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-

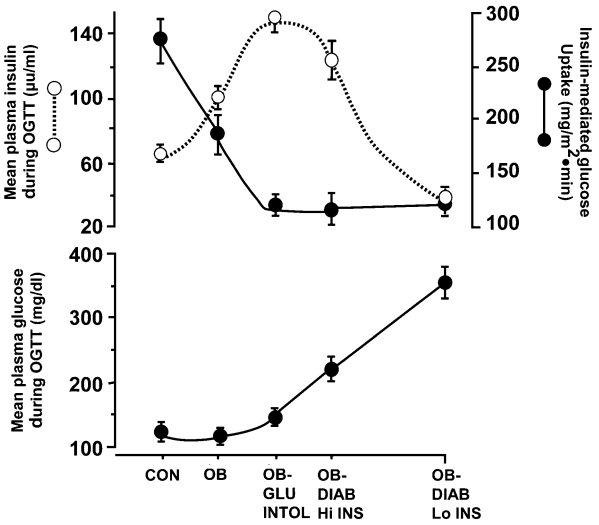

Fig. 1 Summary of the plasma insulin (top) (dashed line, open circles) and plasma glucose (bottom) responses during a 100-g OGTT and tissue sensitivity to insulin in obese (OB) normal glucose tolerant, obese glucose intolerant (OB-GLU INTOL), obese hyperinsulinemic diabetic (OB-DIAB Hi INS), and obese 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

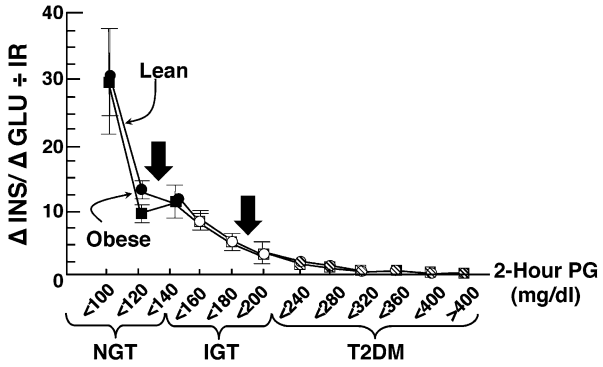

Fig. 2 Insulin secretion/insulin resistance (disposition) index (defined as increment in insulin/increment in glucose ÷ insulin resistance [ΔINS/ΔGLU ÷ IR]) in individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) as a function of the 2-h 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)

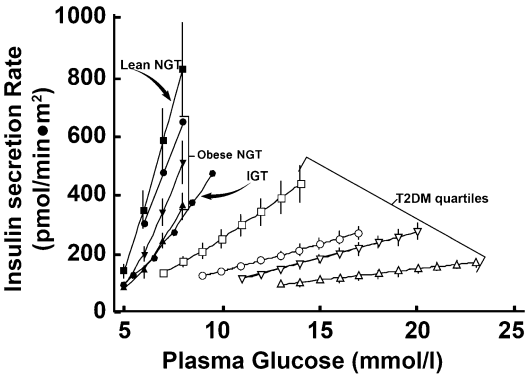

Fig. 3 Plot of insulin secretion rate against the concomitant plasma glucose concentration in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (T2D) by quartile of fasting hyperglycemia. The mean slope of the fitting functions measures β-cell glucose 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

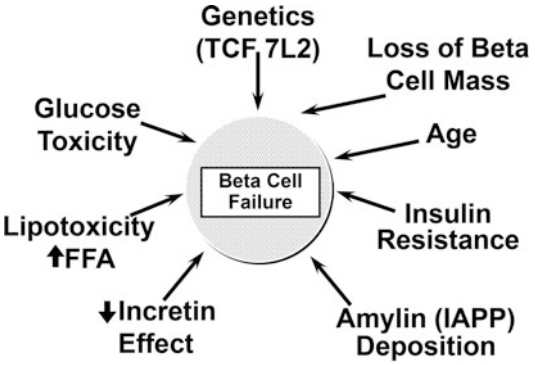

Fig. 4 Pathogenetic factors implicated in the progressive impairment in insulin secretion in T2DM

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

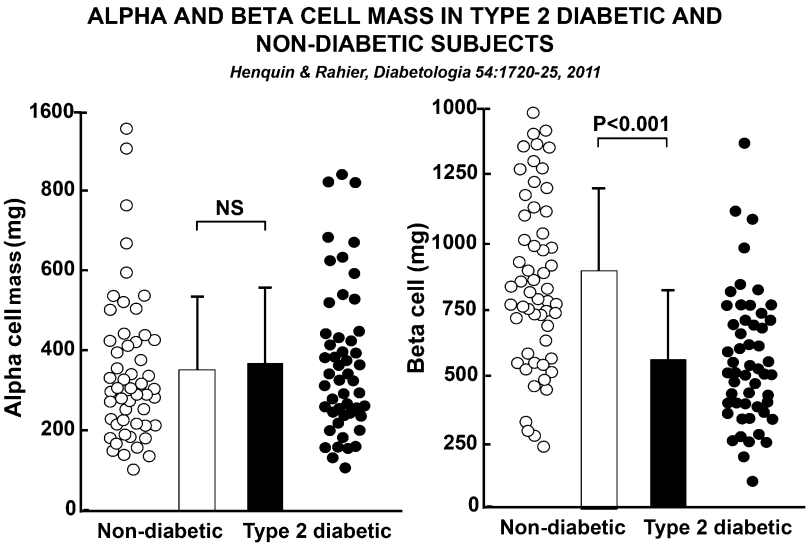

Fig. 5 Beta cell and alpha cell mass in 57 type 2 diabetic and 52 nondiabetic individuals. On mean, beta cell mass was decreased by ~35% in diabetic subjects. There was no difference in alpha cell mass. (From Henquin et al., *Diabetologia* 54:1720–1725, 2011)

