insulin detemir as well appears to have a flatter profile and less variability as compared to human NPH insulin, but, due to its duration of action shorter than glargine, it more often needs to be administered not in one but in two daily injections (Home et al. 2004; Le Floch et al. 2009).

Insulin glargine was therefore by far the insulin to be preferred as basal insulin in type 1 diabetes. However, some recent trials have provided evidence that use of the recently marketed insulin degludec allows the same degree of optimization of blood glucose profiles and HbA1c levels as insulin glargine, but with a lower risk of hypoglycemia (mainly nocturnal hypoglycemia) (Davies et al. 2014). This might be due to the flatter insulin profile achievable with insulin degludec and/or to the lower intra-subject day-to-day variability in pharmacodynamics demonstrated with degludec versus glargine (Nakamura et al. 2015). Moreover, it has been demonstrated that, contrary to insulin glargine, changing daily the time of administration of insulin degludec does not affect its efficacy and safety. Thus, use of insulin degludec gives patients more flexibility in the management of their insulin regimen.

Use of the more concentrated insulin glargine U300 has also resulted, as compared to insulin glargine U100, in a lower hypoglycemia risk for the same degree of glucose control (Ritzel et al. 2015). In this case as well, this might be related to the more prolonged half-life due to the reduced subcutaneous depot area. Studies are ongoing to investigate whether the advantage obtainable with the use of insulin glargine U300 is comparable to those obtainable by the use of insulin degludec.

Regardless of the type of basal insulin used, basal insulin needs to be titrated. Several titration algorithms have been proposed, mostly based on measurement of fasting blood glucose in the morning, when blood glucose is least affected by prandial glucose excursions. Algorithms vary in frequency of dose adjustments, in suggested targets, and in magnitude of insulin dose variations. There is no evidence that one among the proposed algorithms is better than any other, but one algorithm needs to be decided upon, together with the patient. The patient has to be instructed in measuring and recording fasting blood glucose values and in titrating her/his basal insulin dose accordingly (Arnolds et al. 2013).

As to the insulin to be used at mealtime, presently rapid-acting insulin analogues are recommended in MDI. Insulin lispro, insulin aspart, and insulin glulisine pharmacokinetic and pharmacodynamics are so similar that no compelling reason exists to prefer one over the other. As to the titration of mealtime insulin, the bolus should

be proportional to the carbohydrate content of the meal to be ingested. Patients need therefore to be instructed in carbohydrate counting (Son et al. 2014). However, no study has been able to individuate the insulin to carbohydrate factor to be used to calculate the insulin dose, and different recommendations exist on how to calculate this (Bevier et al. 2007). Furthermore, the optimal insulin to carbohydrate factor is likely to be different from patient to patient, and it has to be "discovered" in each patient by trial and error. Self blood glucose measurement obtained before and 2 h after each meal, together with accurate recording of meal composition, will serve as guidance to titrate prandial rapid-acting insulin dose, considering that it has become clear that also fat and protein component of the meal do affect the post-meal glycemic rise. Titration of prandial insulin dose based solely on blood glucose levels prior to injection (the so-called sliding scale) might lead to amplification of blood glucose excursions and should be abandoned (Umpierrez et al. 2007). Starting a type 1 diabetic patient on MDI requires an initial "guessing" of the insulin doses, since patients are likely to be insulin-naïve. A diabetes "team" should ideally take the patient under care, where a dietician and a nurse trained in diabetes education work together with the physician on the optimization of insulin regimens, meal plans, and work/physical activity schedules. The total 24 h insulin requirement for a normal weight type 1 diabetic subject should be around 0,3–0,4 U insulin per Kg. Of this, 40–50% would be basal insulin and the remaining 50–60% should be covered by prandial insulin subdivided in the meals taken by the patient. These are of course average estimates, which can vary widely from patient to patient according to individual insulin sensitivity, age, activity, degree of glucose control, etc. It is usually suggested to start with lower doses, also to avoid severe hypoglycemia in the early stage of the diabetes education process, and then up-titrate both basal and prandial insulin as needed. It has to be kept in mind that poor glucose control increases insulin requirements (Vuorinen-Markkola et al. 1992). Thus, once glucose profiles ameliorate following treatment, insulin requirements are likely to drop and insulin doses might need to be down-titrated accordingly.

Insulin Treatment in Type 2 Diabetes

While in type 1 diabetes insulin treatment is mandatory and cannot be postponed or avoided without endangering patient life, an increasing number of alternative treatment options are presently available for patients with type 2 diabetes. However, in type 2 diabetes, as in type 1 diabetes, optimal glucose control is instrumental in preventing and/or delaying the occurrence of complications. Nevertheless, the vast majority of patients in treatment for T2D have suboptimal glycemic control, with HbA1c levels exceeding the suggested targets. Several studies have indeed determined that only 30–50% of patients achieve an HbA1c $<7\%$, as recommended by the EASD/ADA guidelines, with a single antidiabetic agent, and that glycemic control tends to worsen over time, mostly because treatment is not timely and properly intensified (Karter et al. 2007; Turner et al. 1999; Benoit et al. 2005). Furthermore, type 2 diabetes is a progressive disease, characterized by decreasing

insulin levels due to gradual deterioration in pancreatic beta cell function. As a matter of fact, beta cell function, already likely to be severely impaired at diagnosis, reduces rapidly over a period of just a few years (U.K. prospective diabetes study 16 1995). This rapid beta cell decline means that insulin replacement will eventually become necessary in order to achieve and maintain glycemic control, since other available therapies, with the exception of SGLT2 inhibitors, rely on the body's ability to produce insulin (U.K. prospective diabetes study 16 1995). On the other hand, insulin is an injective treatment (and, as such, often reluctantly accepted by patients) needing careful titration, and insulin treatment is associated with increased risk of hypoglycemia and often with weight gain. Pros and cons of insulin treatment need therefore to be skillfully balanced in type 2 diabetic, and the main questions to be correctly answered are when and how to initiate it, how to optimize it, and how to intensify it.

There is no unanimous consensus as to when to start in insulin therapy in type 2 diabetes. However, when glycemic control cannot be achieved using the maximum-tolerated dose of metformin or other oral agents, insulin initiation must be considered as a next step. Insulin treatment should not anymore be considered as a last resort, as most scientific societies recommend that insulin therapy be started sooner rather than later. In the ADA/EASD joint position statement, initiation of basal insulin treatment ranks equally to other treatments (sulfonylureas, pioglitazone, DPP-IV inhibitors, SGLT2 inhibitors, and GLP-1 Rx agonists) as the option to be preferred for dual therapy in add-on to metformin when metformin monotherapy fails (Inzucchi et al. 2015). Furthermore, temporary insulin therapy to rapidly restore glucose control and favor amelioration of insulin sensitivity and insulin secretion has also been proposed in type 2 diabetes with elevated (>9%) HbA1c at baseline (Pozzilli et al. 2010). Early introduction of insulin therapy might also help restore beta cell function, as insulin has anti-apoptotic effects on beta cell in vitro. Furthermore, a study has shown increased remission chances in type 2 diabetes subjects temporarily treated with MDI or insulin pump at diagnosis (Bernard-Kargar and Ktorza 2001; Weng et al. 2008). Furthermore, as compared to sulfonylureas, early insulin treatment resulted in better endogenous insulin secretion in a study in Scandinavian subjects (Alvarsson et al. 2003). Sadly, however, real-world retrospective observational studies have shown that more than 8 years might elapse while patients are in poor glycemic control before insulin treatment is introduced (Dailey 2008).

One must also consider, however, that other injectable agents, namely, GLP-1 Rx agonists, are nowadays an alternative to initiating insulin treatment in type 2 diabetic patients failing metformin monotherapy or metformin plus another oral agent treatment. Trials have compared insulin glargine versus GLP-1 Rx agonists already licensed or in development (exenatide (Heine et al. 2005), exenatide LAR (Diamant et al. 2012), liraglutide (Russell-Jones et al. 2009), dulaglutide (Giorgino et al. 2015), and semaglutide (Zaccardi et al. 2016)). They have consistently shown that GLP-1 Rx agonists were non-inferior or, most often, superior to insulin glargine in terms of decrease in HbA1c or in terms of number of treated subjects achieving an HbA1c goal of <7% or <6.5%. Furthermore, insulin treatment was associated with

weight gain, while GLP-1 Rx agonist treatment was associated with weight loss, and hypoglycemia risk was generally lower with GLP-1 Rx agonists. The issue could be raised that in all the above head-to-head studies comparing GLP-1 Rx agonists and glargine, the latter might have been under-titrated and that a more aggressive insulin titration might have led to different results. However, frequency of hypoglycemia tended to be greater in the insulin arms, and more aggressive insulin titration might have resulted in patient harm. Of course, individual patient preferences and characteristics, as well as cost, need to be taken into consideration when deciding which injectable treatment to prefer in patients failing oral antihyperglycemic drugs. Also, preparation of basal insulin + GLP-1 receptor agonist in fixed dose combination has recently been licensed for use. In registration trials, this combination has obtained better results as compared to either of the two agents alone in patients failing oral agents (Gough et al. 2014).

However, patients do exist in whom endogenous insulin secretion is so greatly impaired that insulin treatment is the only viable alternative to bring them under control. In any case, being relative or absolute insulin deficiency the first of the many culprits involved in type 2 diabetes pathogenesis, insulin treatment is always an option to consider in type 2 diabetes and its initiation should not be unduly delayed (Defronzo 2009).

As to how to initiate insulin therapy in type 2 diabetes, the general consensus is nowadays that to initiate with basal insulin is preferable and easier for the patient (Inzucchi et al. 2015). Indeed, particularly in patients with large post-meal glycemic excursion, it could be conceivable to start with prandial insulin. However, in the APOLLO study (Bretzel et al. 2008), where administration of glargine at bedtime was compared to administration of lispro insulin at mealtime, the use of glargine resulted in better fasting blood glucose (although postprandial excursions were obviously better controlled by lispro insulin) and, for the same reduction in HbA1c, in lower hypoglycemia risk and less weight gain. Similar results were obtained in the INITATE and in the 4 T studies where rapid-acting insulin three times a day and premixed insulins twice a day lead to a minimally better reduction in HbA1c as compared to basal insulin, but basal insulin was associated with significantly less hypoglycemia and weight gain (Raskin et al. 2005; Holman et al. 2007). It appears therefore that initiating insulin therapy with basal insulin is a safe and easy way to start insulin treatment, exposing the patient to a lesser hypoglycemia risk and a slightly inferior risk of gaining weight. As to which basal insulin to use in type 2 diabetic subjects, since these subjects usually have some residual endogenous insulin secretion, the use of basal insulin analogues in lieu of NPH insulin is less compelling. However, the treat-to-target study has demonstrated that for an equally intensive titration, aimed at obtaining HbA1c levels $<7\%$, the risk of hypoglycemia is significantly lower with insulin glargine than for NPH insulin (Riddle et al. 2003). By the same token, studies performed in type 2 diabetic patients in the course of insulin degludec registration phase have shown a modest but significant advantage of degludec over glargine in terms of less overall and nocturnal hypoglycemia risk (Rodbard et al. 2013). This has been ascribed to the longer duration of action and to the lower day-to-day variability in insulin profiles of degludec versus glargine. One

study has demonstrated a slight advantage also of glargine U300 versus glargine U100 in type 2 diabetes relatively to the hypoglycemia risk (Riddle et al. 2015).

Factoring in also cost considerations, it appears reasonable to start basal insulin therapy in type 2 diabetic subjects with U100 insulin glargine. One should then resort to the newer and more expensive insulin formulations in patients presenting a pronounced rise in blood glucose later in the afternoon and/or exhibiting a large day-to-day variability in morning fasting blood glucose and/or in the 24 h blood glucose profile. Regardless of which insulin is used to start basal insulin therapy, insulin doses need to be titrated. Several titration algorithms have been proposed: they seem equivalent and they all use morning fasting blood glucose as the value upon which to up-titrate or down-titrate the basal insulin dose (Strange 2007). This allows implementation of insulin therapy in type 2 diabetes by measurement of a single value of blood glucose in the morning (which in some algorithms does not even need to be measured every day): this makes insulin therapy easier and more readily acceptable by patients. Basal insulin titration needs to be implemented in all patients, so to avoid exposing patients to the possible hazards linked to insulin therapy without achieving the benefit of the optimal glucose control insulin therapy is meant to provide.

In most occasions, insulin treatment is started in type 2 diabetes when oral treatment fails to maintain adequate glucose control. Thus the question is about whether or not to continue oral antihyperglycemic agent when starting insulin treatment. Metformin should always be continued, since randomized controlled trial has shown modest improvement in glucose control, reduction of insulin doses, and a less pronounced weight gain when metformin is added on to basal insulin treatment (Pradhan et al. 2009; Kooy et al. 2009).

Much more uncertainty exists about potential advantages of using sulfonylurea together with basal insulin. This might be associated with a slight reduction of insulin doses and may be a small improvement in glucose control, but it increases the risk of hypoglycemia and weight gain (Raskin 2008). One study has shown lower HbA1c and fasting blood glucose values when pioglitazone versus placebo was used on the top of insulin therapy (Mattoo et al. 2005). However increased weight gain and increased risk of peripheral edema and of heart failure were associated with the use of insulin+pioglitazone in a meta-analysis. More recently, improved glycemic control and/or decreased risk of hypoglycemia has been shown with the use of DPP-IV inhibitor in add-on to basal insulin therapy (Fonseca et al. 2007; Vilsbøll et al. 2010). Finally, SGLT2 inhibitors have recently been introduced in the treatment of type 2 diabetic patients. Being these drugs mechanism of action totally independent on insulin secretion and insulin action, there is a strong rationale for their use in combination with insulin. Studies with the use of SGLT2 inhibitors in add-on to basal insulin therapy have shown efficacy and safety of this approach (Rosenstock et al. 2015b; Fioretto et al. 2015; Neal et al. 2015).

Treatment with a basal insulin, even when carefully titrated, might not be sufficient in a proportion of patients to obtain the desirable level of glucose control. Thus, insulin treatment might need to be intensified. Even in this case, no unanimous consensus exists as to how to intensify basal insulin treatment in type 2 diabetes. The different options have to be carefully evaluated according to patient characteristics.

Failure of treatment with basal insulin to obtain acceptable HbA1c levels is usually due to excessive postprandial glucose excursions. Thus, intensifying basal insulin therapy by using rapid-acting insulin analogues at mealtime has a strong rationale. This involves starting a basal-bolus therapy, very much like that usually implemented in type 1 diabetic patient. When starting basal-bolus therapy, patients need to be carefully instructed on the ratio between premeal insulin dose and carbohydrate content of the meal and instructed as well on self blood glucose measurement and recording. Basal-bolus therapy is thus the most complex regimen and, as such, has a strong impact on type 2 diabetic patient quality of life (Davis et al. 2001).

An alternative to basal-bolus treatment is to add to the basal insulin a shot of a rapid-acting insulin analogue administered right before the main meal of the day (the one followed by the larger blood glucose excursion). This has been called "basal plus" approach and has been shown to be an effective way to intensify basal insulin therapy minimizing patient inconvenience (Lankisch et al. 2008).

An alternative way to intensify basal insulin therapy could be adding to basal insulin treatment the administration of a GLP-1 receptor agonist. Indeed, a number of studies have compared safety and efficacy of addition of GLP-1 receptor agonists versus addition of premeal rapid-acting insulin in patients failing basal insulin therapy (Diamant et al. 2014; Mathieu et al. 2014). A meta-analysis of these studies has shown that addition of GLP-1 receptor agonists was slightly better in terms of metabolic control and significantly better in terms of weight gain and risk of hypoglycemia as compared to basal-bolus treatment (Eng et al. 2014). As noted above, preparation of basal insulin + GLP-1 receptor agonist in fixed dose combination has recently been licensed for use. Switching to treatment with these combinations in patients failing basal insulin therapy has been proven efficacious and safe in registration trials (Lingvay et al. 2016). Therefore, use of basal insulin + GLP-1 receptor agonists in fixed dose combination might become in the near future an easy and safe way to intensify basal insulin therapy in type 2 diabetes.

Insulin Treatment in Pregnant Women

Insulin treatment has dramatically changed the outcome of pregnant diabetic women. Before the advent of insulin therapy, fetal mortality was more than 90% and mother mortality was about 30% in pregnant diabetic women. Insulin therapy is thus pivotal for treating diabetic women (both type 1 and type 2) during pregnancy, and it is highly recommended in gestational diabetes mellitus (GDM) when enforcing appropriate eating habits and lifestyle fails to achieve and maintain appropriate glycemic targets (Gestational diabetes mellitus 2004; Rodbard et al. 2007). Management of insulin treatment during pregnancy might be complex, and it could be accomplished either by multiple daily injections of insulin analogues or by the use of continuous subcutaneous insulin infusion by mini-pumps. Either approach needs to be guided by careful and intensive self blood glucose monitoring

and/or, when feasible, by continuous glucose monitoring via a subcutaneous glucose sensor (CGM).

In women affected by type 1 or by type 2 diabetes, appropriate preconception counseling, including optimization of insulin therapy, is of the outmost importance to obtain adequate glucose control during the first gestational weeks, thus decreasing fetal malformation risk (Wahabi et al. 2012). In diabetic women, congenital malformation frequency (which is a function of the degree of glucose control very early in the course of pregnancy) is still three times as high as in nondiabetic women. However, due to the implementation of a more intensive approach to glucose control, unfavorable fetal outcomes have significantly decreased in the latest years (Higgins et al. 2011). In diabetic women, the suggested HbA1c target to achieve before and during pregnancy is 6.5%. Besides, treatment must aim at reducing as much as possible glycemic swings and blood glucose variability, since instability of glucose control, even in the presence of target HbA1c values, might increase fetal malformation risk (Dalfrà et al. 2011; Kerssen et al. 2006a, 2007, 2006b). It must also be kept in mind that, regardless of malformation risk, the intrauterine environment is likely to affect metabolic features not only in the fetus but also in the neonate and probably throughout the life of the individual (Yessoufou and Moutairou 2011). Pregnancy must therefore be adequately planned, and the woman must be involved in choosing the most appropriate insulin treatment strategy apt to achieve the best possible glucose control.

Insulin regimen to be implemented must consider the need to provide adequate basal insulinization as well as proper insulin boluses to cover meal-related insulin needs. It must be kept in mind that during pregnancy glucose homeostasis undergoes a host of changes in order to guarantee proper substrate delivery to the fetus (Kalhan et al. 1997). These changes are even more pronounced in diabetic pregnant women and need to be accounted for when choosing an insulin treatment strategy which needs to be flexible and adaptable to the individual patient needs (Murphy et al. 2007, 2012). In this way, ambitious targets might be reached with an as low as possible risk of hypoglycemia. Glycemic targets are generally more stringent during pregnancy as compared to the prepregnancy period: besides, both pre- and postprandial desirable blood glucose levels change over the course of pregnancy. Thus, insulin requirements tend to decrease during the first trimester of pregnancy, and this might expose the patient to a greater hypoglycemia risk. Insulin requirements then increase progressively to a final increase of about 30–40% around gestational week 30, reaching a total insulin 24 h requirement between 0.7 and 1.0 units for kilogram of body weight (Parretti et al. 2001; Yogev et al. 2004).

In the attempt to mimic as close as possible physiological 24 h glucose profile, to limit day-to-day glucose variability and to have the lowest possible hypoglycemia risk, rapid-acting and long-acting insulin analogue should be used. However, besides hypoglycemia risk, other factors needing to be considered when implementing insulin treatment in pregnancy are the mitogen and the teratogen risk. The first is linked to the specific insulin type selectivity for the insulin receptor versus the IGF-1 receptor. The teratogen risk is instead linked to the potential transplacental passage of insulin analogues. Lispro, aspart, and detemir insulin exhibit higher selectivity for

the insulin receptor and have a mitogen activity similar to that of human insulin (Vigneri et al. 2010). On the other hand, after subcutaneous injection, glargine insulin is degraded into two active metabolites, M1 and M2. M1 represents more than 90% of circulating glargine insulin, and, as compared to human insulin, it exhibits lower mitogen activity and lower affinity for the IGF-1 receptor (Vigneri et al. 2010).

As to the teratogen risk, it only comes into play in the case antibodies develop against the insulin molecule. These might bind the insulin molecule and help vehicle it across the placental barrier. Lispro and aspart insulin do not seem able to induce antibodies formation, and antibodies against these molecule have not been found in the cord blood of women using them (McCance et al. 2008). Besides, at the highest doses used, neither lispro nor glargine insulin seems to be able to cross the placental barrier (Pollex et al. 2010). The safety of using lispro or aspart insulin during pregnancy has been proven in several studies where use of these molecules was not associated with any increase in unfavorable fetal and/or neonatal outcomes (Bhattacharyya et al. 2001; Heller et al. 2010; Hod et al. 2008). Although no theoretical reason exists for this not being the same with glulisine insulin, no data are at present available about the use of this insulin analogue during pregnancy. As to basal insulin analogues, insulin glargine and insulin detemir should be preferred since they, as compared to NPH human insulin, seem to ensure a better nocturnal blood glucose control with lower hypoglycemia risk and to favor a lower day-to-day glucose variability (Lepore et al. 2000; Porcellati et al. 2007; Heise et al. 2004). No difference in maternal and or fetal/neonatal outcome has been observed with the use of glargine, detemir, or NPH human insulin (Negrato et al. 2010; Mathiesen et al. 2012; Callesen et al. 2013).

Finally, insulin therapy by continuous subcutaneous insulin infusion (CSII) might be the first choice in pregnant type 1 diabetic women (Misso et al. 2010). However, studies comparing CSII versus multiple daily injections, insulin therapy in pregnant type 1 diabetic women have failed to show any difference in glucose control, hypoglycemia frequency, and maternal or fetal/neonatal outcome (Cummins et al. 2010; Talaviya et al. 2013).

Insulin Treatment-Related Risks

Events related to the insulin effects often experienced by patients treated with insulin are hypoglycemia and weight gain. These will be discussed below together with the once much discussed hypotheses that insulin treatment might be linked to increased cardiovascular risk and/or increased cancer risk.

Hypoglycemia

Hypoglycemia is the most prominent barrier to intensification of insulin treatment toward optimal targets. On the basis of the results of the DCCT trial (DCCT 1986),

an HbA1c target not inferior to 6,5% is currently recommended in subjects with type 1 diabetes. This is because an HbA1c level between 6,5 and 7,0% seems to represent the best compromise between the need to prevent complications and an unacceptable risk of hypoglycemia. In type 2 diabetes subjects, national and international guidelines call for a careful individualization of glycemic targets.

Hypoglycemia might be severe and lead to different degree of cognitive function impairments up to hypoglycemic coma. It needs to be promptly treated by oral glucose administration (in the case of minimal cognitive impairment) or by administration of IV glucose or intramuscular glucagon. Severe hypoglycemia often requires hospital admission.

Hypoglycemia is associated with electrical alterations in the cardiac tissue, with acute alterations in the coagulation system, with a catecholamine surge, and with acute release of inflammation molecules (Desouza et al. 2010; Chow et al. 2014; Gogitidze Joy et al. 2010). All of these might predispose to an acute cardiovascular event. Besides, hypoglycemia might have a deleterious impact on everyday life by making very dangerous simple activities such as driving a motor vehicle or climbing stairs.

Insulin treatment-related hypoglycemia is more frequent in type 1 than in type 2 diabetic patients. In a study on a Scottish cohort, the overall hypoglycemia event rate was 42.9 and 16.37 events per patient per year in type 1 and in type 2 diabetic subjects (Donnelly et al. 2005). The main predictors of hypoglycemia were previous history of hypoglycemia in type 1 diabetes and duration of insulin treatment in type 2 diabetes. Other recognized hypoglycemia risk factors are HbA1c $<6\%$, autonomic neuropathy, and hypoglycemia unawareness. Renal insufficiency is also associated with increased risk of hypoglycemia, although the mechanisms have not been fully elucidated (Alsahli and Gerich 2015). In type 2 diabetes, another risk factor for hypoglycemia during insulin treatment is the concomitant use of sulfonylureas. As to the possible triggers of a hypoglycemic event, the most significant are unplanned physical activity and skipped meals (or ingestion of meals with carbohydrate content much lower than it was supposed to be) (McCrimmon and Sherwin 2010).

Any possible measure needs to be taken to minimize hypoglycemia risk in insulin-treated patients. These include throughout considerations of all risk factors, individualization of treatment targets, optimal choice of the types of insulin to use, and careful patient instruction on how to avoid, how to recognize, and how to treat hypoglycemic episodes.

Weight Gain

Insulin treatment is almost invariably associated with weight gain. This represents more of a problem in type 2 diabetic patients. In type 1 diabetic patients, intensive insulin treatment resulted in a weight gain of 2,1 Kg during the first year in the DCCT (Adverse events and their association 1995). In the same cohort, weight gain continued during the follow-up but seemed to reach a plateau at a total weight gain of about 4 Kg after 5 years of treatment (Purnell et al. 1998). Insulin treatment induces

weight gain in subject with type 2 diabetes as well. In these subjects, weight gain can be especially undesirable because of its psychological effects and the potential negative effects on cardiovascular risk factors. A weight gain of as much as 8 Kg has been reported in subjects with type 2 diabetes started on insulin therapy (Larger et al. 2001). Much of the weight gain, however, occurs within the first 2 years following initiation of insulin treatment (Larger 2005).

As to the mechanisms for the weight gain, insulin favors glucose utilization at adipose tissue and skeletal muscle tissue level, and, more importantly, the hormone suppresses lipolysis and favors lipid synthesis. Much less convincing are the evidence supporting the hypothesis that insulin prompts orexin stimuli and thus it increases appetites (Griffond et al. 1999). Most of the weight gain following initiation of insulin therapy appears indeed related to the reduction in glycosuria following improvement of metabolic control (Russell-Jones and Khan 2007). Cessation of the calorie loss due to glucose elimination in the urine, in the absence of a parallel decrease in food intake, could be responsible for most of the insulin therapy-related weight gain (Mäkimattila et al. 1999). Furthermore, insulin might induce water retention, and this might contribute to weight gain as well. The magnitude of weight gain seems directly proportional to the insulin dose and to the frequency of hypoglycemia (Biesenbach et al. 2006).

Indeed, fear of hypoglycemia might induce a "defensive eating" pattern which definitely increases caloric intake. In type 2 diabetes, concomitant diabetes treatment might affect the impact of insulin therapy on weight gain. Thus, use of metformin together with insulin seems to limit weight gain, which is instead enhanced by concomitant use of sulfonylurea or pioglitazone (Yki-Järvinen 2001). In any case, weight gain observed with insulin treatment does not justify to avoid or to unduly delay implementation of insulin treatment when needed to obtain acceptable glycemic control. This is also because there is no evidence that weight gained is such to have a significant impact on related cardiovascular risk factors. Still, attention must be payed to this aspect, proper counseling on eating habits and lifestyle has to be enforced, and combination therapy able to contain weight gain (metformin, GLP-1 receptor agonists, SGLT2 inhibitors) need to be carefully considered.

Cardiovascular Risk and Cancer Risk

A large debate has been going on in the past about a potential negative impact of insulin treatment on cardiovascular risk. This was mainly stemming from observations linking elevated circulating insulin levels to increased risk of cardiovascular events and from data showing a larger mortality in critical patients receiving insulin infusion in the ICU (Muis et al. 2005; Finfer et al. 2009). In the first case, however, elevated endogenous insulin levels are the marker of insulin resistance, and insulin resistance (which actually implies a lack of insulin action) is per se associated with increased cardiovascular risk. In the second case, the group in intensive insulin treatment had a frequency of hypoglycemia 30 times larger than the control group, and hypoglycemia is per se a risk factor for cardiovascular events, especially in frail

subjects (Abdelhafiz et al. 2015). The whole argument has been finally put to rest by the ORIGIN study where no difference in cardiovascular events was observed in a relatively long follow-up between type 2 diabetes patients treated with basal insulin early in the course of the disease and patients kept on oral hypoglycemic agents (mostly metformin and sulfonylurea) treatment (Gerstein et al. 2012). It is therefore now accepted that insulin treatment does not cause an increase in cardiovascular risk.

As to the potential increase in the risk of cancer with insulin therapy, insulin is not only a major regulator of cell metabolism but it is also a growth factor. It has been observed that, in many cancer cells, the insulin receptor is overexpressed and the A isoform, which has a predominant mitogen effect, is more represented than the B isoform. These characteristics provide a selective growth advantage to malignant cells when exposed to insulin. Thus, it is theoretically possible that hyperinsulinemia, either endogenous or exogenous, might increase the risk of cancer (Vigneri et al. 2016). In vivo, in man, a concern has been raised particularly in relation to the use of long-acting insulin analogue (Hemkens et al. 2009). However, the available evidence from observational studies is at best inconclusive (Wu et al. 2016). Thus, the US Food and Drug Administration has stated that currently available evidence is insufficient to draw definitive conclusions regarding the association between long-acting insulin analogues and cancer (US Food and Drug Administration n.d.; FDA Drug Safety Communication n.d.; FDA Drug Safety Podcast for Healthcare Professionals n.d.), while the European Medicines Agency concluded that insulin glargine does not increase the risk of cancer (European Medicines Agency n.d.). The only randomized controlled trial to have directly addressed the issue, the ORIGIN trial, did not find any evidence of increased cancer risk associated with glargine treatment: however, the follow-up period (>7 years) might have been too short, given the latency of cancer, to pick up an eventual signal. Furthermore, it was powered to have a 90% chance of detecting a 20% increase in risk: it was therefore underpowered to detect any smaller risk increase (Gerstein et al. 2012).

Insulin Therapy Side Effects

The side effects of insulin therapy include local skin reactions, insulin allergy, lipoatrophy, and lipohypertrophy.

Localized skin reaction at the site of injection was less uncommon before the use of purified insulins. Now they are indeed very rare and are generally associated with the non-insulin component of the injection, such as the latex in the needle (and this is why most needles are now latex free) (Radermecker and Scheen 2007). Generally, it is possible to find alternative insulin preparations or products not causing the reaction. Occurrence of systemic insulin allergy is indeed rare (0,1% of patients) and its manifestation are usually seen within 1 or 2 weeks from starting or, more often, from resuming insulin therapy. Usually a local reaction occurs within an hour after injection, gradually increasing to involve large areas of the body. The reaction might evolve into a generalized urticarial pattern. Angioneurotic edema and even

anaphylactic shock have been sporadically reported (Kaya et al. 2007). The reaction is clinically similar to penicillin allergy, and about one-third of patients with insulin allergy have a history of penicillin allergy. Insulin allergy should be treated by desensitization.

It is defined lipoatrophy the loss of subcutaneous fat tissue at the sites of insulin injection. As local skin reactions, insulin lipoatrophy is now much less common thanks to the use of highly purified insulins. Local lipoatrophy is benign in nature but can be disturbing to patients due to cosmetic reasons. The precise cause of lipoatrophy is still uncertain. In some instances, it resolves itself by switching to a different insulin preparation, but this is not always the case.

Lipohypertrophy of the subcutaneous tissue at the site of insulin injection is a much more common problem, found in as many as one out of two patients on insulin treatment (Mattoo et al. 2005). The occurrence of lipohypertrophy seems to be related to frequency in the change of needles, frequency in changing injection sites, and duration of insulin use. Injection of insulin always on the same site predisposes to lipohypertrophy. The phenomenon might tend to perpetuate itself since patients tend to prefer lipohypertrophy areas to inject insulin, being injections in these areas less painful than at other sites. One must keep in mind, however, that absorption of insulin from lipohypertrophy areas is decreased, is delayed, and is more variable as compared to non-lipohypertrophy areas. The problem is even worst with the development of fibrocollagenous nodules at the injection sites. These might greatly hamper insulin absorption if insulin keeps to be injected at that site with the consequent significant deterioration of glucose control (Duckworth et al. 2009). Changing frequently and regularly insulin injection sites and avoiding injecting insulin in lipohypertrophy areas or nodules which might form are the proper ways to prevent these local insulin injection side effects and to limit their impact on glucose control.

References

Abdelhafiz AH, Rodríguez-Mañas L, Morley JE, Sinclair AJ. Hypoglycemia in older people – a less well recognized risk factor for frailty. Aging Dis. 2015;6(2):156–67.

Adverse events and their association with treatment regimens in the diabetes control and complications trial. Diabetes Care. 1995;18(11):1415–27.

Alsahli M, Gerich JE. Hypoglycemia in patients with diabetes and renal disease. J Clin Med. 2015;4(5):948–64.

Alvarsson M, Sundkvist G, Lager I, et al. Beneficial effects of insulin versus sulphonylurea on insulin secretion and metabolic control in recently diagnosed type 2 diabetic patients. Diabetes Care. 2003;26(8):2231–7.

Arnolds S, Heise T, Flacke F, Sieber J. Common standards of basal insulin titration in type 2 diabetes. J Diabetes Sci Technol. 2013;7(3):771–88.

Becker RHA, Frick AD, Nosek L, Heinemann L, Rave K. Dose-response relationship of insulin glulisine in subjects with type 1 diabetes. Diabetes Care. 2007;30(10):2506–7.

Becker RHA, Nowotny I, Teichert L, Bergmann K, Kapitza C. Low within- and between-day variability in exposure to new insulin glargine 300 U/ml. Diabetes Obes Metab. 2015;17(3):261–7.

Benoit SR, Fleming R, Philis-Tsimikas A, Ji M. Predictors of glycemic control among patients with type 2 diabetes: a longitudinal study. BMC Public Health. 2005;5:36.
Bernard-Kargar C, Ktorza A. Endocrine pancreas plasticity under physiological and pathological conditions. Diabetes. 2001;50(Suppl 1):S30–5.
Bevier WC, Zisser H, Palerm CC, et al. Calculating the insulin to carbohydrate ratio using the hyperinsulinaemic-euglycaemic clamp-a novel use for a proven technique. Diabetes Metab Res Rev. 2007;23(6):472–8.
Bhattacharyya A, Brown S, Hughes S, Vice PA. Insulin lispro and regular insulin in pregnancy. QJM. 2001;94(5):255–60.
Biesenbach G, Raml A, Alsaraji N. Weight gain and insulin requirement in type 2 diabetic patients during the first year after initiating insulin therapy dependent on baseline BMI. Diabetes Obes Metab. 2006;8(6):669–73.
Blevins TC, Dahl D, Rosenstock J, et al. Efficacy and safety of LY2963016 insulin glargine compared with insulin glargine (Lantus®) in patients with type 1 diabetes in a randomized controlled trial: the ELEMENT 1 study. Diabetes Obes Metab. 2015;17(8):726–33.
Blundell TL, Cutfield JF, Cutfield SM, et al. Three-dimensional atomic structure of insulin and its relationship to activity. Diabetes. 1972;21(2 Suppl):492–505.
Bolli GB, Owens DR. Insulin glargine. Lancet (London). 2000;356(9228):443–5.
Bretzel RG, Nuber U, Landgraf W, Owens DR, Bradley C, Linn T. Once-daily basal insulin glargine versus thrice-daily prandial insulin lispro in people with type 2 diabetes on oral hypoglycaemic agents (APOLLO): an open randomised controlled trial. Lancet (London). 2008;371 (9618):1073–84.
Brunton S, Carmichael B, Funnell M, et al. Type 2 diabetes: the role of insulin. J Fam Pract. 2005;54 (May):445–52.
Callesen NF, Damm J, Mathiesen JM, Ringholm L, Damm P, Mathiesen ER. Treatment with the long-acting insulin analogues detemir or glargine during pregnancy in women with type 1 diabetes: comparison of glycaemic control and pregnancy outcome. J Matern Fetal Neonatal Med. 2013;26(6):588–92.
Caputo S, Andersen H, Kaiser M, Karnieli E, Meneghini LF, Svendsen AL. Effect of baseline glycosylated hemoglobin A1c on glycemic control and diabetes management following initiation of once-daily insulin detemir in real-life clinical practice. SOLVE Study Group. Endocr Pract. 2013;19(3):462–70.
Chow E, Bernjak A, Williams S, et al. Risk of cardiac arrhythmias during hypoglycemia in patients with type 2 diabetes and cardiovascular risk. Diabetes. 2014;63(5):1738–47.
Cobry E, McFann K, Messer L, et al. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. Diabetes Technol Ther. 2010;12(3):173–7.
Cummins E, Royle P, Snaith A, et al. Clinical effectiveness and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes: systematic review and economic evaluation. Health Technol Assess. 2010;14(11):iii–v, xi-xvi, 1–181.
Dailey G. Optimum management of type 2 diabetes – timely introduction, optimization and intensification of basal insulin. Diabetes Obes Metab. 2008;10(Suppl 2):5–13.
Dalfrà MG, Sartore G, Di Cianni G, et al. Glucose variability in diabetic pregnancy. Diabetes Technol Ther. 2011;13(8):853–9.
Davies MJ, Gross JL, Ono Y, et al. Efficacy and safety of insulin degludec given as part of basal-bolus treatment with mealtime insulin aspart in type 1 diabetes: a 26-week randomized, open-label, treat-to-target non-inferiority trial. Diabetes Obes Metab. 2014;16(10):922–30.
Davis TM, Clifford RM, Davis WA. Effect of insulin therapy on quality of life in type 2 diabetes mellitus: The Fremantle Diabetes Study. Diabetes Res Clin Pract. 2001;52(1):63–71.
Defronzo RA, Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58(4):773–95.
Del Guerra S, Lupi R, Marselli L, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes. 2005;54(3):727–35.
Desouza CV, Bolli GB, Fonseca V. Hypoglycemia, diabetes, and cardiovascular events. Diabetes Care. 2010;33(6):1389–94.

Diamant M, Van Gaal L, Stranks S, et al. Safety and efficacy of once-weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes over 84 weeks. Diabetes Care. 2012;35(4):683–9.
Diamant M, Nauck MA, Shaginian R, et al. Glucagon-like peptide 1 receptor agonist or bolus insulin with optimized basal insulin in type 2 diabetes. Diabetes Care. 2014;37(10):2763–73. https://doi.org/10.2337/dc14-0876.
Donnelly LA, Morris AD, Frier BM, et al. Frequency and predictors of hypoglycaemia in type 1 and insulin-treated type 2 diabetes: a population-based study. Diabet Med. 2005;22(6):749–55.
Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med. 2009;360(2):129–39.
Eng C, Kramer CK, Zinman B, Retnakaran R. Glucagon-like peptide-1 receptor agonist and basal insulin combination treatment for the management of type 2 diabetes: a systematic review and meta-analysis. Lancet (London). 2014;384(9961):2228–34.
European Medicines Agency. Outcome of review of new safety data on insulin glargine. 2013.
FDA Drug Safety Communication. Update to ongoing safety review of Lantus (insulin glargine) and possible risk of cancer. 2011.
FDA Drug Safety Podcast for Healthcare Professionals. Update to ongoing safety review of Lantus (insulin glargine) and possible risk of cancer. 2011.
Finfer S, Chittock DR, SY-S S, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med. 2009;360(13):1283–97.
Fioretto P, Giaccari A, Sesti G. Efficacy and safety of dapagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, in diabetes mellitus. Cardiovasc Diabetol. 2015;14:142.
Fonseca V, Schweizer A, Albrecht D, Baron MA, Chang I, Dejager S. Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes. Diabetologia. 2007;50(6):1148–55.
Gerstein HC, Bosch J, Dagenais GR, et al. Basal insulin and cardiovascular and other outcomes in dysglycemia. N Engl J Med. 2012;367(4):319–28.
Gestational diabetes mellitus. Diabetes Care. 2004;27(Suppl 1):S88–90.
Giorgino F, Benroubi M, Sun J-H, Zimmermann AG, Pechtner V. Efficacy and safety of once-weekly Dulaglutide versus insulin glargine in patients with type 2 diabetes on metformin and glimepiride (AWARD-2). Diabetes Care. 2015;38(12):2241–9.
Gogitidze Joy N, Hedrington MS, Briscoe VJ, Tate DB, Ertl AC, Davis SN. Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals. Diabetes Care. 2010;33(7):1529–35.
Gough SCL, Bode B, Woo V, et al. Efficacy and safety of a fixed-ratio combination of insulin degludec and liraglutide (IDegLira) compared with its components given alone: results of a phase 3, open-label, randomised, 26-week, treat-to-target trial in insulin-naive patients with type 2 di. Lancet Diabetes Endocrinol. 2014;2(11):885–93.
Griffond B, Risold PY, Jacquemard C, Colard C, Fellmann D. Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. Neurosci Lett. 1999;262(2):77–80.
Heine RJ, Van Gaal LF, Johns D, Mihm MJ, Widel MH, Brodows RG. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. Ann Intern Med. 2005;143(8):559–69.
Heise T, Nosek L, Rønn BB, et al. Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. Diabetes. 2004;53(6):1614–20.
Heise T, Hermanski L, Nosek L, Feldman A, Rasmussen S, Haahr H. Insulin degludec: four times lower pharmacodynamic variability than insulin glargine under steady-state conditions in type 1 diabetes. Diabetes Obes Metab. 2012;14(9):859–64.
Heller S, Damm P, Mersebach H, et al. Hypoglycemia in type 1 diabetic pregnancy: role of preconception insulin aspart treatment in a randomized study. Diabetes Care. 2010; 33(3):473–7.
Hemkens LG, Grouven U, Bender R, et al. Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. Diabetologia. 2009;52(9):1732–44.

Higgins M, Galvin D, McAuliffe F, et al. Pregnancy in women with type 1 and type 2 diabetes in Dublin. Ir J Med Sci. 2011;180(2):469–73. https://doi.org/10.1007/s11845-011-0682-8.
Hirsch IB. Insulin analogues. N Engl J Med. 2005;352(2):174–83.
Hod M, Damm P, Kaaja R, et al. Fetal and perinatal outcomes in type 1 diabetes pregnancy: a randomized study comparing insulin aspart with human insulin in 322 subjects. Am J Obstet Gynecol. 2008;198(2):186.e1–7. https://doi.org/10.1016/j.ajog.2007.08.005.
Holman RR, Thorne KI, Farmer AJ, et al. Addition of biphasic, prandial, or basal insulin to oral therapy in type 2 diabetes. N Engl J Med. 2007;357(17):1716–30.
Home P, Bartley P, Russell-Jones D, et al. Insulin detemir offers improved glycemic control compared with NPH insulin in people with type 1 diabetes: a randomized clinical trial. Diabetes Care. 2004;27(5):1081–7.
Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38(1):140–9.
Jacobs MA, Keulen ET, Kanc K, et al. Metabolic efficacy of preprandial administration of Lys (B28), Pro(B29) human insulin analog in IDDM patients. A comparison with human regular insulin during a three-meal test period. Diabetes Care. 1997;20(8):1279–86.
Janssen MM, Casteleijn S, Devillé W, Popp-Snijders C, Roach P, Heine RJ. Nighttime insulin kinetics and glycemic control in type 1 diabetes patients following administration of an intermediate-acting lispro preparation. Diabetes Care. 1997;20(12):1870–3.
Kalhan S, Rossi K, Gruca L, Burkett E, O'Brien A. Glucose turnover and gluconeogenesis in human pregnancy. J Clin Invest. 1997;100(7):1775–81.
Karter AJ, Moffet HH, Liu J, et al. Glycemic response to newly initiated diabetes therapies. Am J Manag Care. 2007;13(11):598–606.
Kaya A, Gungor K, Karakose S. Severe anaphylactic reaction to human insulin in a diabetic patient. J Diabetes Complications. 2007;21(2):124–7.
Kerssen A, de Valk HW, Visser GHA. Do HbA1c levels and the self-monitoring of blood glucose levels adequately reflect glycaemic control during pregnancy in women with type 1 diabetes mellitus? Diabetologia. 2006a;49(1):25–8.
Kerssen A, de Valk HW, Visser GHA. Forty-eight-hour first-trimester glucose profiles in women with type 1 diabetes mellitus: a report of three cases of congenital malformation. Prenat Diagn. 2006b;26(2):123–7.
Kerssen A, de Valk HW, Visser GHA. Increased second trimester maternal glucose levels are related to extremely large-for-gestational-age infants in women with type 1 diabetes. Diabetes Care. 2007;30(5):1069–74.
Kooy A, de Jager J, Lehert P, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. Arch Intern Med. 2009;169(6):616–25.
Lankisch MR, Ferlinz KC, Leahy JL, Scherbaum WA. Introducing a simplified approach to insulin therapy in type 2 diabetes: a comparison of two single-dose regimens of insulin glulisine plus insulin glargine and oral antidiabetic drugs. Diabetes Obes Metab. 2008;10(12):1178–85.
Larger E. Weight gain and insulin treatment. Diabetes Metab. 2005;31(4 Pt 2):4S51–6.
Larger E, Rufat P, Dubois-Laforgue D, Ledoux S. Insulin therapy does not itself induce weight gain in patients with type 2 diabetes. Diabetes Care. 2001;24(10):1849–50.
Le Floch J-P, Lévy M, Mosnier-Pudar H, et al. Comparison of once- versus twice-daily administration of insulin detemir, used with mealtime insulin aspart, in basal-bolus therapy for type 1 diabetes: assessment of detemir administration in a progressive treat-to-target trial (ADAPT). Diabetes Care. 2009;32(1):32–7.
Lepore M, Pampanelli S, Fanelli C, et al. Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes. 2000;49(12):2142–8.
Lingvay I, Pérez Manghi F, García-Hernández P, et al. Effect of insulin glargine up-titration vs insulin Degludec/Liraglutide on glycated hemoglobin levels in patients with uncontrolled type

2 diabetes: the DUAL V randomized clinical trial. JAMA. 2016;315(9):898–907. https://doi.org/10.1001/jama.2016.1252.
Mäkimattila S, Nikkilä K, Yki-Järvinen H. Causes of weight gain during insulin therapy with and without metformin in patients with type II diabetes mellitus. Diabetologia. 1999;42(4):406–12.
Mathiesen ER, Hod M, Ivanisevic M, et al. Maternal efficacy and safety outcomes in a randomized, controlled trial comparing insulin detemir with NPH insulin in 310 pregnant women with type 1 diabetes. Diabetes Care. 2012;35(10):2012–7.
Mathieu C, Rodbard HW, Cariou B, et al. A comparison of adding liraglutide versus a single daily dose of insulin aspart to insulin degludec in subjects with type 2 diabetes (BEGIN: VICTOZA ADD-ON). Diabetes Obes Metab. 2014;16(7):636–44.
Mattoo V, Eckland D, Widel M, et al. Metabolic effects of pioglitazone in combination with insulin in patients with type 2 diabetes mellitus whose disease is not adequately controlled with insulin therapy: results of a six-month, randomized, double-blind, prospective, multicenter, parallel-g. Clin Ther. 2005;27(5):554–67.
McCance DR, Damm P, Mathiesen ER, et al. Evaluation of insulin antibodies and placental transfer of insulin aspart in pregnant women with type 1 diabetes mellitus. Diabetologia. 2008;51(11):2141–3.
McCrimmon RJ, Sherwin RS. Hypoglycemia in type 1 diabetes. Diabetes. 2010;59(10):2333–9.
Meneghini LF, Rosenberg KH, Koenen C, Merilainen MJ, Lüddeke H-J. Insulin detemir improves glycaemic control with less hypoglycaemia and no weight gain in patients with type 2 diabetes who were insulin naive or treated with NPH or insulin glargine: clinical practice experience from a German subgroup of the PREDICTIVE st. Diabetes Obes Metab. 2007;9(3):418–27.
Misso ML, Egberts KJ, Page M, O'Connor D, Shaw J. Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. Cochrane Database Syst Rev. 2010;1:CD005103.
Muis MJ, Bots ML, Grobbee DE, Stolk RP. Insulin treatment and cardiovascular disease; friend or foe? A point of view. Diabet Med. 2005;22(2):118–26.
Murphy HR, Rayman G, Duffield K, et al. Changes in the glycemic profiles of women with type 1 and type 2 diabetes during pregnancy. Diabetes Care. 2007;30(11):2785–91.
Murphy HR, Elleri D, Allen JM, et al. Pathophysiology of postprandial hyperglycaemia in women with type 1 diabetes during pregnancy. Diabetologia. 2012;55(2):282–93.
Nakamura T, Sakaguchi K, So A, et al. Effects of insulin degludec and insulin glargine on day-to-day fasting plasma glucose variability in individuals with type 1 diabetes: a multicentre, randomised, crossover study. Diabetologia. 2015;58(9):2013–9.
Neal B, Perkovic V, de Zeeuw D, et al. Efficacy and safety of canagliflozin, an inhibitor of sodium-glucose cotransporter 2, when used in conjunction with insulin therapy in patients with type 2 diabetes. Diabetes Care. 2015;38(3):403–11.
Negrato CA, Rafacho A, Negrato G, et al. Glargine vs. NPH insulin therapy in pregnancies complicated by diabetes: an observational cohort study. Diabetes Res Clin Pract. 2010;89(1):46–51.
Owens DR. Pharmacokinetics and pharmacodynamics of insulin glargine 300 U/mL in the treatment of diabetes and their clinical relevance. Expert Opin Drug Metab Toxicol. 2016;12(8):977–87.
Parretti E, Mecacci F, Papini M, et al. Third-trimester maternal glucose levels from diurnal profiles in nondiabetic pregnancies: correlation with sonographic parameters of fetal growth. Diabetes Care. 2001;24(8):1319–23.
Pollex EK, Feig DS, Lubetsky A, Yip PM, Koren G. Insulin glargine safety in pregnancy: a transplacental transfer study. Diabetes Care. 2010;33(1):29–33.
Porcellati F, Rossetti P, Busciantella NR, et al. Comparison of pharmacokinetics and dynamics of the long-acting insulin analogs glargine and detemir at steady state in type 1 diabetes: a double-blind, randomized, crossover study. Diabetes Care. 2007;30(10):2447–52.
Pozzilli P, Leslie RD, Chan J, et al. The A1C and ABCD of glycaemia management in type 2 diabetes: a physician's personalized approach. Diabetes Metab Res Rev. 2010;26(4):239–44.
Pradhan AD, Everett BM, Cook NR, Rifai N, Ridker PM. Effects of initiating insulin and metformin on glycemic control and inflammatory biomarkers among patients with type 2 diabetes: the LANCET randomized trial. JAMA. 2009;302(11):1186–94.

Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Diabetes control and complications trial. JAMA. 1998;280(2):1140–6.

Radermecker RP, Scheen AJ. Allergy reactions to insulin: effects of continuous subcutaneous insulin infusion and insulin analogues. Diabetes Metab Res Rev. 2007;23(5):348–55.

Raskin P. Why insulin sensitizers but not secretagogues should be retained when initiating insulin in type 2 diabetes. Diabetes Metab Res Rev. 2008;24(1):3–13.

Raskin P, Guthrie RA, Leiter L, Riis A, Jovanovic L. Use of insulin aspart, a fast-acting insulin analog, as the mealtime insulin in the management of patients with type 1 diabetes. Diabetes Care. 2000;23(5):583–8.

Raskin P, Allen E, Hollander P, et al. Initiating insulin therapy in type 2 diabetes: a comparison of biphasic and basal insulin analogs. Diabetes Care. 2005;28(2):260–5.

Riddle MC, Rosenstock J, Gerich J. The treat-to-target trial: randomized addition of glargine or human NPH insulin to oral therapy of type 2 diabetic patients. Diabetes Care. 2003; 26(11):3080–6.

Riddle MC, Yki-Järvinen H, Bolli GB, et al. One-year sustained glycaemic control and less hypoglycaemia with new insulin glargine 300 U/ml compared with 100 U/ml in people with type 2 diabetes using basal plus meal-time insulin: the EDITION 1 12-month randomized trial, including 6-month extension. Diabetes Obes Metab. 2015;17(9):835–42.

Ritzel R, Roussel R, Bolli GB, et al. Patient-level meta-analysis of the EDITION 1, 2 and 3 studies: glycaemic control and hypoglycaemia with new insulin glargine 300 U/ml versus glargine 100 U/ml in people with type 2 diabetes. Diabetes Obes Metab. 2015;17(9):859–67.

Rodbard HW, Blonde L, Braithwaite SS, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of diabetes mellitus. Endocr Pract. 2007;13(Suppl 1):1–68. https://doi.org/10.4158/EP.13.S1.1.

Rodbard HW, Cariou B, Zinman B, et al. Comparison of insulin degludec with insulin glargine in insulin-naive subjects with type 2 diabetes: a 2-year randomized, treat-to-target trial. Diabet Med. 2013;30(11):1298–304.

Rosenstock J, Hollander P, Bhargava A, et al. Similar efficacy and safety of LY2963016 insulin glargine and insulin glargine (Lantus®) in patients with type 2 diabetes who were insulin-naïve or previously treated with insulin glargine: a randomized, double-blind controlled trial (the ELEMENT 2 study). Diabetes Obes Metab. 2015a;17(8):734–41.

Rosenstock J, Jelaska A, Zeller C, Kim G, Broedl UC, Woerle HJ. Impact of empagliflozin added on to basal insulin in type 2 diabetes inadequately controlled on basal insulin: a 78-week randomized, double-blind, placebo-controlled trial. Diabetes Obes Metab. 2015b;17(10):936–48.

Russell-Jones D, Khan R. Insulin-associated weight gain in diabetes – causes, effects and coping strategies. Diabetes Obes Metab. 2007;9(6):799–812.

Russell-Jones D, Vaag A, Schmitz O, et al. Liraglutide vs insulin glargine and placebo in combination with metformin and sulfonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+SU): a randomised controlled trial. Diabetologia. 2009;52(10):2046–55.

Shah RB, Patel M, Maahs DM, Shah VN. Insulin delivery methods: past, present and future. Int J Pharm Investig. 2016;6(1):1–9.

Son O, Efe B, Son NE, Akalin A, Kebapçi N. Investigation on carbohydrate counting method in type 1 diabetic patients. Biomed Res Int. 2014;2014:176564.

Standards of Medical Care in Diabetes-2016: Summary of Revisions. Diabetes Care. 2016;39 (Suppl 1):S4–5.

Strange P. Treat-to-target insulin titration algorithms when initiating long or intermediate acting insulin in type 2 diabetes. J Diabetes Sci Technol. 2007;1(4):540–8.

Talaviya PA, Saboo BD, Joshi SR, et al. Pregnancy outcome and glycemic control in women with type 1 diabetes: a retrospective comparison between CSII and MDI treatment. Diabetes Metab Syndr. 2013;7(2):68–71.

The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. The DCCT Research Group. Diabetes. 1986;35(5):530–45.

Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. JAMA. 1999;281(21):2005–12.

U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes. 1995;44(11):1249–58.

U.S. Food and Drug Administration. Early Communication About Safety of Lantus (insulin glargine).

Umpierrez GE, Palacio A, Smiley D. Sliding scale insulin use: myth or insanity? Am J Med. 2007;120(7):563–7.

Vigneri R, Squatrito S, Sciacca L. Insulin and its analogs: actions via insulin and IGF receptors. Acta Diabetol. 2010;47(4):271–8.

Vigneri R, Goldfine ID, Frittitta L. Insulin, insulin receptors, and cancer. J Endocrinol Investig. 2016;39:1365.

Vilsbøll T, Rosenstock J, Yki-Järvinen H, et al. Efficacy and safety of sitagliptin when added to insulin therapy in patients with type 2 diabetes. Diabetes Obes Metab. 2010;12(2):167–77.

Vuorinen-Markkola H, Koivisto VA, Yki-Jarvinen H. Mechanisms of hyperglycemia-induced insulin resistance in whole body and skeletal muscle of type I diabetic patients. Diabetes. 1992;41(5):571–80.

Wahabi HA, Alzeidan RA, Esmaeil SA. Pre-pregnancy care for women with pre-gestational diabetes mellitus: a systematic review and meta-analysis. BMC Public Health. 2012;12:792.

Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet (London). 2008;371(9626):1753–60.

Wu JW, Filion KB, Azoulay L, Doll MK, Suissa S. Effect of long-acting insulin analogs on the risk of cancer: a systematic review of observational studies. Diabetes Care. 2016;39(3):486–94.

Yessoufou A, Moutairou K. Maternal diabetes in pregnancy: early and long-term outcomes on the offspring and the concept of "metabolic memory". Exp Diabetes Res. 2011;2011:218598.

Yki-Järvinen H. Combination therapies with insulin in type 2 diabetes. Diabetes Care. 2001;24(4):758–67.

Yogev Y, Ben-Haroush A, Chen R, Rosenn B, Hod M, Langer O. Diurnal glycemic profile in obese and normal weight nondiabetic pregnant women. Am J Obstet Gynecol. 2004;191(3):949–53.

Zaccardi F, Htike ZZ, Webb DR, Khunti K, Davies MJ. Benefits and harms of once-weekly glucagon-like Peptide-1 receptor agonist treatments: a systematic review and network meta-analysis. Ann Intern Med. 2016;164(2):102–13.

Insulin Pumps

22

John C. Pickup

Contents

Abstract

Insulin pump therapy (continuous subcutaneous insulin infusion, CSII) is a form of intensified insulin treatment involving subcutaneous infusion of short-acting insulin from a portable pump. There is a well-established evidence base for the effectiveness of CSII in type 1 diabetes, which includes reduction in HbA_{1c}, blood glucose variability, and all grades of hypoglycemia compared to MDI, but more research is needed on how new long-acting insulin preparations and more effective diabetes education will reduce the number who do not achieve target levels of control on MDI and who are thus candidates for CSII. Insulin pump therapy is an affordable, cost-effective therapeutic option for most healthcare

J. C. Pickup (✉)
Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Guy's Hospital, London, UK
e-mail: john.pickup@kcl.ac.uk

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_22

settings. There is an increasing role for CSII in patients with poorly controlled type 2 diabetes who are not adequately managed on MDI, but smaller, cheaper, and simpler "patch" pumps are likely to be needed to make insulin pump therapy cost-effective in this type of diabetes.

Keywords
Insulin pump therapy · Continuous subcutaneous insulin infusion · CSII · Type 1 diabetes · Type 2 diabetes · Hypoglycemia · Glycemic control · Intensified insulin therapy

Introduction

The term "insulin pump therapy" is now synonymous with continuous subcutaneous insulin infusion (CSII), a type of intensified insulin treatment based on variable-rate infusion of short-acting insulin from a portable pump and delivered via a cannula implanted in the subcutaneous tissue. CSII was originally developed in the 1970s (Pickup et al. 1978) as an experimental procedure to test the effects of prolonged near-normoglycemia on diabetic microvascular complications (injection regimens of the time were not able to maintain strict glycemic control in type 1 diabetes), but it quickly entered routine clinical practice as a therapeutic option for selected people with type 1 diabetes. CSII use was particularly encouraged by the results of the DCCT (Diabetes Control and Complications Trial 1993) in the 1990s, which showed the importance of strict glycemic control in preventing diabetic microangiopathy and where the intensified arm in the trial consisted of either CSII or multiple daily insulin injections (MDI). The uptake of insulin pump therapy in the last 20 years or so has also been encouraged by the commercial availability of more reliable and flexible insulin pumps with adjustable infusion rates and alarm systems for malfunctions. The increasing evidence base for insulin pump effectiveness compared to MDI, and the appearance of several national and international guidelines that advise on the best use of CSII in clinical practice have also promoted the increasing use of insulin pump therapy.

The principle of CSII is to obtain better metabolic control in diabetes by mimicking non-diabetic insulin administration with a slow delivery of short-acting insulin throughout the day and night (basal insulin) and boosts at meal times (prandial insulin or boluses). The slow basal infusion of insulin with CSII (about 1 unit/h for an adult) has several pharmacological advantages that help in improving glycemic control. There is a much lower variability of subcutaneous insulin absorption with CSII (coefficient of variation [cv] about ±5%) compared to depot injections of long-acting insulin injections like isophane insulin (cv about ±50%) (Lauritzen et al. 1983), accounting for a reduction in within- and between-day blood glucose variability (see below). The constant and controllable basal infusion also produces flatter circulating insulin levels than many long-acting insulin formulations, especially at night, resulting in less risk of nocturnal hypoglycemia. The facility to automatically alter the basal rate at a preset time enables, for example, an

increase in rate during the hours before breakfast to counter the elevated blood glucose levels at this time in some patients (the "dawn phenomenon") (Koivisto et al. 1986), or the basal rate can be reduced to avoid hypoglycemia during and after exercise, not possible when depot insulin has been injected before exercise (Perkins and Ridell 2006).

The current practice of CSII is to use short-acting monomeric insulin in the pump (aspart, lispro or glulisine). It is recommended that CSII is initiated and supervised by a specialist team consisting of a physician with an interest and training in insulin pump therapy, a diabetes nurse educator and a dietician. Patients who are candidates for CSII should be motivated and willing to undertake CSII procedures, particularly frequent self-monitoring of blood glucose and carbohydrate counting. Further practical details of how to start and manage patients on CSII and some recent advances in pump therapy can be found elsewhere (Grunberger et al. 2014; Pickup 2012; Pozzilli et al. 2016).

The Benefits of Insulin Pump Therapy in Type 1 Diabetes

Reduction in HbA_{1c}

There is still some controversy about the magnitude of the likely improvement in glycemic control when CSII is compared to modern MDI regimens. This is partly because in some trials both MDI and CSII might have been used suboptimally and because inappropriate types of patients have been entered in some trials (see below). The majority of randomized controlled trials (RCTs) of CSII vs. MDI have employed isophane-based MDI rather than long-acting insulin formulations with more predictable absorption like glargine, detemir, and degludec, which may offer improved control in their own right, at least in some patients. Also, the intensity of structured diabetes education applied during MDI in some trials has been variable, leading some to question whether the strict control of CSII could not be matched by best contemporary MDI regimens that include appropriate insulin regimens and educational approaches such as carbohydrate counting and insulin dosage adjustment. Equally, there are several measures to optimize CSII that are not always applied, including the use of bolus calculators, appropriate bolus profiles and timing of meal insulin, computer download of pump data to detect therapeutic errors and adjust infusion rates, and so on.

A number of meta-analyses of RCTs comparing glycemic control during CSII and MDI have shown that HbA_{1c} is on average about 0.3–0.6% lower on CSII than on MDI (Pickup et al. 2002; Weissberg-Benchell et al. 2003; Pickup and Sutton 2008; Misso et al. 2010). However, some of these analyses included RCTs from early insulin pump trials with now obsolete pumps or where non-monomeric insulin was used, or trials where there was a near-normal baseline (MDI) HbA_{1c}. This last point is important because there is clear evidence from pooled individual patient data from RCTs (Retnakaran et al. 2004), from meta-regression of the effect size in RCTs (Pickup and Sutton 2008), and from individual patient responses in clinic patients

(Pickup et al. 2006) that the greatest fall in HbA_{1c} is in those with worst control at baseline. Thus, a rather modest mean effect size of, say, 0.5% (5 mmol/mol) in a meta-analysis does not reflect the much larger expected difference of about 1.5% (16 mmol/mol) in those with an elevated baseline HbA_{1c} of, say, 9% (75 mmol/mol).

In the long-term (at least 5 years of pump therapy), some 90% of patients with type 1 diabetes maintain a lower HbA_{1c} on CSII than their starting HbA_{1c} on MDI but not all subjects achieve optimal control (Nixon et al. 2014) (Fig. 1). In about 30% of subjects switched from MDI to CSII because of elevated HbA_{1c}, HbA_{1c} improves over 1–2 years and good control is maintained over the entire period. In about 60% of patients, the HbA_{1c} improves on CSII reaching a nadir after 1–2 years, but then control starts to deteriorate somewhat. Some 10% of subjects do not improve at any time on insulin pump therapy.

It is unclear why a small proportion of patients with type 1 diabetes and poor glycemic control on MDI fail to benefit from CSII. These nonresponders do not appear to have an excessive fear of hypoglycemia that prevent them from tightening control, but they are more likely to have a higher BMI than responders (Nixon et al. 2014), suggesting a lack of dietary compliance and insulin resistance might be issues. Study of the psychological characteristics of CSII patients has also shown that nonresponders as a group have a high external locus of control, believing that their diabetes is dependent on external events and beyond their control (Aberle et al. 2009). It is not known whether psychological intervention can help to improve control in this group.

These varying long-term outcomes of CSII have emphasized the need for regular follow-up in the clinic, where worsening control can be detected at an early stage and measures instigated to re-establish near-normoglycemia. A check list of the main targets for review in poorly controlled pump patients is useful and covers bolus insulin timing, profiles and missed boluses, basal insulin, infusion set practice, diet review, and a consideration of sensor-augmented pump therapy.

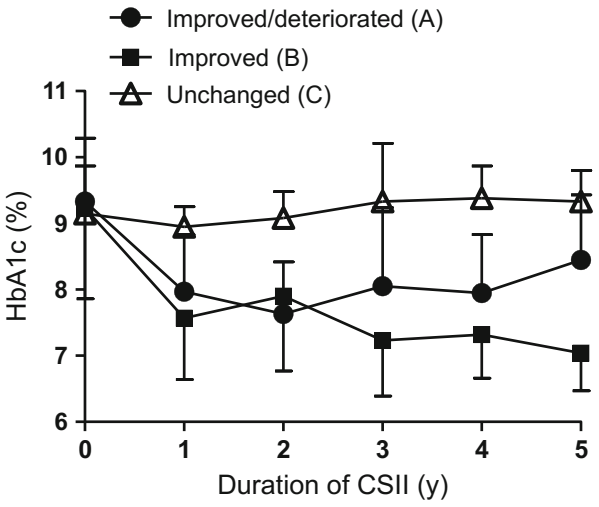


Fig. 1 Long-term changes in HbA_{1c} levels in people with poorly controlled type 1 diabetes switched from MDI to CSII. Patients can be divided into those where HbA_{1c} improves markedly and then worsens somewhat after about 2 years (A), those where HbA_{1c} improvement is maintained over at least 5 years (B), and those where HbA_{1c} does not significantly improve at any time (C). Data from Nixon et al. (2014)

Timing of bolus insulin before meals. Many patients treated by CSII continue to administer the meal insulin bolus at the start of the meal (and a few give the insulin after the meal), encouraged perhaps by healthcare professionals who believe that the short-acting monomeric insulins are sufficiently quickly absorbed to control adequately meal-induced hyperglycemia when delivered at this time. But this practice can lead to excessive post-prandial blood glucose increases, and studies have shown that giving the bolus 15–20 min before the meal is an optimal timing that can reduce the blood glucose by 2–3 mmol/l compared to immediate pre-meal bolusing (Cobry et al. 2010).

Appropriate meal insulin profiles. High-fat meals cause late and excessive post-prandial hyperglycemia because fat delays gastric emptying and causes insulin resistance. The extended/square wave feature on modern pumps is an option for the bolus to be administered over some hours, instead of the usual immediate delivery. Square wave or dual wave (the combination of immediate and extended) meal insulin profiles have been shown to manage some high-fat meals better than traditional bolusing (Jones et al. 2005), and it is worth re-educating patients on the value and appropriate use of this technology.

Missed boluses. Missing meal boluses is common, especially in children and adolescents (Olinder et al. 2009), and a low number of boluses per day is highly correlated with elevated HbA_{1c} in pump patients. Reasons for missing boluses may include forgetting, not bothering, attempting to avoid hypoglycemia or avoiding weight gain. Missed boluses can be detected by computer downloads of pump data and advice to give the bolus 20 min before meals may help to remind the patient about giving meal boluses.

Basal insulin review. In addition to checking that the overnight and daytime basal rates are appropriate, it is worth noting that frequent basal rate changes throughout the day can be associated with poor and erratic control (Laimer et al. 2016), perhaps because several hours are needed for a new steady state circulating insulin to be reached after each step change in rate. In clinical practice, reducing the number of basal rate changes can often improve control, and most patients with type 1 diabetes can be managed by no more than two or three basal rates per day.

Infusion set practice. Infusion site lipohypertrophy is common: we found in a survey of non-metabolic complications of CSII that about 25% of patients reported obvious lipohypertrophy, most frequently in those with a long duration of CSII (Pickup et al. 2014), and it is probably much more frequent if careful examination for lipohypertrophy were made by healthcare professionals. Lipohypertrophy is a known cause of impaired insulin absorption and poor and erratic control and is caused by insulin administration, either by injection or infusion, over a period of time at the same site. We also found that use of the set for more than 3 days was associated more often with infusion set blockage, presumably due to insulin aggregation. It should be recommended, therefore, that patients rotate each new infusion set to a different anatomical site and limit use of each set to no more than 3 days.

Diet. Although overall the weight does not change in type 1 diabetic patients switched to CSII, about one third of type 1 diabetic patients gain weight on CSII and this makes optimal control more difficult. Some patients may believe the new dietary

freedom on the pump allows them to "eat anything," or calories formally lost as glycosuria in the hyperglycemic patient may be retained when CSII is started and better control is achieved. Review by the dietician is very helpful in limiting weight gain.

Reduction in Blood Glucose Variability and Hypoglycemia

Both within-day and between-day blood glucose variability are reduced by switching from MDI to CSII (Pickup et al. 2005). High glycemic variability is associated with a high frequency of hypoglycemia, and reducing variability is a major way in which CSII reduces hypoglycemia. All grades of hypoglycemia are reduced by switching from MDI to CSII. For example, meta-analysis of RCTs and observational studies of hypoglycemia-prone type 1 diabetic subjects shows that severe hypoglycemia is reduced by about 75% on CSII versus MDI (Pickup and Sutton 2008). Those subjects with the most frequent hypoglycemia during MDI have the largest improvement on CSII, and the reduction in severe hypoglycemia with CSII is maintained over several years (Quirós et al. 2016). Lesser degrees of hypoglycemia ("mild to moderate") are less well studied in RCTs but the percentage of self-monitored blood glucose levels <3.5 mmol/l is also reported to be about 75% less on CSII than MDI in some observational studies (Pickup et al. 2005).

In patients where hypoglycemia persists with CSII, the addition of continuous glucose monitoring (CGM), often called "sensor-augmented pump therapy," should be offered. The most advanced form of this is the use of low-glucose insulin-suspend (LGS) pumps, where the basal infusion rate is automatically suspended for up to 2 h when CGM-measured glucose concentrations fall below a preset threshold or when hypoglycemia is predicted to occur over some horizon, usually 30 min. Several, observational studies and RCTs indicate that the duration of nocturnal hypoglycemia and the frequency of severe hypoglycemia are further reduced with LGS pumps compared to traditional CSII (Bergenstal et al. 2013; Choudhary et al. 2011, 2013; Ly et al. 2013).

Reduced Mortality

Comparatively little is known about long-term clinical outcomes such as vascular disease in patients treated by CSII vs. MDI, but recent information on mortality from the Swedish National Diabetes Registry is of note (Steineck et al. 2015). Here, data on 2441 type 1 diabetic patients on CSII were compared with 15,727 on MDI, and cardiovascular events or deaths were studied over a mean 6.8-year follow-up. All-cause mortality was reduced by 27% on CSII, coronary heart disease (CHD) mortality by 45%, and stroke and CHD mortality by 42%. There are many reasons why mortality may be less on CSII; HbA_{1c} levels were similar in the two groups (a mean of 7.9 vs. 8.0% [63 vs. 64 mmol/mol], CSII vs. MDI), but the number of patients experiencing ≥ 3 episodes of hypoglycemia was significantly less on CSII,

possibly pointing to less risk of hypoglycemia-induced cardiac arrhythmias. A high frequency of severe hypoglycemia is strongly related to increased mortality in both type 1 and type 2 diabetes (McCoy et al. 2012). Other risk factors that may influence cardiovascular disease such as glycemic variability and lifestyle factors such as diet and exercise were not measured in this study.

Improved Quality of Life and Treatment Satisfaction

The discontinuation rate for CSII is low, less than 5% at most centers (Pickup 2012), indicating a good overall level of satisfaction with the treatment, though it is somewhat higher in adolescents and females (de Vries et al. 2011). Some RCTs comparing glycemic control and quality of life during CSII and MDI (for example, as assessed by measures such as the SF-36 score) show a clear benefit with insulin pumps (deVries et al. 2002), but other studies have shown little or no improvement in quality of life with CSII. One may speculate that this may be because patients in some trials were relatively well controlled with little hypoglycemia and therefore had a good quality of life at baseline, and thus were expected to show little improvement in quality of life on switching to CSII. Probably the largest improvement in quality of life with CSII then is with patients suffering from frequent severe hypoglycemia and prolonged elevated HbA_{1c} on MDI.

CSII in Children

Insulin pump therapy has been used safely and effectively in children and adolescents since CSII first entered clinical practice in the 1970s (Tamborlane et al. 1979) and it continues to be a popular therapy in this age group (Kordonouri et al. 2011). However, uptake of pumps in young people with type 1 diabetes varies markedly between countries (as it does in adults): a recent survey of data from more than 54,000 type 1 diabetic patients in three large registries in Germany/Austria, the US Type 1 Diabetes Exchange and in England and Wales showed that uptake was 41% in Germany/Austria, 47% in USA but only 14% in England and Wales (Sherr et al. 2016). Interestingly, HbA_{1c} was highest in the low-use countries: 8.9% versus 8.0% and 8.3% (74 vs. 64 vs. 67 mmol/mol), England and Wales versus Germany/Austria versus USA, though there may be several reasons (such as socioeconomic status) why patients in some countries have a poorer diabetes control than others.

Special considerations for the use of CSII in young people include the fact that children are often unwilling or unable to perform MDI, particularly with the need for supervised midday injections at school, so many practitioners and guidelines consider it is appropriate to start CSII in children without them having first “failed” on MDI (National Institute for Health and Care Excellence 2008). Also, adolescents are more likely to discontinue the pump (de Vries et al. 2011) and may achieve somewhat worse control than adults, perhaps related to the known insulin resistance of adolescence, and to erratic sleep and exercise patterns, and adherence issues.

CSII in Pregnancy

Insulin pumps may be used effectively in pregnancy and in the preconception period, under the same guidelines for nonpregnant subjects – when an elevated HbA_{1c} or hypoglycemia persists with MDI (see below) – though since glycemic targets are lower in pregnancy, an appropriate indication might be when an $HbA_{1c} < 6.1\%$ (43 mmol/mol) (or according to national pregnancy guidelines) cannot be achieved on MDI without disabling hypoglycemia. There is no evidence that glycemic control or pregnancy outcomes such as pre-eclampsia, congenital abnormalities, birth weight, neonatal hypoglycemia, and stillbirths are different on MDI vs. CSII, though there are comparatively few RCTs available on this topic (Mukhopadhyay et al. 2007). More research is needed, particularly in pregnant diabetic women who have failed to achieve glycemic targets on MDI before being randomized to CSII.

Cost-Effectiveness of CSII

A systematic review of 11 formal cost-effectiveness studies of CSII vs. MDI in type 1 diabetes in eight countries has shown that it may be considered value for money for healthcare systems in all or most settings (Roze et al. 2015). CSII was on average 1.4 times more costly than MDI in this review but the higher lifetime costs are partially offset by cost-savings from reduced diabetes-related complications. With a base case HbA_{1c} of 8.7% (72 mmol/mol), the mean incremental cost-effectiveness ratio (ICER) was Euros 30,862 (US $40,143) per quality-adjusted life year (QALY) gained. The results were highly sensitive to the degree of reduction in HbA_{1c} and frequency of hypoglycemia, with the best affordability in those with worst control at baseline. What is considered value for money will differ between countries and healthcare systems, but since the unofficial willingness-to-pay threshold used by the widely influential UK National Institute for Health and Care Excellence (NICE) is <£30,000 (Euros 36,158) per QALY, CSII will be considered cost-effective in most countries.

Insulin Pump Therapy in Type 2 Diabetes

Until recently, CSII was usually reserved for selected patients with type 1 diabetes, and many guidelines (e.g., NICE 2008) do not recommend insulin pump therapy in type 2 diabetes because of the poor and conflicting evidence of effectiveness in the limited number of RCTs that have been published (Raskin et al. 2003; Wainstein et al. 2005). However, a number of observational studies in the last decade or so have indicated that many patients with type 2 diabetes who are poorly controlled on MDI may achieve a significant improvement in HbA_{1c} on switching to CSII (Edelman et al. 2010; Leinung et al. 2013), and the reduction appears to be maintained over many years (Morera et al. 2016).

A recent large, multicenter RCT has added further weight to the evidence base for use of CSII in type 2 diabetes. In the OpT2mise trial (Reznik et al. 2014), patients underwent a pre-randomization period of optimization designed to improve control on MDI, and only those with a persistently elevated HbA_{1c} (8–12%, 64–108 mmol/mol) and insulin dose of 0.7–1.8 units/kg were randomized to continued MDI or CSII. After 6 months, the mean HbA_{1c} difference between CSII and MDI was 0.7% (8 mmol/mol), favoring pump therapy, with a 20% insulin dose reduction and no increased hypoglycemia. Those with the highest baseline HbA_{1c} enjoyed the greatest reduction on CSII, a difference of 1.1% (12 mmol/mol) for those with an HbA_{1c} of 9.3–11.5% (78–102 mmol/mol) on MDI.

There are several reasons why control in type 2 diabetes may be better on CSII. For example, there is evidence that large depot doses of long-acting insulin formulations like glargine that are given in the insulin-resistant type 2 diabetic patient are more poorly absorbed than the same dose of insulin administered as the slow infusion of CSII (Parkner et al. 2008). Treatment satisfaction also tends to be better with CSII than MDI in type 2 diabetes (Raskin et al. 2003), so adherence to treatment may be improved with pump therapy.

Trials to date of insulin pumps in type 2 diabetes have used the traditional pumps used for type 1 diabetes, but there is increasing evidence that sophisticated pumps with flexible basal rate and bolus dose adjustment, and bolus calculators are not required for type 2 diabetes. Most patients with type 2 diabetes can be managed with a single basal rate throughout the 24 h (Edelman et al. 2010) with a simple meal-time insulin delivery. A number of manufacturers are now developing simpler, cheaper "patch" pumps which use one of a limited number of preset basal rates and simple (say 2-unit amount) meal-insulin delivery. These are likely to be more suitable and cost-effective for the large number of potential candidates for CSII in the type 2 diabetes community.

Guidelines and Indications for Best Use of CSII (Table 1)

In the UK, NICE considers that CSII is a treatment option in adults with type 1 diabetes either: when HbA_{1c} remains elevated ($\geq$8.5%, 69 mmol/mol) after best attempts with MDI or when there is continued disabling hypoglycemia on MDI (National Institute for Health and Care Excellence 2008). In children, in addition to the above indications, CSII may be used when in the opinion of the physician MDI is considered impractical. The cut-off HbA_{1c} of 8.5% (69 mmol/mol) in these guidelines is the level at which CSII is thought to be cost-effective and affordable for the National Health Service in the UK, rather than the level below which microvascular complications are not thought to occur and lowering of HbA_{1c} not thought to be worthwhile. Other healthcare systems may set this level at a lower HbA_{1c} value, say 7.5% (58 mmol/mol), though this recommendation is not always based on formal cost-effectiveness calculations. The American Association of Clinical Endocrinologists and the American College of Endocrinology have recommended CSII in type 1

Table 1 Suggested indications for a trial of insulin pump therapy in diabetes

In type 1 diabetes
When there is continued elevated HbA_{1c} after best attempts with MDI, including basal-bolus insulin injection therapy with long-acting insulin analogues such as glargine, detemir, and degludec), frequent SMBG, structured diabetes education, and frequent contact with a multidisciplinary team of healthcare professionals. Note: CSII has been shown to be cost-effective for most healthcare systems when the baseline $HbA_{1c} \geq 8.5\%$ (69 mmol/mol), but this cut-off may vary between national guidelines, re-imbursement and healthcare systems, according to the "willingness-to-pay" threshold.
When there is continued disabling hypoglycemia after best attempts with MDI. Note: Usually, this is in the judgment of the physician, as the definition of "disabling" is not agreed, but for most healthcare systems it refers to frequent episodes of severe hypoglycemia, requiring third party assistance.
In children and adolescents, when there is elevated HbA_{1c} and disabling hypoglycemia on MDI, as above, but also when in the judgment of the physician MDI is considered inappropriate of impractical CSII may be started without having first "failed" on MDI.
In the first trimester of pregnancy or pre-conceptually when target HbA_{1c} levels (<6.1%, 43 mmol/mol, or according to national guidelines) cannot be achieved without disabling hypoglycemia.
When funding is available and a specialist team of trained healthcare professionals is available to initiate and supervise follow-up, CSII may be trialed for those who may not necessarily have grossly elevated HbA_{1c} levels or frequent severe hypoglycemia but may have a personal preference for this therapy because of potential benefits in lifestyle flexibility, well-being, and ability to perform confidently and effectively.
In type 2 diabetes
When there is continued elevated HbA_{1c} in spite of best attempts to reach target glycemic levels with MDI and structured diabetes education and other adjunctive therapy such as GLP-1 inhibitors. Note: Many national guidelines do not yet recommend the routine use of CSII in type 2 diabetes or have not established a cut-off HbA_{1c} level above which CSII is cost-effective, but guidance is under active review

CSII continuous subcutaneous insulin infusion, *GLP* glucagon-like peptide, *MDI* multiple daily insulin injections, *SMBG* self-monitoring of blood glucose

diabetes when patients "do not reach glycemic goals despite adherence to maximum MDI."

There is continued debate on whether the use of CSII should be expanded to include patients with lesser degrees of poor diabetes control who just prefer insulin pump therapy as their form of intensive insulin therapy or who wish to enjoy the improved quality of life and flexibility of lifestyle associated with CSII. When funding and the specialist team of healthcare professionals are available for supervision, there seems no reason to exclude such patients (Table 1).

Summary

There is a well-established evidence base for the effectiveness of CSII in type 1 diabetes, which includes reduction in HbA_{1c} and all grades of hypoglycemia compared to MDI, but more research is needed on how new long-acting insulin

preparations and more effective diabetes education will reduce the number who do not achieve target levels of control on MDI. There is an increasing role for CSII in patients with poorly controlled type 2 diabetes who are not managed on MDI, but smaller, cheaper, and simpler patch pumps are likely to be needed to make insulin pump therapy cost-effective in this type of diabetes.

References

Aberle I, Scholz U, Bach-Kliegel B, Fischer C, Gorny M, Langer K, Kliegel M. Psychological aspects in continuous subcutaneous insulin infusion: a retrospective study. J Psychol. 2009;14:147–60.

Bergenstal RM, Klonoff DC, Garg SK, Bode BW, Meredith M, Slover RH, Ahmann AJ, Welsh JB, Lee SW, Kaufman FR, ASPIRE In-Home Study Group. Threshold-based insulin-pump interruption for reduction of hypoglycemia. NEJM. 2013;369:224–32.

Choudhary P, Shin J, Wang Y, Evans ML, Hammond PJ, Kerr D, Shaw JA, Pickup JC, Amiel SA. Insulin pump therapy with automated insulin suspension in response to hypoglycemia: reduction in nocturnal hypoglycemia in those at greatest risk. Diabetes Care. 2011;34:2023–5.

Choudhary P, Ramasamy S, Green L, Gallen G, Pender S, Brackenridge A, Amiel SA, Pickup JC. Real-time continuous glucose monitoring reduces severe hypoglycemia in hypoglycemia-unaware patients with type 1 diabetes. Diabetes Care. 2013;36:4160–2.

Cobry E, McFann K, Messer L, Gage V, VanderWel B, Horton L, Chase PH. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. Diabet Technol Ther. 2010;12:173–7.

de Vries L, Grushka Y, Lebenthal Y, Shalitin S, Phillip M. Factors associated with increased risk of insulin pump discontinuation in pediatric patients with type 1 diabetes. Pediatr Diabetes. 2011;12:506–12.

DeVries JH, Snoek FJ, Kostense PJ, Masurel N, Heine RJ. A randomized trial of continuous subcutaneous insulin infusion and intensive injection therapy in type 1 diabetes for patients with long-standing poor glycemic control. Diabetes Care. 2002;25:2074–80.

Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. NEJM. 1993;329:977–86.

Edelman S, Bode BW, Bailey TS, Kipnes MS, Brunelle R, Chen X, Frias JP. Insulin pump therapy in patients with type 2 diabetes. Safely improved glycemic control using a simple insulin dosing regimen. Diabet Technol Ther. 2010;12:627–33.

Grunberger G, Abelseth J, Bailey T, Bode B, Handelsman Y, Hellman R, Jovanovič L, Lane W, Raskin P, Tamborlane W, Rothermel C. Consensus statement by the American Association of Clinical Endocrinologists/American College of Endocrinology Insulin Pump Management Task Force. Endocr Pract. 2014;20:463–89.

Jones SM, Quarry JL, Caldwell-McMillan M, Mauger DT, Gabbay RA. Optimal insulin pump dosing and postprandial glycemia following a pizza meal using the continuous glucose monitoring system. Diabet Technol Ther. 2005;7:233–40.

Koivisto VA, Yki-Järvinen H, Helve E, Pelkonen R. Pathogenesis and prevention of the dawn phenomenon in diabetic patients treated with CSII. Diabetes. 1986;35:78–82.

Kordonouri O, Hartmann R, Danne T. Treatment of type 1 diabetes in children and adolescents using modern insulin pumps. Diabetes Res Clin Pract. 2011;93(Suppl):S118–24.

Laimer M, Melmer A, Mader JK, Schütz-Fuhrmann I, Engels H-R, Götz G, Pfeifer M, Hermann JM, Stettler C, Holl RW. Variability of basal rate profiles in insulin pump therapy and association with complications in type 1 diabetes mellitus. PLoS One. 2016;11(3):e0150604. https://doi.org/10.1371/journal.pone.0150604.

Lauritzen T, Pramming S, Deckert T, Binder C. Pharmacokinetics of continuous subcutaneous insulin infusion. Diabetologia. 1983;24:326–9.
Leinung M, Thompson S, Mingfrei Luo PA-C, Leykina L, Nardacci E. Use of insulin pump therapy in patients with type 2 diabetes after failure of multiple daily injections. Endocr Pract. 2013;19:9–13.
Ly TT, Nicholas JA, Retterath A, Lim EM, Davis EA, Jones TW. Effect of sensor-augmented insulin pump therapy and automated insulin suspension vs standard insulin pump therapy on hypoglycemia in patients with type 1 diabetes: a randomized clinical trial. JAMA. 2013;310: 1240–7.
McCoy RG, Van Houten HK, Ziegenfuss JY, Shah ND, Wermers RA, Smith SA. Increased mortality of patients with diabetes reporting severe hypoglycemia. Diabetes Care. 2012;35: 1897–901.
Misso ML, Egberts KJ, Page M, O'Connor D, Shaw J. Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. Cochrane Database Syst Rev. 2010;(1):Art. No.: CD005103. https://doi.org/10.1002/14651858.CD005103.pub2.
Morera J, Joubert M, Morello R, Rod A, Lireux B, Reznik Y. Sustained efficacy of insulin pump therapy in type 2 diabetes: 9-year follow-up in a cohort of 161 patients. Diabetes Care. 2016;39: e74–5.
Mukhopadhyay A, Fraser RB, Bolarinde O. Continuous subcutaneous insulin infusion vs. intensive conventional insulin therapy in pregnant diabetic women: a systematic review and meta-analysis of randomized, controlled trials. Am J Obstet Gynecol. 2007;197:447–56.
National Institute for Health and Care Excellence. Continuous subcutaneous insulin infusion for the treatment of diabetes mellitus. In: Technology appraisal guidance 151 (review of technology appraisal guidance 57). London, UK: NICE; 2008.
Nixon R, Folwell R, Pickup JC. Variations in the quality and sustainability of long-term glycaemic control with continuous subcutaneous insulin infusion. Diabet Med. 2014;31:1174–7.
Olinder AL, Kernell A, Smide B. Missed bolus doses: devastating for metabolic control in CSII-treated adolescents with type 1 diabetes. Pediatr Diabetes. 2009;10:142–8.
Parkner T, Laursen T, Vestergaard ET, Hartvig H, Smedegaard JS, Lauritzen T, Christiansen JS. Insulin and glucose profiles during continuous subcutaneous insulin infusion compared with injection of a long-acting insulin in type 2 diabetes. Diabet Med. 2008;25:585–91.
Perkins BA, Ridell MC. Type 1 diabetes and exercise: using the insulin pump to maximum advantage. Can J Diabet. 2006;30:72–9.
Pickup JC. Insulin-pump therapy for type 1 diabetes mellitus. NEJM. 2012;366:1616–24.
Pickup JC, Sutton AJ. Severe hypoglycaemia and glycaemic control in type 1 diabetes: meta-analysis of multiple daily insulin injections versus continuous subcutaneous insulin infusion. Diabet Med. 2008;25:765–74.
Pickup JC, Keen H, Parsons JA, Alberti KGMM. Continuous subcutaneous insulin infusion: an approach to achieving normoglycaemia. BMJ. 1978;i:204–7.
Pickup JC, Mattock MB, Kerry S. Glycaemic control with continuous subcutaneous insulin infusion compared to intensive insulin injection therapy in type 1 diabetes: meta-analysis of randomised controlled trials. BMJ. 2002;324:705–8.
Pickup JC, Kidd J, Burmiston S, Yemane N. Effectiveness of continuous subcutaneous insulin infusion in hypoglycaemia-prone type 1 diabetes: implications for NICE guidelines. Pract Diabet Int. 2005;22:10–4.
Pickup JC, Kidd J, Burmiston S, Yemane N. Determinants of glycaemic control in type 1 diabetes during intensified therapy with multiple daily insulin injections or continuous subcutaneous insulin infusion: importance of blood glucose variability. Diabet Metab Res Rev. 2006;22: 232–7.
Pickup JC, Yemane N, Brackenridge A, Pender S. Nonmetabolic complications of continuous subcutaneous insulin infusion: a patient survey. Diabet Technol Ther. 2014;16:145–9.

Pozzilli P, Battelino T, Danne T, Hovorka R, Jarosz-Chobot P, Renard E. Continuous subcutaneous insulin infusion in diabetes: patient populations, safety, efficacy, and pharmacoeconomics. Diabetes Metab Res Rev. 2016;32:21–39.

Quirós C, Giménez M, Ríos R, Careaga M, Roca D, Vidal M, Conget I. Long-term outcome of insulin pump therapy: reduction of hypoglycaemia and impact on glycaemic control. Diabet Med. 2016;33:1422. https://doi.org/10.1111/dme.13094.

Raskin P, Bode BW, Marks JB, Hirsch IB, Weinstein RL, McGill JB, Peterson GE, Mudaliar SR, Reinhardt RR. Continuous subcutaneous insulin infusion and multiple daily injection therapy are equally effective in type 2 diabetes: a randomized, parallel-group, 24-week study. Diabetes Care. 2003;26:2598–603.

Retnakaran R, Hochman J, DeVries JH, Hanaire-Broutin H, Heine RJ, Melki V, Zinman B. Continuous subcutaneous insulin infusion versus multiple daily injections. The impact of baseline A1c. Diabetes Care. 2004;27:2590–6.

Reznik Y, Cohen O, Aronson R, Conget I, Runzis S, Castaneda J, Scott W Lee. Insulin pump treatment compared with multiple daily injections for treatment of type 2 diabetes (OpT2mise): a randomised open-label controlled trial. The Lancet. 2014;384(9950):1265–1272.

Roze S, Smith-Palmer J, Valentine W, de Portu S, Nørgaard K, Pickup JC. Cost-effectiveness of continuous subcutaneous insulin infusion versus multiple daily injections of insulin in type 1 diabetes: a systematic review. Diabet Med. 2015;32:1415–24.

Sherr JL, Hermann JM, Campbell F, Foster NC, Hofer SE, Allgrove J, Maahs DM, Kapellen TM, Holman N, Tamborlane WV, Holl RW, Beck RW, Warner JT, T1D Exchange Clinic Network, DPV Initiative, National Paediatric Diabetes Audit, Royal College of Paediatrics and Child Health registries. Use of insulin pump therapy in children and adolescents with type 1 diabetes and its impact on metabolic control: comparison of results from three large, transatlantic paediatric registries. Diabetologia. 2016;59:87–91.

Steineck I, Cederholm J, Eliasson B, Rawshani A, Eeg-Olofsson K, Svensson A-M, Zethelius B, Avdic T, Landin-Olsson M, Jendle J, Gudbjörnsdóttir S, the Swedish National Diabetes Register. Insulin pump therapy, multiple daily injections, and cardiovascular mortality in 18,168 people with type 1 diabetes: observational study. BMJ. 2015;350:h3234.

Tamborlane WV, Sherwin RS, Genel M, Felig P. Reduction to normal of plasma glucose in juvenile diabetes by subcutaneous administration of insulin with a portable infusion pump. NEJM. 1979;300:573–8.

Wainstein J, Metzger M, Boaz M, Minuchin O, Cohen Y, Yaffe A, Yerushalmy Y, Raz I, Harman-Boehm I. Insulin pump therapy vs. multiple daily injections in obese type 2 diabetic patients. Diabet Med. 2005;22:1037–46.

Weissberg-Benchell J, Antisdel-Lomaglio J, Seshadri R. Insulin pump therapy. A meta-analysis. Diabetes Care. 2003;26:1079–87.

Islet Cell or Pancreas Transplantation

23

Lorenzo Piemonti, Carlo Socci, Rita Nano, Paola Maffi, and Antonio Secchi

Contents

L. Piemonti (✉)
Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

Internal Medicine and Transplant Unit, Vita-Salute San Raffaele University, Milan, Italy
e-mail: piemonti.lorenzo@hsr.it

C. Socci
Unit of Transplant Surgery, IRCCS San Raffaele Scientific Institute, Milan, Italy

R. Nano
Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

P. Maffi
Diabetes Research Institute (SR-DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy

Clinical Transplant Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

A. Secchi
Internal Medicine and Transplant Unit, Milan, Italy

Internal Medicine and Transplant Unit, Vita-Salute San Raffaele University, Milan, Italy
e-mail: antonio.secchi@hsr.it

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5_23

Abstract

Islet cell or pancreas transplantation is the only long-term diabetes treatment that consistently results in normal hemoglobin A_{1c} levels without the risk of severe hypoglycemia. Additionally, islet cell or pancreas transplantation may prevent, halt, or even reverse the complications of diabetes. Consequently, it is an important part of the management of a subset of patients with diabetes, namely, those in renal failure and those with life-threatening complications of their diabetes such as hypoglycemic unawareness. Here, we explore the indications, options, and outcomes of islet cell or pancreas transplantation as a treatment for diabetes mellitus. The morbidity of solid-organ pancreas transplantation restricts pancreas transplantation to relatively younger and fitter patients. Islet cell transplantation is less invasive and, therefore, more appealing to patients, endocrinologists, and diabetologists. Pancreas transplants and islet transplants should be considered complementary, not mutually exclusive, procedures that are chosen on the basis of the individual patient's surgical risk. As the mortality and morbidity of solid pancreas transplantation diminish and the longer-term outcomes of both solid-organ and islet transplantation improve, the appropriate indications for both procedures will expand, particularly with the increasing incidence of diabetes as well as evidence that transplantation is suitable not only for type 1 diabetics but also for selected insulin-dependent patients with type 2 diabetes.

Keywords

Pancreas transplantation · Islet transplantation

Beta-Cell Replacement Therapies as Treatment Options for Type 1 Diabetes Patients

The ultimate treatment goal for type 1 diabetes is to re-create normal (nondiabetic) or nearly normal blood sugar levels to assure a long healthy life. In healthy subjects under everyday life conditions, the mean 24-hour interstitial fluid glucose concentration is about 90 mg/dl, and the mean fasting glucose concentration is about 80 mg/dl with a mean peak tissue glucose concentration of about 120–130 mg/dl and mean time to peak glucose between 46 and 50 min (Freckmann et al. 2007). Moreover, tissue glucose concentrations in nondiabetic subjects were below 100 mg/dl and 140 mg/dl during 80% and 99.2% of the total day, respectively (Freckmann et al. 2007). In the last decades, significant improvements in insulin therapy thanks to new preparations (i.e., ultrafast and long-lasting insulin analogues) and the adoption of intensive diabetes management have resulted in an overall improvement of patients' glycemic control and a decreased incidence of chronic complications of diabetes

(Gregg et al. 2014). In spite of this, the treatment is still far from being optimal. Studies investigating continuous glucose profiles in diabetic patients demonstrated that glucose concentrations were above 140 mg/dl during about 60% of the total day (Garg et al. 2006) or above 180 mg/dl during about 30% of the total day (Bode et al. 2005). The majority of people with type 1 diabetes have higher HbA_{1c} levels than recommended in guidelines based on the published evidence from registries (McKnight et al. 2015), and roughly only about one-fourth of patients with type 1 gains therapeutic target (Mannucci et al. 2014). High proportions of individuals not achieving glycemic targets with current therapies are also reported for patients treated at centers that focus on the care of type 1 diabetes (Miller et al. 2015). In fact, only a minority of children and adults with type 1 diabetes participating in the T1D Exchange clinic registry achieve HbA_{1c} targets, despite insulin pump being used by 60% of participants (Miller et al. 2015). Moreover, the data indicate that acute complications of insulin treatment remain a problem (Weinstock et al. 2013) in a substantial percentage of patients: 6% reported having had a seizure or loss of consciousness due to hypoglycemia, and 3% reported having DKA event in the prior 3 months. Advanced technologies [insulin pumps (CSII), bolus calculators (BC), real-time continuous glucose monitors (RT-CGM), sensor-augmented pumps (SAP), low-glucose threshold suspend (LGTS) systems, low-glucose predictive suspend (LGPS) systems, and artificial pancreas (AP) systems] are becoming more prevalent in diabetes management, but the limitations of advanced technologies in reducing both A1c and hypoglycemia rates are relevant (Vigersky 2015). CSII reduces A1c in some (Pickup and Sutton 2008; Fatourechi et al. 2009; Bonfanti et al. 2015; Ross et al. 2015) but not all studies (Golden and Sapir 2012), while it improves hypoglycemia in patients with high baseline rates (Pickup and Sutton 2008). BC improve A1c and improve the fear of hypoglycemia but not hypoglycemia rates (Schmidt and Norgaard 2014). RT-CGM alone and when combined with CSII improves A1c with a neutral effect on hypoglycemia rates (Floyd et al. 2012; Golden and Sapir 2012). SAP improves A1c but not hypoglycemia rates (Bergenstal et al. 2010). LGTS reduces hypoglycemia with a neutral effect on A1c (Bergenstal et al. 2013), and LGPS reduces hypoglycemia with a small increase in plasma glucose levels (Maahs et al. 2014). In short-term studies, artificial pancreas systems reduce both hypoglycemia rates and plasma glucose levels (Phillip et al. 2013; Russell et al. 2014). CSII and RT-CGM are cost-effective technologies, but their wide adoption is limited by cost and psychosocial and educational factors. Exogenous insulin administration cannot avoid the long-term complications of diabetes in all patients, and the life expectancy of patients with diabetes is still much shorter compared to that of the general population (Hu et al. 2001; Franco et al. 2007; Lind et al. 2014; Livingstone et al. 2015). Diabetes is one of the leading causes of end-stage renal disease, blindness, and amputation (Gregg et al. 2014). In principle, the treatment for type 1 diabetes, type 3c diabetes, and many cases of type 2 diabetes lies in the possibility of replacing destroyed or exhausted beta-cell mass in order to restore two essential functions: sensing blood sugar levels and secreting appropriate amounts of insulin in the vascular bed, ideally into the portal system. Currently, the only available clinical approach of restoring beta-cell mass in patients with diabetes is the transplantation of

beta cells (i.e., pancreas or islet transplantation). The goals of beta-cell replacement are to restore glucose-regulated endogenous insulin secretion with normalization of glucose levels, arresting the progression of the complications of diabetes and improving quality of life. In the allogenic setting, both pancreas and islet transplantation require lifelong immunosuppression to prevent rejection of the graft and recurrence of the autoimmune process. Current immunosuppressive regimens are capable of preventing beta-cell failure for months to years, but the agents used expose to several side effects and increase the risk for specific malignancies (Geissler 2015) and opportunistic infections (Helfrich and Ison 2015). In addition the most commonly used agents – calcineurin inhibitors and rapamycin – are also known to impair normal islet function and/or insulin action (Rangel 2014). Furthermore, these agents have other toxicities, including the harmful effect on renal function (de Mattos et al. 2000). Differently that for uremic patients in which chronic immunosuppression is already present because of concurrent or previous kidney transplantation, a specific assessment of the risk of the initiation of a long-term immunosuppressive therapy in islet or pancreas transplantation alone should be considered only in patients with serious progressive complications of diabetes in whom the quality of life is very limited by a poor glycemic control or a high number of severe hypoglycemic events notwithstanding optimized intensive insulin therapy or clinical and emotional problems with exogenous insulin therapy that are so severe as to be incapacitating.

Pancreas and Islet Cell Transplantation: Indication

Pancreas or islet transplantation can be performed simultaneously with kidney transplantation, after kidney transplantation or alone, depending on the clinical condition of the patient and on organ availability. A general overview of indications for pancreas or islet transplantation is provided in the Table 1. Within these general indications, some differences are observed in different countries, and there are important issues that are still to be addressed.

Table 1 General indications for the three types of pancreas/islet transplantation.

SPK/SIK	(a) T1D and nonobese T2D in insulin treatment (b) Chronic renal failure with GFR <20 mL/min or on dialysis
PAK/IAK	(a) T1D and nonobese T2D in insulin treatment (b) Stable function of previous renal allograft (c) Meet criteria for PTA/ITA
PTA/ITA	(a) T1D (b) Severe diabetic complications but normal or near-normal renal function (c) Frequent and severe episodes of hypoglycemia; assessed by diabetologists to have disabling hypoglycemia or hypoglycemic unawareness or significant impairment of quality of life due to diabetes

SPK simultaneous pancreas–kidney, *SIK* simultaneous islet–kidney, *PAK* pancreas after kidney, *IAK* islet after kidney, *PTA* pancreas transplantation alone, *ITA* islet transplant alone, *T1D* type 1 diabetes mellitus, *GFR* glomerular filtration rate

Indication in Uremic Patient

In general, patients who develop chronic end-stage renal failure, secondary to either type 1 or type 2 diabetes, who are on insulin and are not obese, are considered for simultaneous pancreas–kidney (SPK) transplantation (Robertson et al. 2006; Jahansouz et al. 2011; Gruessner and Gruessner 2013a). This indication is well defined and without controversy as the "gold standard" when the vascular status of the patient can support two organ transplants instead of one (Chiang et al. 2014). In fact diabetic subjects with end-stage renal failure have a high mortality risk (van Dellen et al. 2013). Kidney transplant alone confers a survival benefit compared with dialysis (Port et al. 1993), but the survival after SIK is superior to survival after cadaveric kidney transplant alone (Tyden et al. 1999; Ojo et al. 2001; Mohan et al. 2003), despite added surgical risk (Smets et al. 1999). Survival after living kidney transplantation alone was described to be equivalent to that after SPK (Rayhill et al. 2000), but there is strong evidence that successful pancreas transplantation still increases life expectancy. In fact, although patients undergoing SPK had a higher mortality risk compared with those undergoing living kidney transplantation in the first 18 months, this early survival disadvantage is lost after because of the effects of good metabolic control obtained by SPK (Reddy et al. 2003; Morath et al. 2008). Although stabilization of renal function contributes significantly to improved life expectancy after SPK, studies comparing SPK recipients with functioning grafts, those with either kidney or pancreas graft failure, and recipients of living and cadaveric kidney transplantation have demonstrated that the pancreas graft confers significant additional benefit beyond that offered by the kidney transplant alone (Salvalaggio et al. 2009; Weiss et al. 2009; Norman et al. 2011). Pancreas after kidney (PAK) is an alternative option to SPK. Patients who undergo PAK transplantation commonly have an identified living kidney donor and undergo cadaveric pancreas transplantation later on. PAK is also an option for diabetic patients with unstable glycemic control who have already had kidney transplantation with stable graft function and sufficient cardiac reserve to receive a second transplantation or in patients who received SPK and lose the pancreas for technical reasons, mainly thrombosis, shortly after transplantation. PAK is becoming increasingly used due to shorter waiting lists for kidney availability compared to SPK (Jahansouz et al. 2011). The main advantage of SPK is the high success rate of the pancreas graft, which contrasts with PAK and mainly pancreas transplant alone (PTA). One of the main reasons suggested for this success rate is the possibility of an early detection of acute rejection in concomitant transplanted kidney (which often is associated with rejection in the transplanted pancreas) by monitoring serum creatinine; this allows a quick treatment with immunosuppressant therapy (Jahansouz et al. 2011). Where a living donor option for kidney transplantation is available, PAK may be preferred in order to achieve earlier independence from dialysis; however, in light of the inferior pancreas graft survival outcomes of PAK compared with those of SPK, the former remains controversial, and the decision on how best to proceed must be made with careful consideration of the individual patient's circumstances and their likely waiting time on the combined pancreas–kidney list. Data on the outcomes of SPK

and PAK transplantation compared directly with simultaneous islet–kidney (SIK) and islet-after-kidney (IAK) transplantation are rare. There are no direct, randomized trials comparing the outcomes, and there are few observational studies (Gerber et al. 2008; Maffi et al. 2011; Lehmann et al. 2015; Moassesfar et al. 2016).

The available data on a long-term follow-up suggest that the combination of kidney transplantation with pancreas transplantation as well as with isolated islet transplantation results in significant and sustained improvement of glucose control without the occurrence of severe hypoglycemia. While insulin independence is more common in SPK/PAK recipients, SIK/IAK can be conducted with a lower complication rate. No difference in the decline of kidney function between the two groups is evident (Lehmann et al. 2015). A careful selection of the adequate procedure by interdisciplinary transplantation teams may help to ensure optimal care for patients with diabetes undergoing combined transplantation. The decision should be influenced by the assessment of pretransplant surgical risk and the definition of treatment goals. Both SPK and PAK should be undertaken in patients who are relatively young (<50 years) and nonobese (<30 kg/m^2) and who do not have coronary artery disease and with vascular conditions capable to support double transplantation. These patient selection criteria minimize operative mortality (<1%) and reduce early technical pancreas graft loss (~10%). Patients beyond 50 years require critical evaluation, because benefit for survival is not evident for this group (Ojo et al. 2001). Islet transplantation (SIK and IAK), a minimally invasive procedure, allows for inclusion of older patients and patients with coronary and peripheral artery disease who would be ineligible for a whole-pancreas transplant. Moreover islet recipients must consider glycemic control and absence of hypoglycemia as their primary therapeutic goal rather than insulin independence. Malignancies, chronic infections, and insufficient compliance are contraindications for both SPK/PAK and SIK/IAK transplantation.

Indication in Non-Uremic Patients

There has been debate about beta-cell replacement therapies (PTA and ITA) in the absence of an indication for kidney transplantation because of the risks of mortality, morbidity, and immunosuppression. Established indications for PTA and ITA have been developed by the American Diabetes Association (ADA) in 2004 (Robertson et al. 2004, 2006): "In the absence of indications for kidney transplantation, pancreas transplantation should only be considered a therapy in patients who exhibit these three criteria: (1) a history of frequent, acute, and severe metabolic complications (hypoglycemia, hyperglycemia, ketoacidosis) requiring medical attention; (2) clinical and emotional problems with exogenous insulin therapy that are so severe as to be incapacitating; and (3) consistent failure of insulin-based management to prevent acute complications." The indications for PTA and ITA reported by ADA are generally associated to the concept of "brittle" diabetes. A proportion of T1D patients experience a highly instable form of the disease known as "brittle" and characterized by a severe instability of blood glucose levels with frequent and

unpredictable episodes of severe hypoglycemia and/or ketoacidosis (Voulgari and Tentolouris 2011; Voulgari et al. 2012). Due to this unpredictable large variability in blood glucose levels, brittle T1D patients often report difficulties in finding an optimal insulin dosing schedule to normalize glucose levels (Bertuzzi et al. 2007). The definition of brittle diabetes has evolved since it was first introduced in the 1930s by Woodyatt to describe patients with excessive fluctuations of blood sugar which could not be explained by patient or physician errors and that unpredictability and unexpectedly led to hypoglycemic reactions (Tattersall 1997). Nowadays, a generally accepted definition of brittle diabetes could be a severe instability of blood glucose levels with frequent and unpredictable episodes of severe hypoglycemia and/or ketoacidosis that disrupts quality of life. The high incidence of severe hypoglycemia episodes observed in the brittle population and, in general, the metabolic instability lead to a reduction in the physiological response to these events and to a certain degree of impairment in the ability to identify further episodes, which is known as hypoglycemia unawareness (Cryer 2013). Several different etiologies have been described for brittle diabetes, although in a significant number of patients, the cause remains unknown. Organic causes explain brittleness in some occasions, and psychosocial factors have also been described in some patients (Gill 1992; Vantyghem and Press 2006; Voulgari et al. 2012). The main organic causes of brittleness include malabsorption, certain drugs (including alcohol and antipsychotics), defective insulin absorption or accelerated degradation, defect of hyperglycemic hormones especially glucocorticoids and glucagon, and above all autonomic neuropathy resulting in changed (delayed or fastened) gastric emptying and hypoglycemic unawareness. Apart from organic causes, psychosocial factors that seem to cause brittle diabetes are complex and diverse. The deliberate induction of factitious brittleness (i.e., hypoglycemia and/or ketoacidosis events) has been described as a response to intolerable life stress (Gill 1992). Furthermore obsessive control and frequent doses adjustments can in some instances increase blood glucose instability, instead of improving metabolic control. Eating disorders like anorexia nervosa in patients with T1D might lead to insulin dose reduction or omission by the patient as a method of weight control. The natural history of the condition remains largely unknown. A few long-term follow-up studies of brittle patients provide some insight on the course of the disease (Tattersall et al. 1991; Kent et al. 1994; Cartwright et al. 2011). According to these studies, the high frequency of hypoglycemia and/or diabetic ketoacidosis events in brittle patients translates in the development of diabetes complications in the long term including nephropathy, retinopathy, and neuropathy, which show an increased incidence compared to non-brittle patients. Unnoticed severe hypoglycemia events are life-threatening and one of the major determinants of quality of life impairment in brittle diabetes patients. Diabetes-related complications are also the main cause of death of brittle patients (Cartwright et al. 2011). Available literature on the epidemiology of brittle T1D is scarce. One of the main reasons for this is probably the lack of a clear definition of diagnostic criteria for the condition. A prevalence rate of 1.2/1,000 diabetic patients and of 2.9/1,000 insulin-treated diabetic patients was reported (Gill et al. 1996). Brittle diabetes is associated with a substantial humanistic burden to patients, caregivers, and family.

The frequency of acute events like hypoglycemia or diabetic ketoacidosis and the subsequent hospital admissions, as well as the incidence of complications, have a major impact in the quality of life of patients. When compared to patients with "stable" T1D, the number of emergency admissions and the length of in-hospital stay due to poor diabetic control are much more frequent in brittle patients, in some cases resulting in patients spending up to several months each year in the hospital (Voulgari and Tentolouris 2011). Lifestyle disruption is also induced by other aspects like pregnancy complications and a higher risk of death due to diabetes complications (Voulgari et al. 2012). Most studies assessing the characteristics of brittle diabetes have also identified a high prevalence of psychosocial disruptions and psychiatric disorders, especially mood and anxiety disorders (Tattersall et al. 1991). Patients with brittle diabetes are generally terrified by the condition and resist with psychotic-type defense reactions when psychotherapeutic approaches are performed commonly driving to deep regression, suicidal feelings, and mistreatment of diabetes. Several measures and methodologies have been introduced in order to quantify metabolic instability, including the assessment of the mean amplitude of the largest glycemic excursions or the mean of daily differences between blood glucose values, among others (McDonnell et al. 2005; Baghurst 2011). Recently, beta-cell replacement therapies were indicated in the treatment algorithm of "problematic hypoglycemia" (Choudhary et al. 2015). Hypoglycemia is a common and greatly feared complication of T1D (Seaquist et al. 2013; Frier 2014). The term severe hypoglycemia is used for episodes with such a degree of cognitive impairment that the patient needs assistance from another person in order to achieve normal glycemia (Workgroup on Hypoglycemia, American Diabetes Association 2005). Many severe hypoglycemia events are single episodes caused by insulin dosing errors, exercise, and alcohol. Conversely, problematic hypoglycemia is a condition in which episodes of severe hypoglycemia are unpredictable, cannot be easily explained or prevented, and, therefore, have a significant negative impact on health and quality of life. The criteria of problematic hypoglycemia include two or more episodes of severe hypoglycemia in the past 12 months or one episode of severe hypoglycemia in the past 12 months associated with impaired awareness of hypoglycemia, extreme glycemic lability, or major fear and maladaptive behavior. Simple tools are available clinically to quantitate awareness of hypoglycemia (Gold et al. 1994; Clarke et al. 1995), hypoglycemia severity (Ryan et al. 2004b), and glycemic lability (Ryan et al. 2004b). Scores and indexes have been developed to quantify hypoglycemic frequency and hypoglycemic awareness, including the low blood glucose index (LBGI), the Clarke score, or the HYPO score:

- The LBGI is a summary statistic used to assess the risk for severe hypoglycemia based on the percentage of low self-monitored blood glucose readings and their magnitude in the lower blood glucose range, thus integrating the frequency and severity of hypoglycemia events. Based on the LBGI score, patients are classified as having a low (<2.5), moderate (2.5–5), and high (>5) risk of severe hypoglycemia (Kovatchev et al. 1998).

- The Clarke score is based on an eight-question survey to patients aimed at assessing patients' hypoglycemia awareness. The final score may range between 0 and 7. A score ≤ 2 would classify patients as aware, while a score ≥ 4 indicates reduced awareness of hypoglycemia and therefore an increased risk of severe hypoglycemic episodes (Clarke et al. 1995).
- The HYPO score used a complex scoring system that takes into account the frequency, the severity, and the loss of symptoms of hypoglycemia. It combines the data obtained from records of capillary blood glucose levels over a 4-week period and the number of self-reported hypoglycemic events during this period and during the past year. Points are awarded for each documented episode of hypoglycemia with extra points depending on the severity of the associated neurologic symptoms and if additional help was required within the episode. If autonomic symptoms provided adequate warning of impending hypoglycemia, no points are awarded to the episode. Normal subjects usually show a HYPO score of 0, while stable diabetes patients' scores are around 200. A HYPO score ≥ 1047 (ninetieth percentile) indicate that the patient has severe problems with hypoglycemia (Ryan et al. 2004b).

The epidemiology of severe hypoglycemia in T1D patients has been widely described in the literature. Studies identified in a comprehensive review of evidence report a yearly prevalence of severe hypoglycemia of 7–66% in T1D patients (Pedersen-Bjergaard et al. 2003; Giorda et al. 2015), although in most cases, prevalence ranges from 30% to 40% (Pedersen-Bjergaard et al. 2004; UK Hypoglycaemia Study Group 2007; Gruden et al. 2012; Weinstock et al. 2013; Frier 2014). In terms of incidence, the number of severe hypoglycemia episodes per patient-year generally ranges between 1.0 and 1.7, although some variability exists, and one episode of severe hypoglycemia is experienced by one-third of patients with T1D at least once a year. Comparatively fewer studies have been identified specifically addressing hypoglycemia unawareness, in part, due to a lack of an agreed definition (Graveling and Frier 2010; Hoi-Hansen et al. 2010). Hypoglycemia unawareness is found in 20–40% of patients with T1D (Gold et al. 1994; Geddes et al. 2008; Choudhary et al. 2010; Ogundipe et al. 2011; Hopkins et al. 2012) and increases the risk of severe hypoglycemia by 6–20-fold (Gold et al. 1994; Clarke et al. 1995; Pedersen-Bjergaard et al. 2004). Prevalence of impaired awareness of hypoglycemia increased with diabetes duration and ageing (Olsen et al. 2014). Recurrent hypoglycemia can cause significant morbidity (Frier 2004, 2014) and mortality. Among individuals with T1D, 4–10% of all deaths are attributed to severe hypoglycemia (Skrivarhaug et al. 2006; Feltbower et al. 2008), and risk of death 5 years after an episode of severe hypoglycemia is increased 3.4-fold (McCoy et al. 2012). A four-stage treatment algorithm was recently proposed for "problematic hypoglycemia." All patients with problematic hypoglycemia should undergo structured or hypoglycemia-specific education programs (stage 1). Glycemic and hypoglycemia treatment targets should be individualized and reassessed every 3–6 months. If targets are not met, one diabetes technology – continuous subcutaneous insulin

infusion or continuous glucose monitoring – should be added (stage 2). For patients with continued problematic hypoglycemia despite education (stage 1) and one diabetes technology (stage 2), sensor-augmented insulin pumps preferably with an automated low-glucose suspend feature and/or very frequent contact with a specialized hypoglycemia service can reduce hypoglycemia (stage 3). For patients whose problematic hypoglycemia persists, ITA or PTA should be considered (stage 4). Because PTA (Gruessner and Gruessner 2013b) and ITA (Markmann 2016) outcomes have gradually improved and are both effective in preventing severe hypoglycemia and achieving near-normoglycemia, the optimal treatment option will require an individualized discussion of multiple factors, including the procedural risks (which are higher for a pancreas transplant), importance of insulin independence, waiting time, and sensitization. Some contraindications to a pancreas transplant (age >50 years, high cardiac risk) are common in patients with problematic hypoglycemia; they may only be eligible for an islet transplant. Yet, a small proportion of patients may be ineligible for an islet transplant because of their weight or insulin requirements. The transplant team should consider each patient's preferences and perceptions of risks and benefits. A summary of indications and contraindications of SPK, PAK, PTA, and ITA is provided in Table 2. If the patient has advanced renal disease and is undergoing a renal transplant, a SPK or PAK is reasonable especially if there are problems with lability or hypoglycemia. If the center has local expertise in preparing islets, SIK or IAK transplants could be considered. If the patient has a kidney transplant and has stable diabetes, performing a pancreas transplant, in addition, increases the risk of surgery and requires full discussion with the patient in regard to short- and long-term risks/benefits. If the patient has labile diabetes and no renal disease, the choice between ITA and PTA should be done together with the patients, according to expectations, psychological conditions, and propensity to risk. The burden of procedure-related adverse events, which is clearly higher for PTA than for ITA, should be carefully weighted up, and the patient might be recommended to the more suitable indication, in the center with the best expertise. The most challenging patients are those with unstable diabetes (lability or hypoglycemia problems) and some renal dysfunction. If the renal dysfunction is limited to the presence of microalbuminuria, then islet transplantation is reasonable. If there is macroproteinuria present, the outcomes are less certain, and a pancreas or islet transplant alone can be considered in the light of the possibility that the immunosuppressive drugs may hasten the decline of renal function. A particular subgroup is represented by brittle diabetic patients with chronic kidney disease in an intermediate stage (III and IV, GFR 15–30), when the proposal of SPK seems too early and the proposal of PTA too risky for the progression of kidney disease, thanks to nephrotoxic immunosuppressants. In patients in stage III (GFR 30–60), PTA can be safely and reasonably proposed once assured that a potential kidney living donor is available, useful in case of progression of kidney disease. In patients in stage IV (GFR 15–30), SPK can be proposed if risk equations to predict kidney failure (Tangri et al. 2016) can envisage early and rapid progression of ESRD.

Table 2 Summary of indications and contraindications of SPK, PAK, PTA, and ITA in insulin-treated diabetic patients (Adapted from (Shapiro 2012; Mittal and Gough 2014)

Procedure		SPK	PAK	PTA	ITA
		Age > 18, <50 years	Age > 18, <50 years	Age > 18, <50 years	Age > 18 years
Indications		Chronic renal failure with GFR < 20 mL/min or on dialysis	Stable function of previous renal allograft Non-sensitized (panel reactive antibody <20%) Tolerating maintenance immunosuppression Prednisone ≤5 mg/day	Normal or near-normal renal function (GFR >60 mL/min and absence of macroalbuminuria) C-peptide negative in presence of glucose >4 mmol/L Diabetes duration >5 years	
			Significant diabetic complications Frequent and severe episodes of hypoglycemia (brittle diabetes or problematic hypoglycemia)		
			Refractory hypoglycemia or lability despite: Optimal intensive insulin or insulin pump with appropriate monitoring Supervision by a diabetologist or endocrinologist Increased hypoglycemic risk, evidenced by at least one of the following criteria: Clarke score ≥ 4 HYPO score ≥ 1000 Lability index (LI) ≥ 400 Combined HYPO ≥400 and LI ≥ 300		
Contraindications	**Relative**	Insulin requirements >1.5 units/kg/day Body mass index >30 kg/m2			Insulin requirement >1.0 U/kg/day weight > 90 kg
		High cardiac risk Extensive aorta/iliac and/or peripheral vascular disease Cerebrovascular accident with long-term impairment			
	Absolute	Excessive cardiovascular risk (significant non-correctable coronary artery disease; left ventricular ejection fraction <50%; myocardial infarction within 6 months) Non-curable malignancy (excluding localized skin cancer) Active sepsis or peptic ulcer Major psychiatric history likely to result in non-adherence Inability to withstand surgery and immunosuppression			
Benefits		Insulin independence Good pancreas and kidney graft outcomes	Insulin independence Early dialysis independence	Insulin independence Cure of hypoglycemia	Cure of hypoglycemia Less invasive
Risks		Operative morbidity and mortality	Sensitization Poorer pancreas graft outcomes	Sensitization Risk of graft failure Higher morbidity procedure	Sensitization Less likely to achieve insulin independence Often need more than one infusion

SPK simultaneous pancreas–kidney, *PAK* pancreas after kidney, *PTA* pancreas transplantation alone, *ITA* islet transplantation alone, *GFR* glomerular filtration rate

Current Status of Pancreas Transplantation

Surgical Technique and History of Pancreas Transplantation

Most transplant units around the world transplant the whole pancreas together with a segment of donor duodenum (Han and Sutherland 2010). The arterial inflow is usually from the recipient common iliac artery with venous drainage to the common iliac vein. Pancreas transplantation was first used for the treatment of diabetes in humans in 1966 (Kelly et al. 1967). In the 1970s the pancreas transplant development was continued with the first urinary drainage via the ureter (Gliedman et al. 1973), segmental PTA with end-to-side ductoenterostomy (Merkel et al. 1973), and injection of neoprene (Dubernard et al. 1978). In the 1980s, the bladder drainage technique was reported and developed (Cook et al. 1983; Starzl et al. 1984; Nghiem and Corry 1987). From the mid-1980s to mid-1990s, the anastomosis of the donor duodenum was usually to the bladder drainage and was the most common technique worldwide (Prieto et al. 1987). This technique has the advantage of enabling urinary amylase to be used as a biochemical marker of pancreatic function and fewer complications with regard to contamination from enterotomy or duodenal leaks (Sollinger et al. 2009); however, bladder drainage has the disadvantage of being associated with metabolic and urological complications including dehydration, metabolic acidosis, and irritation from cystitis. For this reason, the enteric drainage became the routine method in the late 1990s (Gruessner and Sutherland 2000). This technique is more physiological but renders the pancreas less easily monitored. Despite this, as a result of improvements in surgical technique, radiological imaging and antimicrobial prophylaxis, outcomes after pancreas transplantation with enteric drainage, are equivalent to those after bladder drainage. The first large case series of living donor segmental transplantation – a technique started in the late 1970s (Sutherland et al. 1980) – was reported in the 1990s (Gaber et al. 1995b). The use of portal drainage in recipients of enterically drained whole-organ pancreaticoduodenal transplants was described in the 1990s (Rosenlof et al. 1992). This approach, although associated with more physiological systemic levels of insulin, is not supported by evidence of substantial benefit with respect to graft or patient survival (Bazerbachi et al. 2012). Although there has been concern that the hyperinsulinemia associated with systemic venous drainage may be associated with adverse events such as an increased risk of atherosclerosis, there is no convincing evidence that systemic venous drainage places pancreas recipients at a disadvantage (Stadler et al. 2010).

Clinical Outcomes of Pancreas Transplantation

From 1966 to 2012, >42,000 pancreas transplants performed worldwide were reported to the International Pancreas Transplant Registry (IPTR), the majority of which reported diabetes as underlying disease (over 90% T1D) (Gruessner 2011; Gruessner and Gruessner 2013a). The most frequently used modality of pancreatic

transplant was SPK (75%), followed by PAK (12%) and PTA (7%). The number of pancreatic transplants grew until 2004, and since then, it has gradually diminished (Gruessner and Gruessner 2014; Kandaswamy et al. 2015).

Patient and Graft Survival

Patient survival is equivalent after SPK, PAK, and PTA (Gruessner and Gruessner 2013a). Patient survival rates have continued to improve over time in all three categories, reaching 96% at 1 year and 80% at 5 years posttransplantation (Gruessner and Gruessner 2013a). In all three recipient categories, cardiovascular and/or cerebrovascular problems and infections were the leading causes of early (<3 months posttransplant) and late (>1 year posttransplant) death after transplant surgery (Gruessner and Gruessner 2012). Pancreas graft survival (defined as insulin independence) rates have also improved significantly over time in all three categories but remains higher with SPK transplantation. Graft survival rates at 1 year were 89% (SPK), 86% (PAK), and 82% (PTA). The figures at 5 years were 71% (SPK), 65% (PKT), and 58% (PTA). The estimated half-life (50% function) of pancreas grafts is 14 years (SPK), 7 years (PAK), and 7 years (PTA). In case of pancreas failure, the organ can be removed, when necrosis or colliquation is envisaged or maintained when it becomes fibrotic, without further risk of colliquation. In case of failure, a second pancreas transplant can be considered. In this case immunosuppression should be maintained in order to avoid appearance of DSA. In this case retransplantation must be done in a timely manner. Absolute contraindications to pancreas retransplantation are poor cardiovascular conditions, impairment of kidney function, and high percentage of **panel reactive antibody** (PRA) or high level of donor-specific alloantibodies (DSA).

Complications

Pancreas transplantation is a major surgical procedure associated with several technical complications. In general, the primary complication related to pancreatic graft loss is technical failure, followed by acute or chronic rejection. The rate of technical failure has declined across all recipient categories and is currently about 9% (Kandaswamy et al. 2016). Considering transplants performed between 2007 and 2011, technical complications were the most common reasons for graft loss posttransplant in all three categories (63% for SIK, 75% for PAK, and 77% for PTA). Technical failure is understood as the loss of the graft in the first 3 months of transplant due to vascular thrombosis (50%), pancreatitis (20%), infection (18%), fistulas (6.5%), and hemorrhage (2.4%). The rate of graft loss owing to acute rejection peaked between 3 and 12 months posttransplant, while the rate of graft loss owing to chronic rejection constantly increased from time since surgery (18% for SIK, 14% for PAK, and 36% for PTA >1 year posttransplant). Chronic rejection (18% for SIK, 14% for PAK, and 36% for PTA) and death with a functioning graft (38% for SIK, 18% PAK, and 13% for PTA) are the two most common causes of long-term graft loss (>1 year posttransplant) (Gruessner and Gruessner 2012). Pancreatic transplant presents 10–20% of surgical complications that require review laparotomy. The risk factors for surgical complications include prolonged time in peritoneal dialysis, donor or recipient with a body mass index >28 kg/m^2, donor or

recipient age over 45 years, cerebrovascular disease as cause of donor death, prolonged preservation time (>20 h), retransplantation, and prior abdominal surgery (Sutherland et al. 2001; Gruessner and Gruessner 2012, 2014). One of the most feared complications in pancreatic transplant with enteric drainage is intestinal leak, since it poses risks to patient's survival (Jahansouz et al. 2011). The incidence of intestinal leak ranges from 5% to 8%, and most occur during the immediate postoperative period. The early leak is related to technical problems, such as impaired blood irrigation and ischemia. The potential risk factors for the occurrence of early intestinal leak are prolonged cold ischemia time, duodenal trauma, post-reperfusion pancreatitis, and intra-abdominal infection. Its treatment generally leads to the removal of the pancreatic graft (Nath et al. 2005). Pancreatic transplants with bladder drainage imply frequent and severe urological and metabolic complications. Approximately 10–25% of patients submitted to pancreatic transplant with bladder drainage need to be submitted to intestinal conversion of the graft's exogenous drainage (Stratta 2005). The main metabolic complications are metabolic acidosis and dehydration due to loss of water and sodium bicarbonate in the urine. These patients should receive adequate fluid and bicarbonate replacement in the follow-up of pancreatic transplant with bladder drainage. Despite better pancreatic transplant results, infectious complications continue to be the primary causes of morbidity and mortality. In fact, the use of immunosuppressant drugs in pancreas transplantation recipients is associated with an increased incidence of infections. Infections are more common in the first months following transplantation. The main pathogens involved are bacterial (*Staphylococcus* sp., *Pseudomonas aeruginosa*, *Clostridium difficile*) and viral (mainly *Cytomegalovirus*), although some fungal infections may be observed (*Candida* sp.). The urinary tract and abdominal wall are the most affected sites. Infection rates tend to decline after the first 3 months. In the long term, a retrospective cohort study including 216 pancreas transplant recipients identified a 63% incidence of infections (mainly of bacterial origin) requiring hospitalization during a >5-year follow-up period resulting in an increased risk of mortality (Rostambeigi et al. 2010). Patients submitted to pancreas transplant have a high risk of developing infection by *Cytomegalovirus* due to the use of antilymphocyte serum in immunosuppression protocols. The mean incidence is 25%. The incidence of malignancies secondary to immunosuppression is also increased in pancreas or kidney–pancreas transplant recipients. Data reported in the literature show similar long-term figures compared to those reported for other solid organ transplantation recipients (Stratta 1998). A retrospective single-center study including 360 diabetic patients who had undergone SPK transplantation reported an overall incidence of malignant tumors of 6.2% ($n = 25$) after a median follow-up period of 8 years posttransplant. Most common tumor types were non-melanoma skin cancers, lymphomas and lung adenocarcinoma, bladder carcinoma, and peritoneal carcinoma (Girman et al. 2011).

Immunosuppression

Immunosuppressant induction and maintenance regimens to avoid graft rejection in pancreas transplant recipients have evolved over time, resulting in improved outcomes in terms of patient and graft survival. The majority of units use biological

antibody induction (thymoglobulin, alemtuzumab, or basiliximab) to achieve profound immune cell depletion lasting for the first 3 months when the risk of rejection is greatest. There is no difference in patient or graft outcomes according to which induction they received, although a tendency toward less acute rejection with alemtuzumab was described (Hao et al. 2012). Induction is followed by a maintenance combination of tacrolimus (a calcineurin inhibitor, CNI) and mycophenolate mofetil (an antiproliferative agent) to block T-cell activation and expansion, respectively. There is an increasing trend toward the use of steroid-free regimens in all areas of transplantation, and steroids are early or delayed withdrawal or not routinely used at all in either pancreas or islet cell transplantation (Gruessner and Gruessner 2012). The advent of inhibitors of the mammalian target of rapamycin inhibitors (mTORI), such as sirolimus and everolimus, provided an opportunity to reduce both the diabetogenic and nephrotoxic potential of the immunosuppression, although it later transpired that mTORIs do have some nephrotoxicity manifesting as proteinuria (Letavernier and Legendre 2008), as well as a potential to cause diabetes (Johnston et al. 2008). Tolerability of mTORI, particularly their tendency to cause mouth ulcers, rashes, joint pain, and edema, has prevented their wider use (Campistol et al. 2010). Nevertheless, where they are tolerated, they provide a good alternative to CNI-based immunosuppression. The mTORI have a theoretical advantage over tacrolimus for recipients of a pancreas transplant alone (PTA), in whom preservation of renal function is important, and latest data suggest that 18% of PTA recipients are on mTORI (Gruessner and Gruessner 2013b). The combination of CNI and mTORI can provide enhanced immunosuppression (McAlister et al. 2000) but was associated with a risk of enhanced nephrotoxicity and other complications(Feldmeyer et al. 2012) and has proved useful to rescue patients with difficult-to-manage rejection. The other area where mTORI may have a role is in the management of transplant patients who develop a malignancy, since mTORI have been shown to have antineoplastic properties (Hasskarl 2014). The most recent addition to the immunosuppressive armory is belatacept, a biological agent which blocks the CD28 co-stimulatory pathway (Larsen et al. 2005). Results of its use in pancreas transplantation are awaited, but it may be a good alternative to mTORIs in patients with CNI nephrotoxicity (Mujtaba et al. 2014).

Life Expectancy

To this day, both ethical and practical considerations have prevented randomized controlled trials comparing the outcomes of simultaneous pancreas and kidney transplants versus kidney-only transplants, pancreas after kidney transplants versus kidney-only transplants, and pancreas transplants alone versus intensive insulin therapy. The three different modalities of pancreas transplantation (SPK, PAK, and PTA) have been suggested to have long-term mortality benefit compared to continuous insulin treatment in patients who are on waiting list for transplantation (Gruessner et al. 2004; Siskind et al. 2014), although this benefit has been more demonstrated in patients undergoing SPK (Smets et al. 1999; Becker et al. 2000; Ojo et al. 2001; Reddy et al. 2003; Kleinclauss et al. 2009). The survival benefit achieved by SPK, when compared to waiting-list patients, is 14.4 versus 3.7 years, in terms of median survival (propensity score matching) (Rana et al. 2015). In a previous study

conducted in 2004 with data from the UNOS/IPTR database on 13,467 patients, Gruessner et al. (2004) reported significantly decreased mortality after the first year posttransplant among patients who had undergone SPK, PAK, and PTA compared with patients who remained on the waiting lists (SPK, HR 0.04 [*CI*, 0.03–0.04; $p < 0.0001$)]; PAK, HR, 0.18 [*CI*, 0.13–0.25; $p < 0.0001$]; *PTA*, 0.15 [*CI*, 0.08–0.29; $p < 0.0001$] (Gruessner et al. 2004). The patient survival rate at 10 years posttransplant is significantly higher in recipients of a SPK than of a kidney transplant from a deceased donor. Recipients of a SPK had the greatest longevity (23.4 years), as compared with 20.9 years for recipients of a kidney transplant from a living donor and 12.8 years for recipients of a kidney transplant from a deceased donor (Gruessner and Gruessner 2013a). The survival benefit of isolated pancreas transplant (after kidney transplant and alone) is more controversial. Earlier reports stating a survival disadvantage for recipients of solitary pancreas transplants (PAK and PTA) compared with patients on the waiting list for a transplant (Venstrom et al. 2003) now seem to be unsubstantiated (Gruessner et al. 2004; Siskind et al. 2014). Recently UNOS data have shown that pancreas transplantation alone, when compared to waiting list patients, confers a survival benefit of 6.7 years (14.5 vs. 7.8) in terms of median survival (propensity score matching) (Rana et al. 2015). In recipients of PAK, evidence shows that the pancreas transplant improves long-term patient and kidney graft survival rates. Also, glomerular filtration rates appear significantly higher in the kidney graft of recipients of pancreas after kidney transplants than in recipients of kidney transplants alone (Kleinclauss et al. 2009). The survival benefit of PTA is debated. The benefit for the individual patient must be considered by weighing the incapacities experienced with insulin-based treatments against the risks of surgery and immunosuppression. For patients who have experienced frequent and significant hypoglycemic episodes, particularly those requiring third-party assistance, pancreas transplant can be a lifesaving procedure.

No specific quality-of-life questionnaire for use in transplantation currently exists, and so most studies have been limited not only by size but also by the use of generic and heterogeneous quality-of-life measures (Gross and Zehrer 1992; Dew et al. 2000; Speight et al. 2010). A successful simultaneous pancreas and kidney transplant with sustained graft function leads to a large improvement in quality of life, including greater satisfaction with life and health, more feelings of control and independence, and perceptions of better physical, mental, and social health and functioning (Nakache et al. 1994; Isla Pera et al. 2009; Ziaja et al. 2009; Smith et al. 2010). The effect of pancreas after kidney transplants and pancreas transplants alone on quality of life is more difficult to determine because of the much smaller numbers of recipients. Freedom from insulin is exchanged for the complications of immunosuppression, and the short-term difficulties of postoperative recovery are balanced against the long-term benefits.

Metabolic and Functional Outcomes After Pancreas Transplantation

When a segment of the pancreas is transplanted, as it was in the early period of pancreas transplantation, mild metabolic abnormalities were observed, such as impaired glucose tolerance and delayed insulin response to glucose (Pozza et al.

1983). Whole-organ pancreas transplantation achieves a high degree of insulin independence, usually with normalization of many of the frequently measured variables of metabolic function including HbA_{1c} and appropriate insulin, C-peptide, and glucagon responses to circulating blood glucose levels; however, the physiology of glucose homeostasis after pancreas transplantation is not fully understood. A successful pancreas transplant seems to more effectively lower the levels of HbA_{1c} than intensive insulin therapy, and even 10 years posttransplant, a successful pancreas transplant can preserve insulin secretion and provide good glycemic control (Dieterle et al. 2007). Restoration of β-cell secretory capacity, improvement in glucose counter-regulation, and return to hypoglycemia awareness can all be achieved with a successful pancreas transplant (Rickels 2012). Several studies (White et al. 2009; Gruessner and Gruessner 2013a) have reported long-term beneficial effects of the different types of pancreas transplantation on chronic microvascular diabetes complications including diabetic nephropathy (Fioretto et al. 1998, 2006; Fiorina et al. 2007), neuropathy autonomic and peripheral (Kennedy et al. 1990; Martinenghi et al. 1997; Navarro et al. 1997), gastroparesis (Gaber et al. 1991), retinopathy (Koznarova et al. 2000; Giannarelli et al. 2005, 2006), microvascular and macrovascular disease including cerebral vasculopathy and morphology (La Rocca et al. 1995, 2001; Morrissey et al. 1997; Jukema et al. 2002; Larsen et al. 2002, 2004; Biesenbach et al. 2005), cardiac function (Gaber et al. 1995a; Fiorina et al. 2000, 2012; Coppelli et al. 2003; Folli et al. 2010), and sexual function (Salonia et al. 2011). Despite such encouraging results, caution must be exercised for a number of reasons. It has been acknowledged that there is a paucity of long-term, prospective randomized studies of sufficient size to draw meaningful conclusions and that at the present time much of the benefit is circumstantial with most evidence limited to single-center studies. Exposure to calcineurin inhibitors and dehydration can result in impaired kidney function (Boggi et al. 2011); as a result, progression of retinal as well as microvascular lesions has been reported (Ramsay et al. 1988).

Current Status of Pancreatic Islet Transplantation

History of Islet Transplantation

The real father and pioneer of modern-day islet transplantation is Paul E. Lacy. He was the first to describe the method to isolate islets from rodent pancreata in 1969 and few years later carried out successful islet transplantations in rodents for the first time (Ballinger and Lacy 1972). The islet isolation technique developed in the rat by doctor Lacy prompted a surge of experimental studies in rodents. However, for several years the attempts to extend the Lacy isolation protocol to large animal pancreas (i.e., dog, nonhuman primate, and human) yielded poor results. A turning point for clinical islet transplantation was the introduction of the "automated method" of pancreas dissociation by Camillo Ricordi. The method consisted of a mechanically enhanced enzymatic digestion based on a dissociation/filtration

chamber allowing pancreatic fragments and islets freed from the gland to be removed promptly from the system to avoid over-digestion while preserving cluster integrity. The method was first published in 1988 (Ricordi et al. 1988) and has represented ever since the gold standard for virtually all research centers working on human (Ricordi 2003) and large animal islets (Ricordi et al. 1990), besides its application for the isolation of other tissues (Vizzardelli et al. 2001). In 1990, the introduction of novel techniques to improve the efficiency of the isolation techniques resulting in high yields of pancreatic islets prompted the development of numerous clinical islet transplantation programs around the world. The first series of patients with sustained insulin independence was reported in nine patients undergoing excision of the liver and pancreas (that would result in surgery-induced diabetes) and receiving allogeneic liver and islet transplantation from the same cadaveric donor. The first clinical case of sustained insulin independence following allogeneic islet transplantation was a 15-year-old woman whose visceral organs were removed for cancer and who received a multi-visceral organ (liver, small bowel, and islet) transplantation (Tzakis et al. 1990; Ricordi et al. 1992). In 1990, doctors Scharp, Lacy, and colleagues at Washington University reported the first case of transient exogenous insulin independence following transplantation of 800,000 cultured allogeneic islets (pool of two allogeneic islet preparations), isolated using the automated method into a patient with T1D receiving Minnesota antilymphocyte serum, azathioprine and cyclosporine (Scharp et al. 1990). Ten days after transplantation, the patient achieved normoglycemia (albeit with residual glucose intolerance) and discontinued exogenous insulin for 2 weeks (Scharp et al. 1990). Insulin independence following islet transplantation from a single donor obtained using the automated method was reported by Dr. Carlo Socci and colleagues at the San Raffaele Institute in Milan (Italy) in a patient with T1D transplanted in April 1990 (Socci et al. 1991). Subsequently, insulin independence and/or consistent graft function after islet transplantation was reported across the world using cryopreserved (Warnock et al. 1991) along with fresh allogeneic islets, paving the way for the clinical application of cellular therapies to restore beta-cell function in patients with T1D (Hering et al. 1994; Secchi et al. 1997). Unfortunately, despite the advances in this field, between 1990 and 1998, only 8% of the patients receiving and islet transplant remained insulin-independent for more than 1 year (Bretzel 2001). A major advance occurred in 2000, when the University of Alberta group reported that with their protocol (known since then as the "Edmonton Protocol"), they were able to consistently achieve long-term insulin independence −100% at the end of the first year in seven patients with T1D (Shapiro et al. 2000). The Edmonton Protocol included two novel key elements that contributed to those successful results. The first consisted in the intraportal infusion of freshly isolated islets, followed by a second and sometimes a third infusion of additional islets from different donors, to achieve an islet mass [in their experience 10,000 islet equivalents per kilogram (IE/Kg)] necessary to achieve insulin independence. The second was the use of a steroid-free, rapamycin-based protocol of immunosuppression. The interest in islet transplantation was once more refueled, and several centers worldwide resumed their clinical programs (Shapiro et al. 2003). If we consider the 2007–2010 period, the

islet graft survival (C-peptide $\geq$0.3 ng/mL) of 92% at 1 year and 83% at 3 years compares very favorably with whole-pancreas graft survival of 80% at 1 year and 61% at 3 years. In more recent years, these graft survival rates translate to an unconditional 44% insulin independence at 3 years, the highest long-term islet transplant success rate observed to date (Barton et al. 2012). For more information on the history in the field of islet cell transplantation to restore beta-cell function in patients with diabetes, see the recent review (Piemonti and Pileggi 2013).

Process of Islet Transplantation

The process of islet transplantation includes three different stages: (1) pancreas donation and retrieval, (2) islet isolation and culture, (3) islet transplantation.

Pancreas Donation and Retrieval

The selection of a donor pancreas for islet isolation is a key step in the transplantation procedure. Several studies have been conducted to identify the main donor characteristics required for successful islet isolation. Multivariate analyses suggest that donors >20 years of age, with a high body mass index (BMI) and normoglycemic (HbA1c < 6.0%), without hypotension or cardiac arrest and with a minimal inotropic support are optimal for islet isolation (Nano et al. 2005; Shapiro 2012; Balamurugan et al. 2014). However, the decision to allocate a pancreas to islet isolation is generally dependent on the possibility of using the pancreas for a whole-organ procedure, which is normally prioritized given the largest experience with this procedure (Berney and Johnson 2010). The surgical procedure for pancreas retrieval needs to be conducted meticulously. The maintenance of the integrity of the pancreatic capsule and duodenum is crucial for the digestion process later on. The duration of cold ischemia is also critical for a successful procedure as islets are particularly vulnerable to ischemia. It is generally recommended that cold ischemic time should not exceed 8 h (Mittal et al. 2014).

Islet Isolation

The aim of the islet isolation process is to extract the islets of Langerhans (approximately 1–2% of the pancreas) while removing the exocrine/acinar pancreatic tissue. It remains a challenging procedure requiring large expertise and is centrally performed by some highly specialized centers worldwide that generally provide islets for different implanting centers. Even in leading isolation centers, transplantable yields are only achieved in about 50% of pancreases. Islet isolation comprises two different steps: pancreas digestion and islet purification. Pancreas digestion is conducted as a combination of two procedures. Initially, the pancreas is disintegrated through enzymatic digestion by collagenase. Subsequently, a mechanical dissociation process of the already digested pancreas is performed by either manual or automated agitation within a digestion chamber. As a result, a suspension digest containing both the islets and the exocrine and ductal tissue is obtained. After digestion of the pancreas, the suspension digest undergoes purification to decrease

transplanted tissue volume and prevent the activated pancreatic enzymes from being transplanted alongside the islet graft albeit with minimum loss of islet. This process is performed by density gradient purification, as islets are less dense than the exocrine and ductal tissue. Once the islets have been purified, they are counted and assessed for overall percentage purity and percentage viability (Johnson and Jones 2012). A minimal islet mass of 5,000 IEQ/kg is generally required for each transplant and >8,000 IEQ/kg for single-donor success (Shapiro 2012).

Islet Transplantation

The isolated pancreatic islets are subsequently infused into the hepatic portal system of the recipient by transhepatic cannulation of the portal vein with ultrasound and/or fluoroscopic guidance (Gaba et al. 2012). The infusion process lasts for about 1 h, and patients are generally discharged from the hospital within 48 h, once clinically stable and without complications. The initial few days following the islet infusion are critical for the final outcome of the islet transplantation process. Clinical and animal models show that up to 75% of the graft is lost during this period. It is suggested that islet graft loss is mainly related to an activation of the immune system of the recipient, to the ischemia reperfusion injury of the islets, and to relative ischemia in portal venules. Different approaches to improve engraftment are currently under investigation, including the use of anti-TNF agents, anti-IL-1 agents or glucagon-like-peptide 1 (GLP-1) analogues like exenatide.

Clinical Outcomes of Islet Transplantation

Islet transplantation is a minimally invasive treatment that has the potential to reverse diabetes thus resulting in an alternative to whole-pancreas transplantation in diabetic patients. It is estimated that over 1,400 islet transplants have been performed worldwide. Although islet transplantation is extensively considered an experimental procedure, several countries including Canada, the United Kingdom, France, Switzerland, Norway, Sweden, and other European countries fund the procedure as "non-research" standard clinical care. In the United States, major trials funded by the National Institutes of Health are being conducted to obtain a biological license application (BLA) by the Food and Drug Administration (FDA) (Markmann 2016). Islet transplantation may be performed alone (ITA), in simultaneously with renal transplantation (SIK), or following kidney transplantation (IAK). ITA is the most commonly used approach.

Patient and Graft Survival

The main goal of islet transplantation has historically been insulin independence; however, investigators are currently considering additional relevant outcomes, such as the reduction in the frequency of hypoglycemic episodes and the positive effects on complications and quality of life (Robertson 2010). Since the Edmonton Protocol breakthrough, the endocrine outcomes of islet transplantation have substantially improved, and according to the recent report of the Cell Islet Transplantation

Registry, the rate of insulin independence after allogeneic islet infusion (ITA and IAK) is around 66% at 1 year and 44% at 3 years after last infusion (Barton et al. 2012), with 5-year insulin-independent normoglycemia achieved in >50% of patients at the most experienced centers (Bellin et al. 2008, 2012; Vantyghem et al. 2009, 2014a; Maffi et al. 2011; Shapiro et al. 2011). However, multiple infusions are generally required for transplant recipients to achieve insulin independence or to regain insulin independence, as the rate of insulin independence tends to decline over the years. Sixty-four percent of the patients included in the CITR have received more than one islet infusion (Barton et al. 2012). Furthermore, durability of islet graft function, as measured by fasting C-peptide ≥0.3 ng/mL regardless of insulin independence, has been improving significantly over the time, and work from several groups confirms that around 80% of islet-transplanted patients have persistent graft function at 4–5 years after last infusion (Ryan et al. 2005; Barton et al. 2012). Nearly all islet recipients had significant improvements in HbA1c and fasting blood glucose after islet transplantation. Importantly, the presence of insulin-dependent islet graft survival, defined by C-peptide >0.3 ng/mL, is document to protect from severe hypoglycemia (Johnson et al. 2004), and this effect persists even after the islet graft is lost. Available data on severe hypoglycemic events in islet recipients, regardless of graft function, shows that >90% of the patients remained free from severe hypoglycemic events during 5 years of follow-up (Johnson et al. 2004; Barton et al. 2012).

Complication

The procedure of islet transplantation has proven to be very safe, especially when compared with whole-pancreas transplantation (Ryan et al. 2004a; Maffi et al. 2011; Gaba et al. 2012). The incidence of (serious) adverse event related to islet infusions has declined, and the reporting of adverse events has improved over the years. For allogenic islet transplantation bleeding, either intraperitoneal or liver sub capsular is the most common procedure-related complication, occurring with an incidence as high as 13% (Villiger et al. 2005). The exact cause of bleeding in each case is often difficult to determine; however, independent risk factors for hemorrhagic complications include the cumulative number of transplant procedures and heparin dosage of 45 U/kg or more (Villiger et al. 2005). The use of fibrin tissue sealant and embolization coils in the hepatic catheter tract seems to effectively minimize the bleeding risk (Froud et al. 2004; Villiger et al. 2005). Partial portal vein thrombosis complicates fewer than 5% of islet infusion procedures (Ryan et al. 2005), and complete portal venous thrombosis is rare. The use of purer islet preparations, greater expertise in portal vein catheterization, and new radiological devices (catheters medicated with anticoagulation) will continue reducing the risk of portal vein thrombosis, although the risk is unlikely to be completely eliminated. Other complications of islet cell transplantation include transient liver enzyme elevation (50% incidence) (Barshes et al. 2005a), abdominal pain (50% incidence), focal hepatic steatosis (20% incidence) (Bhargava et al. 2004; Maffi et al. 2005), and severe hypoglycemia (<3% incidence). Another complication related to the intrahepatic islet transplantation procedure is portal hypertension that can occur acutely during the islet infusion,

especially in the case of infusions other than the first one (Casey et al. 2002).The portal pressure generally normalizes after the acute phase of the procedure. Finally, severe hypoglycemia is a risk associated with the infusion of islets. Iatrogenic hypoglycemia in the immediate posttransplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognized hypoglycemia in the early posttransplant period. Although islet allotransplantation is a relatively safe procedure, adverse events and serious adverse events are not infrequent. The Eighth Annual Report of the Collaborative Islet Transplant Registry (CITR) reported 1,878 adverse events on 496 out of 864 (57.4%) allograft recipients who underwent islet infusions between 1999 and 2014. The higher than expected incidence of adverse events is in part related to the fact that there is close follow-up of the patients and centers abiding to the strict rules of reporting imposed by the FDA. Moreover, many adverse events seen in this population (30% of recipients) are unrelated to islet transplantation but not unexpected in a cohort of older patients with T1D with significant comorbidity. In any case, many of these events were adjudicated by the investigator as possibly to definitely relate to either the infusion procedure or the immunosuppression. The Eighth Annual Report of the CITR reported that in the first 30 days following islet transplantation, about 31% of recipients experienced a reportable adverse event. Roughly half of these events were adjudicated as possibly or definitely related to either the infusion procedure or the immunosuppression. The vast majority was not unexpected, such as abnormal lymphocyte counts and increased transaminases. About 20% of all recipients experienced a serious adverse event in the first 30 days, which occurred about equally in IAK/SIK as in ITA, and have declined somewhat over the eras. In the first year after islet transplantation, about 48% of all recipients have experienced a reportable adverse event and about one-third have experienced a serious adverse event, with a significant decline in the most recent era. Overall, 16% of all recipients failed to recover completely from an adverse event. The incidence of life-threatening events has declined over time (from 23.9% in 1999–2002 to 3.9% in 2011–2014), and a total of 10.3% of the patients reporting (serious) adverse events in the 2011–2014 period resolved with sequelae. The need to implement antirejection therapy exposes transplant recipients to an increased risk of untoward side effects expected in any immunosuppressed subjects. Opportunistic infections of the urinary tract, upper respiratory tract, and skin are frequent, along with myelosuppressive and gastrointestinal effects of the immunosuppressive drugs. In the majority of the cases, these effects are not severe and resolve without sequelae with medical treatment. Direct organ toxicity of immunosuppressive drugs has been recognized. Symptoms associated with neuro- and/or nephrotoxicity are relatively frequent in subjects receiving chronic immunosuppressive agents currently in use in the clinical arena. In these cases, modification of the antirejection regimen is indicated, with dose reduction or conversion to a different combination of drugs. In the majority of cases, these changes resolve the symptoms without compromising graft survival. The risk of transmission of CMV disease from donor to recipient has been surprisingly low in recipients of islet allografts, particularly in the most recent period with routine use of purified islet preparations (140–144). As with any allogeneic transplant, islet transplant recipients may become sensitized to

islet donor histocompatibility antigens (HLA), leading to the development of panel reactive alloantibodies (PRA). Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centers (148–152). A potential consequence of high PRA levels in recipients of a failed islet transplants is that if these individuals develop diabetic nephropathy in the future, a high PRA may increase their time on a transplant list for a suitable kidney graft. Nephrotoxicity from sirolimus and/or tacrolimus has been described in patients with T1D undergoing islet transplantation, particularly when kidney function is already impaired because of preexisting diabetic nephropathy (Andres et al. 2005; Maffi et al. 2007; Gala-Lopez et al. 2011). CITR ITA recipients exhibited a decline in eGFR of 12.4 ± 19.2 ml/min/1.73 m^2, and IAK/SIK experienced a mean decline of 0.8 ± 32.3 ml/min/1.73 m^2 in 5 years from their first islet infusion, compared to a mean decline of about 9 ml/min/1.73 m^2 over the first 5 years in an age-unadjusted cohort of 1,141 patients with T1D followed by the DCCT and then by EDIC. A total of 41 instances of neoplasm have been reported in 32 of 864 islet transplant recipients during about 5,762 person-years of observed follow-up (0.007 neoplasms per person-year, CITR report). There were 21 instances in 17 patients of basal or squamous cell carcinoma of the skin. There were six instances of malignant ovarian cysts, four instances of breast cancer, two instances of lung cancer, two instances of thyroid cancer, and three instances of PTLD. Of the 14 recipients with non-skin cancer, 8 recovered, 2 recovered with sequelae, 5 have not recovered, and 1 died. Among islet allograft recipients, there have been 25 reports of death to the registry, i.e., a 2.4% crude mortality over a mean follow-up of 6.7 years. Causes of death were cardiovascular ($n = 5$), hemorrhage ($n = 3$), pneumonia ($n = 2$), diabetic ketoacidosis ($n = 1$), infection ($n = 1$), respiratory arrest ($n = 1$), acute toxicity ($n = 1$), pneumopathy ($n = 1$), multi-organ failure of unknown etiology ($n = 1$), lung carcinoma ($n = 1$), and viral meningitis ($n = 1$).

Immunosuppression

Preexisting and transplant-induced auto- and allo-specific cellular immune responses play a crucial role in the loss of islets and islet function infused in the liver (Campbell et al. 2007; Hilbrands et al. 2009; Piemonti et al. 2013) (Bertuzzi and Ricordi 2007) along with nonspecific immune responses predominantly mediated by innate inflammatory processes related to mechanics and site (Moberg et al. 2002; Matsuoka et al. 2010; Citro et al. 2012, 2013). Islet graft rejection occurs without clinical symptoms. Neither guidelines nor formal consensus on the "best" or "standard" immunosuppressive strategy for human islet transplantation is currently available. Multiple induction and maintenance agents are administered peri- and post-every infusion in the same recipient. According to the Collaborative Islet Transplant Registry (CITR) data (Barton et al. 2012), a substantial shift in immunosuppression strategies has been documented during the last 12 years. The 2000–2006 period was dominated by the Edmonton Protocol, which used an interleukin-2 receptor antagonist (e.g., daclizumab) for induction and a mammalian target of rapamycin (mTOR) inhibitor (e.g., sirolimus), together with a calcineurin inhibitor (CNI, e.g., tacrolimus) for maintenance immunosuppression (Shapiro et al. 2006). In the more

recent years, there has been a shift toward the induction with a T-cell-depleting (TCD) antibody, with or without an inhibitor of tumor necrosis factor-α (TNF-α; e.g., etanercept) (Pileggi et al. 2004; Frank et al. 2005; Hering et al. 2005; Marzorati et al. 2007; Alejandro et al. 2008; Bellin et al. 2008; Froud et al. 2008; Gerber et al. 2008; Tan et al. 2008) and maintenance with an mTOR inhibitor or an inosine monophosphate dehydrogenase inhibitor (e.g., mycophenolic acid) combined with a CNI (Shapiro et al. 2000; Froud et al. 2005; Hering et al. 2005; Vantyghem et al. 2009). Moreover, the use of alemtuzumab induction therapy was recently reported and associated with encouraging longer-term function (Shapiro 2011; Nijhoff et al. 2015). New biologic agents with potentially lower islet cell and organ toxicity profiles are currently being evaluated in ongoing clinical trials. Among these are agents that target co-stimulation pathways in immune cells and/or adhesion molecules (CTLA4-Ig, LFA-1 PD-1/PD-L1 CD40) (Badell et al. 2010; Posselt et al. 2010a, b; Turgeon et al. 2010; Fotino and Pileggi 2011; Watanabe et al. 2013; Li et al. 2015) or chemokine receptors (CXCR1/2) (Citro et al. 2012, 2015). Finally, a calcineurin inhibitor-free immunosuppressive regimen was recently reported (Maffi et al. 2014).

Impact of Islet Transplantation on Metabolic Control and Diabetes Complication

Recent clinical trials demonstrated that the effects of islet transplantation on metabolic control are quite reproducible in subjects with unstable type 1 diabetes (Barton et al. 2012). Exogenous insulin requirements needed to attain optimal metabolic control are dramatically reduced immediately after islet transplantation, with a reduction of mean amplitude of glycemic excursions (MAGE) throughout the day and normalization of A1c $<6.5\%$ (Shapiro et al. 2000; Froud et al. 2005). Since the main indications for islet transplantation in subjects with type 1 diabetes are unstable control and frequent severe hypoglycemic episodes, the most remarkable effect of the transplant is the abrogation of severe hypoglycemia (Johnson et al. 2004; Poggioli et al. 2006; Leitao et al. 2008; Tharavanij et al. 2008). Using HYPO score and Lability Index to longitudinally assess islet transplant recipients, a significant reduction in the incidence of severe hypoglycemia over a 4-year follow-up period was demonstrated, a finding suggesting that the intervention can support a better and more physiological metabolic control than medical therapy (Ryan et al. 2004b, 2005). It is noteworthy that the prevention of severe hypoglycemia persists long-term and even in subjects requiring exogenous insulin to maintain optimal glycemic control (such as after implantation of a suboptimal islet mass or after development of graft dysfunction) as far as C-peptide is measurable (Pileggi et al. 2004; Alejandro et al. 2008). Following islet transplantation, the restoration of beta-cell responses to secretagogue stimulation is observed, with improved insulin secretion ("first phase") in response to intravenous glucose, as well as increased C-peptide secretion in response to oral glucose. Normalization of the glycemic threshold triggering the release of counter-regulatory hormones can be demonstrated during hypoglycemic clamp studies, albeit without reaching normalization of the magnitude of the vegetative response. Furthermore, quasi-normal glucagon secretion in response

to hypoglycemia can be observed (Paty et al. 2002, 2006; Rickels et al. 2005a, b, 2007). These observations may, at least in part, explain the significant improvement in metabolic control and recovery of hypoglycemia awareness observed after islet transplantation, which persists after development of graft dysfunction and even several months after graft failure (and loss of detectable C-peptide) (Johnson et al. 2004; Barton et al. 2012). Quality of life dramatically improves after islet transplantation. Improvements include greater satisfaction with life and health, more feelings of control and independence, and perceptions of better physical, mental, and social health and function. T1D patients with insulin independence or partial graft function similarly report reduction of hypoglycemic episodes (Radosevich et al. 2013), improvement of symptom awareness, and the rediscovering of reliability and independence (Barshes et al. 2005b; Poggioli et al. 2006; Toso et al. 2007; Cure et al. 2008; Tharavanij et al. 2008; Benhamou et al. 2009). After transplantation of an adequate islet mass obtained from one or more donor pancreata (estimated $\geq$10.000 islet equivalents (IEq)/kg of recipient's body weight), insulin independence can be reproducibly achieved. By combining donor selection criteria with improved isolation techniques and adequate immunomodulation of the recipient, insulin independence after single-donor islet preparation is becoming more reproducibly possible to achieve. Islet preparations obtained from more than one donor pancreas can be transplanted at once after pooling them or sequentially based on the metabolic needs of each subject. Data from the Clinical Islet Transplant Registry and independent trial reports have shown that insulin independence at 1 year from completion of the transplant is up to 70% with virtually 100% of the subjects maintaining graft function (C-peptide) if adequately immunosuppressed (Alejandro et al. 2008; Barton et al. 2012). A progressive loss of insulin independence with approximately 90% of subjects requiring reintroduction of exogenous insulin at 5 years (most of them with detectable C-peptide) has been reported in clinical trials based on the "Edmonton Protocol" (induction with anti-IL2R antibody; maintenance with sirolimus and tacrolimus) and some variants of it (Shapiro et al. 2000; Hering et al. 2005; Ryan et al. 2005; Shapiro et al. 2006; Vantyghem et al. 2009). More recent trials using more potent lymphodepletion (i.e., thymoglobulin, anti-CD3, or anti-CD52 antibodies) and/or biologics (anti-IL2R, anti-TNF, anti-LFA-1 antibody or CTLA4Ig) have shown great promise with approximately 50% insulin independence at 5 years after islet transplantation (Bellin et al. 2008, 2012; Vantyghem et al. 2009, 2014a; Maffi et al. 2011; Shapiro 2011), which is comparable to some of the data in whole-pancreas transplantation in subjects with type 1 diabetes (Froud et al. 2008; Tan et al. 2008; Vantyghem et al. 2009; Posselt et al. 2010a, b). In light of the results of the last decade of clinical islet transplant trials, achievement of insulin independence, although desirable, no longer should be considered the main goal of islet transplantation. The sizable improvement of metabolic control in the absence of severe hypoglycemic events, the amelioration of diabetes complications, and the achievement of sustained better quality of life, which are quite cumbersome to reproduce by the means of medical treatment, justify the risks associated with the islet transplant procedure and immunosuppression in this high-risk population of subjects with unstable diabetes. Encouraging results have been reported in recent years on the

multiple beneficial effects of islet transplantation on progression of diabetes complications [reviewed in (Bassi and Fiorina 2011)]. Although based on non-randomized pilot studies, which should be cautiously evaluated, they provide the proof of concept of the importance of restoring beta-cell function in patients with diabetes. In particular, improvement of micro- and macroangiopathy (main causes of diabetic nephropathy) (Fiorina et al. 2003c, 2005b; Toso et al. 2006; Fung et al. 2007; Maffi et al. 2007; Senior et al. 2007; Cure et al. 2008; Gerber et al. 2008; Leitao et al. 2009; Thompson et al. 2011; Gillard et al. 2014) and stabilization/reduced progression of retinopathy (Lee et al. 2005; Venturini et al. 2006; Warnock et al. 2008; Thompson et al. 2011) and neuropathy (Lee et al. 2005; Del Carro et al. 2007; D'Addio et al. 2014; Vantyghem et al. 2014b) have been described. Amelioration of cardiovascular and endothelial dysfunction and reduction of atherothrombotic profile, paralleled by reduced incidence of cardiovascular accidents and higher survival rates, were reported in IAK recipients (Fiorina et al. 2003a, b, c, 2005a, b; Del Carro et al. 2007; Danielson et al. 2013; D'Addio et al. 2014). Furthermore, significantly improved longevity of a renal transplant was observed after islet transplantation (Fiorina et al. 2005b). It is likely that these benefits are the consequence of improved metabolic control conferred by the islet transplant. In addition, a contribution of restored C-peptide secretion and its effects on multiple targets has been proposed (Hansen et al. 2002).

Current Challenges in Islet Transplantation

While significant progress has been made in the islet transplantation field, several obstacles remain precluding its widespread use. The clinical experience of islet transplantation has been developed almost exclusively using the intrahepatic infusion through the portal vein (Shapiro et al. 2006). It has been suggested that the loss of as many as 50–75% of islets during engraftment is the reason why a very large number of islets are needed to achieve normoglycemia (Cantarelli and Piemonti 2011; Citro et al. 2013). Moreover, two additional important limitations are the currently inadequate immunosuppression for preventing islet rejection (Piemonti et al. 2013) and the limited oxygen supply to the islet in the engraftment site (Barkai et al. 2013; Lo et al. 2013). Current immunosuppressive regimens are capable of preventing islet failure for months to years, but the agents used in these treatments may increase the risk for specific malignancies and opportunistic infections. In addition the most commonly used agents (like calcineurin inhibitors and rapamycin) are also known to impair normal islet function and/or insulin action. Furthermore, like all medications, these agents have other associated toxicities, including the harmful effect of certain widely employed immunosuppressive agents on renal function. The second very significant factor for early and late loss of islet mass is the critical lack of immediate vascularization and chronic hypoxygenation. Physiological supply of oxygen and nutrients in native islets is maintained by a tight capillary network, destroyed by the islet isolation procedure, restricting supply to diffusion from the portal vein and hepatic arterial capillaries until the revascularization process is completed. Oxygen tension in the liver parenchyma decreases from

approximately 40 to 5 mmHg, eightfold lower compared to the intrapancreatic levels, leading to severe hypoxia and β-cell death. Revascularization of the islet graft in rodent transplant requires 10–14 days and much longer in nonhuman primates and human recipients. Even after the revascularization of the islets is completed, the capillary' density is significantly lower compared to the physiological intrapancreatic situation. The proof of concept for cellular replacement therapy in diabetes has been firmly established with islet transplantation. It represents an extremely promising therapy, but it needs to be improved and made more widely available.

Future Developments in Beta-Cell Replacement Therapies

The field of beta-cell replacement has evolved significantly over the last three decades thanks to the incredible efforts of the research community worldwide with continuous improvements in islet manufacturing process and pancreas transplantation techniques, coupled with better patient management and the development of more effective induction and maintenance immunosuppressive protocols. In addition, islet transplantation represents an excellent platform toward the development of cellular therapies aimed at the restoration of β-cell function using alternative sources of β-cells like xenogeneic islets or insulin-producing cells derived from the differentiation of stem cells. While a wide range of improvements may be implemented in the donor selection and organ allocation scheme to increase pancreas utilization for transplantation, there is increasing new excitement for the use of unlimited alternative sources of transplantable islets, such as xenogeneic (i.e., obtained from other species such as porcine islets) [reviewed in (Marigliano et al. 2011)] or derived from human stem cells (Kroon et al. 2008; Schulz et al. 2012; Pagliuca et al. 2014; Rezania et al. 2014). Currently, the most significant advances come from the stem cell field; in fact it has been described that human ESC and iPSC are able to generate pancreatic progenitors and/or functional β-cells in vitro that can treat diabetic mice, and a clinical trial with ESC-derived cells is ongoing in T1D patients. Moreover, the stem cell approach may synergize well with other developing innovations such as the generation of immune isolating and retrievable devices, fundamental to allow cell therapy without immunosuppression and to overcome the safety concerns about tumorigenic cells. It is likely that altogether, these experiences will change the way we treat T1D and lead to new therapeutic options for patients with diabetes.

References

Alejandro R, Barton FB, et al. 2008 Update from the collaborative islet transplant registry. Transplantation. 2008;86(12):1783–8.

Andres A, Toso C, et al. Impairment of renal function after islet transplant alone or islet-after-kidney transplantation using a sirolimus/tacrolimus-based immunosuppressive regimen. Transpl Int. 2005;18(11):1226–30.

Badell IR, Russell MC, et al. LFA-1-specific therapy prolongs allograft survival in rhesus macaques. J Clin Invest. 2010;120(12):4520–31.

Baghurst PA. Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data: an automated algorithm. Diabetes Technol Ther. 2011;13(3):296–302.
Balamurugan AN, Naziruddin B, et al. Islet product characteristics and factors related to successful human islet transplantation from the collaborative islet transplant registry (CITR) 1999–2010. Am J Transplant. 2014;14(11):2595–606.
Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery. 1972;72(2): 175–86.
Barkai U, Weir GC, et al. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. Cell Transplant. 2013;22(8):1463–76.
Barshes NR, Lee TC, et al. Transaminitis after pancreatic islet transplantation. J Am Coll Surg. 2005a;200(3):353–61.
Barshes NR, Vanatta JM, et al. Health-related quality of life after pancreatic islet transplantation: a longitudinal study. Transplantation. 2005b;79(12):1727–30.
Barton FB, Rickels MR, et al. Improvement in outcomes of clinical islet transplantation: 1999–2010. Diabetes Care. 2012;35(7):1436–45.
Bassi R, Fiorina P. Impact of islet transplantation on diabetes complications and quality of life. Curr Diab Rep. 2011;11(5):355–63.
Bazerbachi F, Selzner M, et al. Portal venous versus systemic venous drainage of pancreas grafts: impact on long-term results. Am J Transplant. 2012;12(1):226–32.
Becker BN, Brazy PC, et al. Simultaneous pancreas-kidney transplantation reduces excess mortality in type 1 diabetic patients with end-stage renal disease. Kidney Int. 2000;57(5):2129–35.
Bellin MD, Kandaswamy R, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. Am J Transplant. 2008;8(11):2463–70.
Bellin MD, Barton FB, et al. Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. Am J Transplant. 2012;12(6):1576–83.
Benhamou PY, Milliat-Guittard L, et al. Quality of life after islet transplantation: data from the GRAGIL 1 and 2 trials. Diabet Med. 2009;26(6):617–21.
Bergenstal RM, Tamborlane WV, et al. Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes. N Engl J Med. 2010;363(4):311–20.
Bergenstal RM, Klonoff DC, et al. Threshold-based insulin-pump interruption for reduction of hypoglycemia. N Engl J Med. 2013;369(3):224–32.
Berney T, Johnson PR. Donor pancreata: evolving approaches to organ allocation for whole pancreas versus islet transplantation. Transplantation. 2010;90(3):238–43.
Bertuzzi F, Ricordi C. Beta-cell replacement in immunosuppressed recipients: old and new clinical indications. Acta Diabetol. 2007;44(4):171–6.
Bertuzzi F, Verzaro R, et al. Brittle type 1 diabetes mellitus. Curr Med Chem. 2007;14(16):1739–44.
Bhargava R, Senior PA, et al. Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. Diabetes. 2004;53(5):1311–7.
Biesenbach G, Konigsrainer A, et al. Progression of macrovascular diseases is reduced in type 1 diabetic patients after more than 5 years successful combined pancreas-kidney transplantation in comparison to kidney transplantation alone. Transpl Int. 2005;18(9):1054–60.
Bode BW, Schwartz S, et al. Glycemic characteristics in continuously monitored patients with type 1 and type 2 diabetes: normative values. Diabetes Care. 2005;28(10):2361–6.
Boggi U, Vistoli F, et al. Results of pancreas transplantation alone with special attention to native kidney function and proteinuria in type 1 diabetes patients. Rev Diabet Stud. 2011;8(2):259–67.
Bonfanti R, Lepore G, et al. Survey on the use of insulin pumps in Italy: comparison between pediatric and adult age groups (IMITA study). Acta Diabetol. 2015;53:403.
Bretzel R, Brendel M, Hering B. International islet transplant registry. Newsletter #9. 2001;8:1.
Campbell PM, Salam A, et al. Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. Am J Transplant. 2007;7(5):1242–8.
Campistol JM, de Fijter JW, et al. mTOR inhibitor-associated dermatologic and mucosal problems. Clin Transpl. 2010;24(2):149–56.

Cantarelli E, Piemonti L. Alternative transplantation sites for pancreatic islet grafts. Curr Diab Rep. 2011;11(5):364–74.
Cartwright A, Wallymahmed M, et al. The outcome of brittle type 1 diabetes – a 20 year study. QJM. 2011;104(7):575–9.
Casey JJ, Lakey JR, et al. Portal venous pressure changes after sequential clinical islet transplantation. Transplantation. 2002;74(7):913–5.
Chiang JL, Kirkman MS, et al. Type 1 diabetes through the life span: a position statement of the American diabetes association. Diabetes Care. 2014;37(7):2034–54.
Choudhary P, Geddes J, et al. Frequency of biochemical hypoglycaemia in adults with Type 1 diabetes with and without impaired awareness of hypoglycaemia: no identifiable differences using continuous glucose monitoring. Diabet Med. 2010;27(6):666–72.
Choudhary P, Rickels MR, et al. Evidence-informed clinical practice recommendations for treatment of type 1 diabetes complicated by problematic hypoglycemia. Diabetes Care. 2015;38 (6):1016–29.
Citro A, Cantarelli E, et al. CXCR1/2 inhibition enhances pancreatic islet survival after transplantation. J Clin Invest. 2012;122(10):3647–51.
Citro A, Cantarelli E, et al. Anti-inflammatory strategies to enhance islet engraftment and survival. Curr Diab Rep. 2013;13(5):733–44.
Citro A, Cantarelli E, et al. The CXCR1/2 pathway: involvement in diabetes pathophysiology and potential target for T1D interventions. Curr Diab Rep. 2015;15(10):638.
Clarke WL, Cox DJ, et al. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care. 1995;18 (4):517–22.
Cook K, Sollinger HW, et al. Pancreaticocystostomy: an alternative method for exocrine drainage of segmental pancreatic allografts. Transplantation. 1983;35(6):634–6.
Coppelli A, Giannarelli R, et al. Pancreas transplant alone determines early improvement of cardiovascular risk factors and cardiac function in type 1 diabetic patients. Transplantation. 2003;76(6):974–6.
Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med. 2013;369(4):362–72.
Cure P, Pileggi A, et al. Improved metabolic control and quality of life in seven patients with type 1 diabetes following islet after kidney transplantation. Transplantation. 2008;85(6):801–12.
D'Addio F, Maffi P, et al. Islet transplantation stabilizes hemostatic abnormalities and cerebral metabolism in individuals with type 1 diabetes. Diabetes Care. 2014;37(1):267–76.
Danielson KK, Hatipoglu B, et al. Reduction in carotid intima-media thickness after pancreatic islet transplantation in patients with type 1 diabetes. Diabetes Care. 2013;36(2):450–6.
de Mattos AM, Olyaei AJ, et al. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis. 2000;35(2):333–46.
Del Carro U, Fiorina P, et al. Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis. Diabetes Care. 2007;30(12):3063–9.
Dew MA, Switzer GE, et al. Psychosocial assessments and outcomes in organ transplantation. Prog Transplant. 2000;10(4):239–59; quiz 260–231.
Dieterle CD, Arbogast H, et al. Metabolic follow-up after long-term pancreas graft survival. Eur J Endocrinol. 2007;156(5):603–10.
Dubernard JM, Traeger J, et al. A new method of preparation of segmental pancreatic grafts for transplantation: trials in dogs and in man. Surgery. 1978;84(5):633–9.
Fatourechi MM, Kudva YC, et al. Clinical review: hypoglycemia with intensive insulin therapy: a systematic review and meta-analyses of randomized trials of continuous subcutaneous insulin infusion versus multiple daily injections. J Clin Endocrinol Metab. 2009;94(3):729–40.
Feldmeyer L, Hofbauer GF, et al. Mammalian target of rapamycin (mTOR) inhibitors slow skin carcinogenesis, but impair wound healing. Br J Dermatol. 2012;166(2):422–4.

Feltbower RG, Bodansky HJ, et al. Acute complications and drug misuse are important causes of death for children and young adults with type 1 diabetes: results from the Yorkshire register of diabetes in children and young adults. Diabetes Care. 2008;31(5):922–6.
Fioretto P, Steffes MW, et al. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med. 1998;339(2):69–75.
Fioretto P, Sutherland DE, et al. Remodeling of renal interstitial and tubular lesions in pancreas transplant recipients. Kidney Int. 2006;69(5):907–12.
Fiorina P, La Rocca E, et al. Reversal of left ventricular diastolic dysfunction after kidney-pancreas transplantation in type 1 diabetic uremic patients. Diabetes Care. 2000;23(12):1804–10.
Fiorina P, Folli F, et al. Long-term beneficial effect of islet transplantation on diabetic macro-/microangiopathy in type 1 diabetic kidney-transplanted patients. Diabetes Care. 2003a;26(4):1129–36.
Fiorina P, Folli F, et al. Islet transplantation improves vascular diabetic complications in patients with diabetes who underwent kidney transplantation: a comparison between kidney-pancreas and kidney-alone transplantation. Transplantation. 2003b;75(8):1296–301.
Fiorina P, Folli F, et al. Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. J Am Soc Nephrol. 2003c;14(8):2150–8.
Fiorina P, Gremizzi C, et al. Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients. Diabetes Care. 2005a;28(6):1358–65.
Fiorina P, Venturini M, et al. Natural history of kidney graft survival, hypertrophy, and vascular function in end-stage renal disease type 1 diabetic kidney-transplanted patients: beneficial impact of pancreas and successful islet cotransplantation. Diabetes Care. 2005b;28(6):1303–10.
Fiorina P, Perseghin G, et al. Altered kidney graft high-energy phosphate metabolism in kidney-transplanted end-stage renal disease type 1 diabetic patients: a cross-sectional analysis of the effect of kidney alone and kidney-pancreas transplantation. Diabetes Care. 2007;30(3):597–603.
Fiorina P, Vezzulli P, et al. Near normalization of metabolic and functional features of the central nervous system in type 1 diabetic patients with end-stage renal disease after kidney-pancreas transplantation. Diabetes Care. 2012;35(2):367–74.
Floyd B, Chandra P, et al. Comparative analysis of the efficacy of continuous glucose monitoring and self-monitoring of blood glucose in type 1 diabetes mellitus. J Diabetes Sci Technol. 2012;6(5):1094–102.
Folli F, Guzzi V, et al. Proteomics reveals novel oxidative and glycolytic mechanisms in type 1 diabetic patients' skin which are normalized by kidney-pancreas transplantation. PLoS One. 2010;5(3):e9923.
Fotino C, Pileggi A. Blockade of leukocyte function antigen-1 (LFA-1) in clinical islet transplantation. Curr Diab Rep. 2011;11(5):337–44.
Franco OH, Steyerberg EW, et al. Associations of diabetes mellitus with total life expectancy and life expectancy with and without cardiovascular disease. Arch Intern Med. 2007;167(11):1145–51.
Frank AM, Barker CF, et al. Comparison of whole organ pancreas and isolated islet transplantation for type 1 diabetes. Adv Surg. 2005;39:137–63.
Freckmann G, Hagenlocher S, et al. Continuous glucose profiles in healthy subjects under everyday life conditions and after different meals. J Diabetes Sci Technol. 2007;1(5):695–703.
Frier BM. Morbidity of hypoglycemia in type 1 diabetes. Diabetes Res Clin Pract. 2004;65(Suppl 1):S47–52.
Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. Nat Rev Endocrinol. 2014;10(12):711–22.
Froud T, Yrizarry JM, et al. Use of D-STAT to prevent bleeding following percutaneous transhepatic intraportal islet transplantation. Cell Transplant. 2004;13(1):55–9.
Froud T, Ricordi C, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005;5(8):2037–46.

Froud T, Baidal DA, et al. Islet transplantation with alemtuzumab induction and calcineurin-free maintenance immunosuppression results in improved short- and long-term outcomes. Transplantation. 2008;86(12):1695–701.
Fung MA, Warnock GL, et al. The effect of medical therapy and islet cell transplantation on diabetic nephropathy: an interim report. Transplantation. 2007;84(1):17–22.
Gaba RC, Garcia-Roca R, et al. Pancreatic islet cell transplantation: an update for interventional radiologists. J Vasc Interv Radiol. 2012;23(5):583–94; quiz 594.
Gaber AO, Oxley D, et al. Changes in gastric emptying in recipients of successful combined pancreas-kidney transplants. Dig Dis. 1991;9(6):437–43.
Gaber AO, el-Gebely S, et al. Early improvement in cardiac function occurs for pancreas-kidney but not diabetic kidney-alone transplant recipients. Transplantation. 1995a;59(8):1105–12.
Gaber AO, Shokouh-Amiri MH, et al. Results of pancreas transplantation with portal venous and enteric drainage. Ann Surg. 1995b;221(6):613–22; discussion 622–614.
Gala-Lopez BL, Senior PA, et al. Late cytomegalovirus transmission and impact of T-depletion in clinical islet transplantation. Am J Transplant. 2011;11(12):2708–14.
Garg S, Zisser H, et al. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor: a randomized controlled trial. Diabetes Care. 2006;29(1):44–50.
Geddes J, Schopman JE, et al. Prevalence of impaired awareness of hypoglycaemia in adults with type 1 diabetes. Diabet Med. 2008;25(4):501–4.
Geissler EK. Post-transplantation malignancies: here today, gone tomorrow? Nat Rev Clin Oncol. 2015;12(12):705–17.
Gerber PA, Pavlicek V, et al. Simultaneous islet-kidney vs pancreas-kidney transplantation in type 1 diabetes mellitus: a 5 year single centre follow-up. Diabetologia. 2008;51(1):110–9.
Giannarelli R, Coppelli A, et al. Effects of pancreas-kidney transplantation on diabetic retinopathy. Transpl Int. 2005;18(5):619–22.
Giannarelli R, Coppelli A, et al. Pancreas transplant alone has beneficial effects on retinopathy in type 1 diabetic patients. Diabetologia. 2006;49(12):2977–82.
Gill GV. The spectrum of brittle diabetes. J R Soc Med. 1992;85(5):259–61.
Gill GV, Lucas S, et al. Prevalence and characteristics of brittle diabetes in Britain. QJM. 1996;89(11):839–43.
Gillard P, Rustandi M, et al. Early alteration of kidney function in nonuremic type 1 diabetic islet transplant recipients under tacrolimus-mycophenolate therapy. Transplantation. 2014;98:451.
Giorda CB, Ozzello A, et al. Incidence and risk factors for severe and symptomatic hypoglycemia in type 1 diabetes. Results of the HYPOS-1 study. Acta Diabetol. 2015;52(5):845–53.
Girman P, Lipar K, et al. Neoplasm incidence in simultaneous pancreas and kidney transplantation: a single-center analysis. Transplant Proc. 2011;43(9):3288–91.
Gliedman ML, Gold M, et al. Clinical segmental pancreatic transplantation with ureter-pancreatic duct anastomosis for exocrine drainage. Surgery. 1973;74(2):171–80.
Gold AE, MacLeod KM, et al. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes Care. 1994;17(7):697–703.
Golden SH, Sapir T. Methods for insulin delivery and glucose monitoring in diabetes: summary of a comparative effectiveness review. J Manag Care Pharm. 2012;18(6 Suppl):S1–17.
Graveling AJ, Frier BM. Impaired awareness of hypoglycaemia: a review. Diabetes Metab. 2010;36(Suppl 3):S64–74.
Gregg EW, Li Y, et al. Changes in diabetes-related complications in the United States, 1990–2010. N Engl J Med. 2014;370(16):1514–23.
Gross CR, Zehrer CL. Health-related quality of life outcomes of pancreas transplant recipients. Clin Transpl. 1992;6(3 part 1):165–71.
Gruden G, Barutta F, et al. Severe hypoglycemia and cardiovascular disease incidence in type 1 diabetes: the EURODIAB prospective complications study. Diabetes Care. 2012;35(7):1598–604.
Gruessner AC. 2011 update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the international pancreas transplant registry (IPTR). Rev Diabet Stud. 2011;8(1):6–16.

Gruessner AC, Gruessner RW. Pancreas transplant outcomes for United States and non United States cases as reported to the united network for organ sharing and the international pancreas transplant registry as of December 2011. Clin Transpl. 2012:23–40. PMID: 23721008.
Gruessner RW, Gruessner AC. The current state of pancreas transplantation. Nat Rev Endocrinol. 2013a;9(9):555–62.
Gruessner RW, Gruessner AC. Pancreas transplant alone: a procedure coming of age. Diabetes Care. 2013b;36(8):2440–7.
Gruessner AC, Gruessner RW. Declining numbers of pancreas transplantations but significant improvements in outcome. Transplant Proc. 2014;46(6):1936–7.
Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) cases reported to the united network for organ sharing (UNOS) and non-US cases reported to the international pancreas transplant registry (IPTR) as of October, 2000. Clin Transpl. 2000:45–72. PMID: 11512358.
Gruessner RW, Sutherland DE, et al. Mortality assessment for pancreas transplants. Am J Transplant. 2004;4(12):2018–26.
Han DJ, Sutherland DE. Pancreas transplantation. Gut Liver. 2010;4(4):450–65.
Hansen A, Johansson BL, et al. C-peptide exerts beneficial effects on myocardial blood flow and function in patients with type 1 diabetes. Diabetes. 2002;51(10):3077–82.
Hao WJ, Zong HT, et al. The efficacy and safety of alemtuzumab and daclizumab versus antithymocyte globulin during organ transplantation: a meta-analysis. Transplant Proc. 2012;44(10):2955–60.
Hasskarl J. Everolimus. Recent Results Cancer Res. 2014;201:373–92.
Helfrich M, Ison MG. Opportunistic infections complicating solid organ transplantation with alemtuzumab induction. Transpl Infect Dis. 2015;17(5):627–36.
Hering BJ, Bretzel RG, et al. New protocol toward prevention of early human islet allograft failure. Transplant Proc. 1994;26(2):570–1.
Hering BJ, Kandaswamy R, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005;293(7):830–5.
Hilbrands R, Huurman VA, et al. Differences in baseline lymphocyte counts and autoreactivity are associated with differences in outcome of islet cell transplantation in type 1 diabetic patients. Diabetes. 2009;58(10):2267–76.
Hoi-Hansen T, Pedersen-Bjergaard U, et al. Classification of hypoglycemia awareness in people with type 1 diabetes in clinical practice. J Diabetes Complicat. 2010;24(6):392–7.
Hopkins D, Lawrence I, et al. Improved biomedical and psychological outcomes 1 year after structured education in flexible insulin therapy for people with type 1 diabetes: the U.K. DAFNE experience. Diabetes Care. 2012;35(8):1638–42.
Hu FB, Stampfer MJ, et al. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. Arch Intern Med. 2001;161(14):1717–23.
Isla Pera P, Moncho Vasallo J, et al. Quality of life in simultaneous pancreas-kidney transplant recipients. Clin Transpl. 2009;23(5):600–5.
Jahansouz C, Kumer SC, et al. Evolution of beta-cell replacement therapy in diabetes mellitus: pancreas transplantation. Diabetes Technol Ther. 2011;13(3):395–418.
Johnson PR, Jones KE. Pancreatic islet transplantation. Semin Pediatr Surg. 2012;21(3):272–80. https://doi.org/10.1053/j.sempedsurg.2012.05.012. PMID: 22800980 [Indexed for MEDLINE]
Johnson JA, Kotovych M, et al. Reduced fear of hypoglycemia in successful islet transplantation. Diabetes Care. 2004;27(2):624–5.
Johnston O, Rose CL, et al. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. J Am Soc Nephrol. 2008;19(7):1411–8.
Jukema JW, Smets YF, et al. Impact of simultaneous pancreas and kidney transplantation on progression of coronary atherosclerosis in patients with end-stage renal failure due to type 1 diabetes. Diabetes Care. 2002;25(5):906–11.

Kandaswamy R, Skeans MA, et al. OPTN/SRTR 2013 annual data report: pancreas. Am J Transplant. 2015;15(Suppl 2):1–20.
Kandaswamy R, Skeans MA, et al. Pancreas. Am J Transplant. 2016;16(Suppl 2):47–68.
Kelly WD, Lillehei RC, et al. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery. 1967;61(6):827–37.
Kennedy WR, Navarro X, et al. Effects of pancreatic transplantation on diabetic neuropathy. N Engl J Med. 1990;322(15):1031–7.
Kent LA, Gill GV, et al. Mortality and outcome of patients with brittle diabetes and recurrent ketoacidosis. Lancet. 1994;344(8925):778–81.
Kleinclauss F, Fauda M, et al. Pancreas after living donor kidney transplants in diabetic patients: impact on long-term kidney graft function. Clin Transpl. 2009;23(4):437–46.
Kovatchev BP, Cox DJ, et al. Assessment of risk for severe hypoglycemia among adults with IDDM: validation of the low blood glucose index. Diabetes Care. 1998;21(11):1870–5.
Koznarova R, Saudek F, et al. Beneficial effect of pancreas and kidney transplantation on advanced diabetic retinopathy. Cell Transplant. 2000;9(6):903–8.
Kroon E, Martinson LA, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008;26(4):443–52.
La Rocca E, Minicucci F, et al. Evolution of carotid vascular lesions in kidney-pancreas and kidney-alone transplanted insulin-dependent diabetic patients. Transplant Proc. 1995;27(6):3072.
La Rocca E, Fiorina P, et al. Cardiovascular outcomes after kidney-pancreas and kidney-alone transplantation. Kidney Int. 2001;60(5):1964–71.
Larsen JL, Ratanasuwan T, et al. Carotid intima media thickness decreases after pancreas transplantation. Transplantation. 2002;73(6):936–40.
Larsen JL, Colling CW, et al. Pancreas transplantation improves vascular disease in patients with type 1 diabetes. Diabetes Care. 2004;27(7):1706–11.
Larsen CP, Pearson TC, et al. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. Am J Transplant. 2005;5(3):443–53.
Lee TC, Barshes NR, et al. The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. Transplant Proc. 2005;37(5):2263–5.
Lehmann R, Graziano J, et al. Glycemic control in simultaneous islet-kidney versus pancreas-kidney transplantation in type 1 diabetes: a prospective 13-year follow-up. Diabetes Care. 2015;38(5):752–9.
Leitao CB, Tharavanij T, et al. Restoration of hypoglycemia awareness after islet transplantation. Diabetes Care. 2008;31(11):2113–5.
Leitao CB, Cure P, et al. Stable renal function after islet transplantation: importance of patient selection and aggressive clinical management. Transplantation. 2009;87(5):681–8.
Letavernier E, Legendre C. mToR inhibitors-induced proteinuria: mechanisms, significance, and management. Transplant Rev (Orlando). 2008;22(2):125–30.
Li T, Ma R, et al. PD-1/PD-L1 costimulatory pathway-induced mouse islet transplantation immune tolerance. Transplant Proc. 2015;47(1):165–70.
Lind M, Svensson AM, et al. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med. 2014;371(21):1972–82.
Livingstone SJ, Levin D, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008–2010. JAMA. 2015;313(1):37–44.
Lo JF, Wang Y, et al. Quantitative and temporal control of oxygen microenvironment at the single islet level. J Vis Exp. 2013;(81):e50616. https://doi.org/10.3791/50616.
Maahs DM, Calhoun P, et al. A randomized trial of a home system to reduce nocturnal hypoglycemia in type 1 diabetes. Diabetes Care. 2014;37(7):1885–91.
Maffi P, Angeli E, et al. Minimal focal steatosis of liver after islet transplantation in humans: a long-term study. Cell Transplant. 2005;14(10):727–33.

Maffi P, Bertuzzi F, et al. Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy. Diabetes Care. 2007;30(5): 1150–5.
Maffi P, Scavini M, et al. Risks and benefits of transplantation in the cure of type 1 diabetes: whole pancreas versus islet transplantation. A single center study. Rev Diabet Stud. 2011;8(1):44–50.
Maffi P, Berney T, et al. Calcineurin inhibitor-free immunosuppressive regimen in type 1 diabetes patients receiving islet transplantation: single-group phase 1/2 trial. Transplantation. 2014;98(12):1301–9.
Mannucci E, Monami M, et al. Achieving HbA1c targets in clinical trials and in the real world: a systematic review and meta-analysis. J Endocrinol Investig. 2014;37(5):477–95.
Marigliano M, Bertera S, et al. Pig-to-nonhuman primates pancreatic islet xenotransplantation: an overview. Curr Diab Rep. 2011;11(5):402–12.
Markmann JF. Isolated pancreatic islet transplantation: a coming of age. Am J Transplant. 2016;16(2):381–2.
Martinenghi S, Comi G, et al. Amelioration of nerve conduction velocity following simultaneous kidney/pancreas transplantation is due to the glycaemic control provided by the pancreas. Diabetologia. 1997;40(9):1110–2.
Marzorati S, Pileggi A, et al. Allogeneic islet transplantation. Expert Opin Biol Ther. 2007;7(11): 1627–45.
Matsuoka N, Itoh T, et al. High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice. J Clin Invest. 2010;120(3):735–43.
McAlister VC, Gao Z, et al. Sirolimus-tacrolimus combination immunosuppression. Lancet. 2000;355(9201):376–7.
McCoy RG, Van Houten HK, et al. Increased mortality of patients with diabetes reporting severe hypoglycemia. Diabetes Care. 2012;35(9):1897–901.
McDonnell CM, Donath SM, et al. A novel approach to continuous glucose analysis utilizing glycemic variation. Diabetes Technol Ther. 2005;7(2):253–63.
McKnight JA, Wild SH, et al. Glycaemic control of type 1 diabetes in clinical practice early in the 21st century: an international comparison. Diabet Med. 2015;32(8):1036–50.
Merkel FK, Ryan WG, et al. Pancreatic transplantation for diabetes mellitus. IMJ Ill Med J. 1973;144(5):477–9 passim.
Miller KM, Foster NC, et al. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D exchange clinic registry. Diabetes Care. 2015;38(6):971–8.
Mittal S, Gough SC. Pancreas transplantation: a treatment option for people with diabetes. Diabet Med. 2014;31(5):512–21.
Mittal S, Johnson P, et al. Pancreas transplantation: solid organ and islet. Cold Spring Harb Perspect Med. 2014;4(4):a015610.
Moassesfar S, Masharani U, et al. A comparative analysis of the safety, efficacy, and cost of islet versus pancreas transplantation in nonuremic patients with type 1 diabetes. Am J Transplant. 2016;16(2):518–26.
Moberg L, Johansson H, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet. 2002;360(9350): 2039–45.
Mohan P, Safi K, et al. Improved patient survival in recipients of simultaneous pancreas-kidney transplant compared with kidney transplant alone in patients with type 1 diabetes mellitus and end-stage renal disease. Br J Surg. 2003;90(9):1137–41.
Morath C, Zeier M, et al. Metabolic control improves long-term renal allograft and patient survival in type 1 diabetes. J Am Soc Nephrol. 2008;19(8):1557–63.
Morrissey PE, Shaffer D, et al. Peripheral vascular disease after kidney-pancreas transplantation in diabetic patients with end-stage renal disease. Arch Surg. 1997;132(4):358–61; discussion 361–352.
Mujtaba MA, Sharfuddin AA, et al. Conversion from tacrolimus to belatacept to prevent the progression of chronic kidney disease in pancreas transplantation: case report of two patients. Am J Transplant. 2014;14(11):2657–61.

Nakache R, Tyden G, et al. Long-term quality of life in diabetic patients after combined pancreas-kidney transplantation or kidney transplantation. Transplant Proc. 1994;26(2):510–1.
Nano R, Clissi B, et al. Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia. 2005;48(5):906–12.
Nath DS, Gruessner A, et al. Late anastomotic leaks in pancreas transplant recipients – clinical characteristics and predisposing factors. Clin Transpl. 2005;19(2):220–4.
Navarro X, Sutherland DE, et al. Long-term effects of pancreatic transplantation on diabetic neuropathy. Ann Neurol. 1997;42(5):727–36.
Nghiem DD, Corry RJ. Technique of simultaneous renal pancreatoduodenal transplantation with urinary drainage of pancreatic secretion. Am J Surg. 1987;153(4):405–6.
Nijhoff MF, Engelse MA, et al. Glycemic stability through islet-after-kidney transplantation using an Alemtuzumab-based induction regimen and long-term triple-maintenance immunosuppression. Am J Transplant. 2015;16:246.
Norman SP, Kommareddi M, et al. Early pancreas graft failure is associated with inferior late clinical outcomes after simultaneous kidney-pancreas transplantation. Transplantation. 2011;92(7):796–801.
Ogundipe OO, Geddes J, et al. Impaired hypoglycaemia awareness and employment in people with type 1 diabetes. Occup Med (Lond). 2011;61(4):241–6.
Ojo AO, Meier-Kriesche HU, et al. The impact of simultaneous pancreas-kidney transplantation on long-term patient survival. Transplantation. 2001;71(1):82–90.
Olsen SE, Asvold BO, et al. Hypoglycaemia symptoms and impaired awareness of hypoglycaemia in adults with type 1 diabetes: the association with diabetes duration. Diabet Med. 2014;31(10):1210–7.
Pagliuca FW, Millman JR, et al. Generation of functional human pancreatic beta cells in vitro. Cell. 2014;159(2):428–39.
Paty BW, Ryan EA, et al. Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. Diabetes. 2002;51(12):3428–34.
Paty BW, Senior PA, et al. Assessment of glycemic control after islet transplantation using the continuous glucose monitor in insulin-independent versus insulin-requiring type 1 diabetes subjects. Diabetes Technol Ther. 2006;8(2):165–73.
Pedersen-Bjergaard U, Pramming S, et al. Recall of severe hypoglycaemia and self-estimated state of awareness in type 1 diabetes. Diabetes Metab Res Rev. 2003;19(3):232–40.
Pedersen-Bjergaard U, Pramming S, et al. Severe hypoglycaemia in 1076 adult patients with type 1 diabetes: influence of risk markers and selection. Diabetes Metab Res Rev. 2004;20(6):479–86.
Phillip M, Battelino T, et al. Nocturnal glucose control with an artificial pancreas at a diabetes camp. N Engl J Med. 2013;368(9):824–33.
Pickup JC, Sutton AJ. Severe hypoglycaemia and glycaemic control in type 1 diabetes: meta-analysis of multiple daily insulin injections compared with continuous subcutaneous insulin infusion. Diabet Med. 2008;25(7):765–74.
Piemonti L, Pileggi A. 25 years of the Ricordi automated method for islet isolation. CellR4. 2013;1(1):e128.
Piemonti L, Everly MJ, et al. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. Diabetes. 2013;62(5):1656–64.
Pileggi A, Ricordi C, et al. Twenty years of clinical islet transplantation at the Diabetes Research Institute – University of Miami. Clin Transpl. 2004:177–204.
Poggioli R, Faradji RN, et al. Quality of life after islet transplantation. Am J Transplant. 2006;6(2):371–8.
Port FK, Wolfe RA, et al. Comparison of survival probabilities for dialysis patients vs cadaveric renal transplant recipients. JAMA. 1993;270(11):1339–43.
Posselt AM, Bellin MD, et al. Islet transplantation in type 1 diabetics using an immunosuppressive protocol based on the anti-LFA-1 antibody efalizumab. Am J Transplant. 2010a;10(8):1870–80.
Posselt AM, Szot GL, et al. Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. Transplantation. 2010b;90(12):1595–601.

Pozza G, Traeger J, et al. Endocrine responses of type 1 (insulin-dependent) diabetic patients following successful pancreas transplantation. Diabetologia. 1983;24(4):244–8.
Prieto M, Sutherland DE, et al. Pancreas transplant results according to the technique of duct management: bladder versus enteric drainage. Surgery. 1987;102(4):680–91.
Radosevich DM, Jevne R, et al. Comprehensive health assessment and five-yr follow-up of allogeneic islet transplant recipients. Clin Transpl. 2013;27(6):E715–24.
Ramsay RC, Goetz FC, et al. Progression of diabetic retinopathy after pancreas transplantation for insulin-dependent diabetes mellitus. N Engl J Med. 1988;318(4):208–14.
Rana A, Gruessner A, et al. Survival benefit of solid-organ transplant in the United States. JAMA Surg. 2015;150(3):252–9.
Rangel EB. Tacrolimus in pancreas transplant: a focus on toxicity, diabetogenic effect and drug-drug interactions. Expert Opin Drug Metab Toxicol. 2014;10(11):1585–605.
Rayhill SC, D'Alessandro AM, et al. Simultaneous pancreas-kidney transplantation and living related donor renal transplantation in patients with diabetes: is there a difference in survival? Ann Surg. 2000;231(3):417–23.
Reddy KS, Stablein D, et al. Long-term survival following simultaneous kidney-pancreas transplantation versus kidney transplantation alone in patients with type 1 diabetes mellitus and renal failure. Am J Kidney Dis. 2003;41(2):464–70.
Rezania A, Bruin JE, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014;32(11):1121–33.
Rickels MR. Recovery of endocrine function after islet and pancreas transplantation. Curr Diab Rep. 2012;12(5):587–96.
Rickels MR, Schutta MH, et al. {beta}-cell function following human islet transplantation for type 1 diabetes. Diabetes. 2005a;54(1):100–6.
Rickels MR, Schutta MH, et al. Islet cell hormonal responses to hypoglycemia after human islet transplantation for type 1 diabetes. Diabetes. 2005b;54(11):3205–11.
Rickels MR, Naji A, et al. Acute insulin responses to glucose and arginine as predictors of beta-cell secretory capacity in human islet transplantation. Transplantation. 2007;84(10):1357–60.
Ricordi C. Islet transplantation: a brave new world. Diabetes. 2003;52(7):1595–603.
Ricordi C, Lacy PE, et al. Automated method for isolation of human pancreatic islets. Diabetes. 1988;37(4):413–20.
Ricordi C, Socci C, et al. Isolation of the elusive pig islet. Surgery. 1990;107(6):688–94.
Ricordi C, Tzakis AG, et al. Human islet isolation and allotransplantation in 22 consecutive cases. Transplantation. 1992;53(2):407–14.
Robertson RP. Islet transplantation a decade later and strategies for filling a half-full glass. Diabetes. 2010;59(6):1285–91.
Robertson P, Davis C, et al. Pancreas transplantation in type 1 diabetes. Diabetes Care. 2004; 27(Suppl 1):S105.
Robertson RP, Davis C, et al. Pancreas and islet transplantation in type 1 diabetes. Diabetes Care. 2006;29(4):935.
Rosenlof LK, Earnhardt RC, et al. Pancreas transplantation. An initial experience with systemic and portal drainage of pancreatic allografts. Ann Surg. 1992;215(6):586–95; discussion 596–587.
Ross PL, Milburn J, et al. Clinical review: insulin pump-associated adverse events in adults and children. Acta Diabetol. 2015;52(6):1017–24.
Rostambeigi N, Kudva YC, et al. Epidemiology of infections requiring hospitalization during long-term follow-up of pancreas transplantation. Transplantation. 2010;89(9):1126–33.
Russell SJ, El-Khatib FH, et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. N Engl J Med. 2014;371(4):313–25.
Ryan EA, Paty BW, et al. Risks and side effects of islet transplantation. Curr Diab Rep. 2004a;4(4):304–9.
Ryan EA, Shandro T, et al. Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. Diabetes. 2004b;53(4):955–62.

Ryan EA, Paty BW, et al. Five-year follow-up after clinical islet transplantation. Diabetes. 2005;54(7):2060–9.
Salonia A, D'Addio F, et al. Kidney-pancreas transplantation is associated with near-normal sexual function in uremic type 1 diabetic patients. Transplantation. 2011;92(7):802–8.
Salvalaggio PR, Dzebisashvili N, et al. Incremental value of the pancreas allograft to the survival of simultaneous pancreas-kidney transplant recipients. Diabetes Care. 2009;32(4):600–2.
Scharp DW, Lacy PE, et al. Insulin independence after islet transplantation into type I diabetic patient. Diabetes. 1990;39(4):515–8.
Schmidt S, Norgaard K. Bolus calculators. J Diabetes Sci Technol. 2014;8(5):1035–41.
Schulz TC, Young HY, et al. A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS One. 2012;7(5):e37004.
Seaquist ER, Anderson J, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care. 2013;36(5):1384–95.
Secchi A, Socci C, et al. Islet transplantation in IDDM patients. Diabetologia. 1997;40(2):225–31.
Senior PA, Zeman M, et al. Changes in renal function after clinical islet transplantation: four-year observational study. Am J Transplant. 2007;7(1):91–8.
Shapiro AM. Strategies toward single-donor islets of Langerhans transplantation. Curr Opin Organ Transplant. 2011;16(6):627–31.
Shapiro AM. Islet transplantation in type 1 diabetes: ongoing challenges, refined procedures, and long-term outcome. Rev Diabet Stud. 2012;9(4):385–406.
Shapiro AM, Lakey JR, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343(4):230–8.
Shapiro AM, Ricordi C, et al. Edmonton's islet success has indeed been replicated elsewhere. Lancet. 2003;362(9391):1242.
Shapiro AM, Ricordi C, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 2006;355(13):1318–30.
Shapiro AT, C, Imes S, Koh A, Kin T, O'Gorman D, Malcolm A, Dinyari P, Owen R, Kneteman RN, Bigam DL, Calne RY, Senior PA, Roep BO. Five-year results of islet-alone transplantation match pancreas-alone transplantation with Alemtuzumab, Tac/MMF, with strong suppression of auto and alloreactivity. In: 13th world congress of the international pancreas and islet transplant association (IPITA), Prague; 2011.
Siskind E, Maloney C, et al. An analysis of pancreas transplantation outcomes based on age groupings – an update of the UNOS database. Clin Transpl. 2014;28(9):990–4.
Skrivarhaug T, Bangstad HJ, et al. Long-term mortality in a nationwide cohort of childhood-onset type 1 diabetic patients in Norway. Diabetologia. 2006;49(2):298–305.
Smets YF, Westendorp RG, et al. Effect of simultaneous pancreas-kidney transplantation on mortality of patients with type-1 diabetes mellitus and end-stage renal failure. Lancet. 1999;353(9168):1915–9.
Smith GC, Trauer T, et al. Prospective quality-of-life monitoring of simultaneous pancreas and kidney transplant recipients using the 36-item short form health survey. Am J Kidney Dis. 2010;55(4):698–707.
Socci C, Falqui L, et al. Fresh human islet transplantation to replace pancreatic endocrine function in type 1 diabetic patients. Report of six cases. Acta Diabetol. 1991;28(2):151–7.
Sollinger HW, Odorico JS, et al. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg. 2009;250(4):618–30.
Speight J, Reaney MD, et al. Patient-reported outcomes following islet cell or pancreas transplantation (alone or after kidney) in type 1 diabetes: a systematic review. Diabet Med. 2010;27(7):812–22.
Stadler M, Anderwald C, et al. Chronic peripheral hyperinsulinemia in type 1 diabetic patients after successful combined pancreas-kidney transplantation does not affect ectopic lipid accumulation in skeletal muscle and liver. Diabetes. 2010;59(1):215–8.
Starzl TE, Iwatsuki S, et al. Pancreaticoduodenal transplantation in humans. Surg Gynecol Obstet. 1984;159(3):265–72.

Stratta RJ. Cardiovascular disease and neoplasms after pancreas transplantation. Lancet. 1998;352 (9121):65–6.
Stratta RJ. Surgical nuances in pancreas transplantation. Transplant Proc. 2005;37(2):1291–3.
Sutherland DE, Goetz FC, et al. Living-related donor segmental pancreatectomy for transplantation. Transplant Proc. 1980;12(4 Suppl 2):19–25.
Sutherland DE, Gruessner RW, et al. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg. 2001;233(4):463–501.
Tan J, Yang S, et al. Simultaneous islet and kidney transplantation in seven patients with type 1 diabetes and end-stage renal disease using a glucocorticoid-free immunosuppressive regimen with alemtuzumab induction. Diabetes. 2008;57(10):2666–71.
Tangri N, Grams ME, et al. Multinational assessment of accuracy of equations for predicting risk of kidney failure: a meta-analysis. JAMA. 2016;315(2):164–74.
Tattersall RB. Brittle diabetes revisited: the third Arnold Bloom memorial lecture. Diabet Med. 1997;14(2):99–110.
Tattersall R, Gregory R, et al. Course of brittle diabetes: 12 year follow up. BMJ. 1991;302 (6787):1240–3.
Tharavanij T, Betancourt A, et al. Improved long-term health-related quality of life after islet transplantation. Transplantation. 2008;86(9):1161–7.
Thompson DM, Meloche M, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. Transplantation. 2011;91(3):373–8.
Toso C, Baertschiger R, et al. Sequential kidney/islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol. Am J Transplant. 2006;6(5 Pt 1):1049–58.
Toso C, Shapiro AM, et al. Quality of life after islet transplant: impact of the number of islet infusions and metabolic outcome. Transplantation. 2007;84(5):664–6.
Turgeon NA, Avila JG, et al. Experience with a novel efalizumab-based immunosuppressive regimen to facilitate single donor islet cell transplantation. Am J Transplant. 2010;10 (9):2082–91.
Tyden G, Bolinder J, et al. Improved survival in patients with insulin-dependent diabetes mellitus and end-stage diabetic nephropathy 10 years after combined pancreas and kidney transplantation. Transplantation. 1999;67(5):645–8.
Tzakis AG, Ricordi C, et al. Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. Lancet. 1990;336(8712):402–5.
UK Hypoglycaemia Study Group. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. Diabetologia. 2007;50(6):1140–7.
van Dellen D, Worthington J, et al. Mortality in diabetes: pancreas transplantation is associated with significant survival benefit. Nephrol Dial Transplant. 2013;28(5):1315–22.
Vantyghem MC, Press M. Management strategies for brittle diabetes. Ann Endocrinol (Paris). 2006;67(4):287–96.
Vantyghem MC, Kerr-Conte J, et al. Primary graft function, metabolic control, and graft survival after islet transplantation. Diabetes Care. 2009;32(8):1473–8.
Vantyghem MC, Defrance F, et al. Treating diabetes with islet transplantation: lessons from the past decade in Lille. Diabetes Metab. 2014a;40(2):108–19.
Vantyghem MC, Quintin D, et al. Improvement of electrophysiological neuropathy after islet transplantation for type 1 diabetes: a 5-year prospective study. Diabetes Care. 2014b;37(6): e141–2.
Venstrom JM, McBride MA, et al. Survival after pancreas transplantation in patients with diabetes and preserved kidney function. JAMA. 2003;290(21):2817–23.
Venturini M, Fiorina P, et al. Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. Transplantation. 2006;81(9):1274–7.
Vigersky RA. The benefits, limitations, and cost-effectiveness of advanced technologies in the management of patients with diabetes mellitus. J Diabetes Sci Technol. 2015;9(2):320–30.

Villiger P, Ryan EA, et al. Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution. Am J Transplant. 2005;5(12):2992–8.
Vizzardelli C, Potter ED, et al. Automated method for isolation of adrenal medullary chromaffin cells from neonatal porcine glands. Cell Transplant. 2001;10(8):689–96.
Voulgari C, Tentolouris N. Brittle diabetes: a contemporary review of the myth and its realization. In: Rigobelo EC, editor. Diabetes – damages and treatments. In: Everlon Cid Rigobelo. Tech; 2011, Janeza Trdine 9, 51000 Rijeka, Croatia. Chapters.
Voulgari C, Pagoni S, et al. 'Brittleness' in diabetes: easier spoken than broken. Diabetes Technol Ther. 2012;14(9):835–48.
Warnock GL, Kneteman NM, et al. Normoglycaemia after transplantation of freshly isolated and cryopreserved pancreatic islets in type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1991;34(1):55–8.
Warnock GL, Thompson DM, et al. A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes. Transplantation. 2008;86(12):1762–6.
Watanabe M, Yamashita K, et al. ASKP1240, a fully human anti-CD40 monoclonal antibody, prolongs pancreatic islet allograft survival in nonhuman primates. Am J Transplant. 2013;13(8):1976–88.
Weinstock RS, Xing D, et al. Severe hypoglycemia and diabetic ketoacidosis in adults with type 1 diabetes: results from the T1D Exchange clinic registry. J Clin Endocrinol Metab. 2013;98(8):3411–9.
Weiss AS, Smits G, et al. Twelve-month pancreas graft function significantly influences survival following simultaneous pancreas-kidney transplantation. Clin J Am Soc Nephrol. 2009;4 (5):988–95.
White SA, Shaw JA, et al. Pancreas transplantation. Lancet. 2009;373(9677):1808–17.
Workgroup on Hypoglycemia, American Diabetes Association. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. Diabetes Care. 2005;28(5):1245–9.
Ziaja J, Bozek-Pajak D, et al. Impact of pancreas transplantation on the quality of life of diabetic renal transplant recipients. Transplant Proc. 2009;41(8):3156–8.

Index

E. Bonora, R. A. DeFronzo (eds.), *Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment*, Endocrinology,
https://doi.org/10.1007/978-3-319-45015-5