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

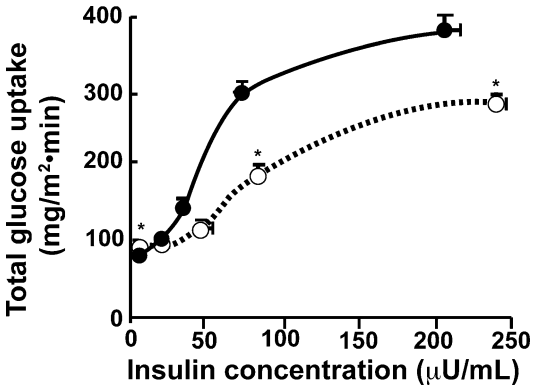

Fig. 6 Dose-response curve relating the plasma insulin concentration to the rate of insulin-mediated whole-body glucose uptake in control (solid circles, solid line) and type 2 diabetic (open circles, dashed line) subjects. *$p < 0.01$ vs. control subjects. (Source: Groop L, et al. *Journal of Clinical 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

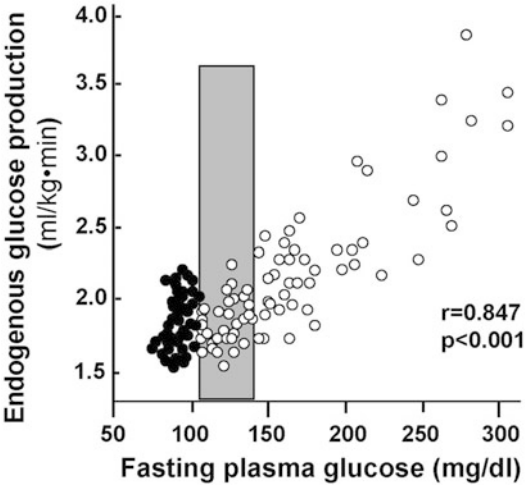

Fig. 7 Summary of HGP in 77 normal-weight type 2 diabetic subjects (open circles) with fasting plasma glucose concentrations ranging from 105 to >300 mg/dl; 72 control subjects matched for age and weight are shown by solid circles. In the 33 diabetic subjects with fasting plasma glucose levels <140 mg/dl (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

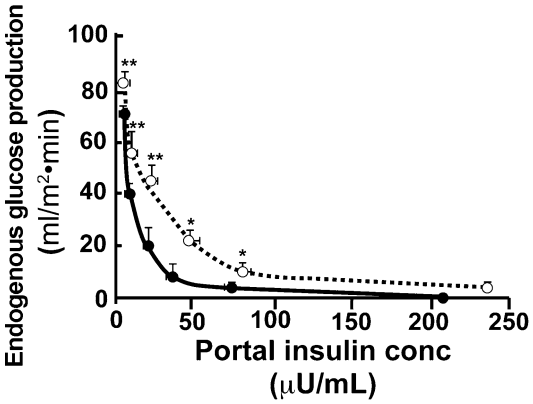

Fig. 8 Dose-response curve relating the plasma insulin concentration to the suppression of HGP in control (solid circles, solid line) and type 2 diabetic (open circles, dashed line) subjects with moderately severe fasting hyperglycemia. *$p < 0.05$, **$p < 0.01$ vs. control subjects. (Source: Groop L, 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

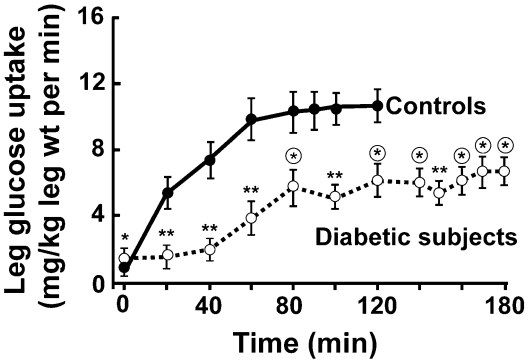

Fig. 9 Time course of change in leg glucose uptake in type 2 diabetic (open circles, dashed line) and control (solid circles, solid line) subjects. In the postabsorptive state, glucose uptake in the diabetic group was significantly greater than that in control subjects. However, the ability of insulin (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

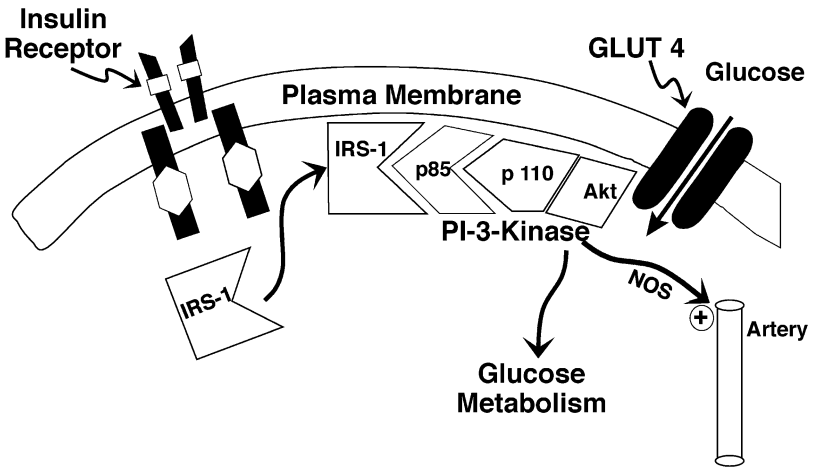

Fig. 10 Insulin signaling pathway in healthy subjects

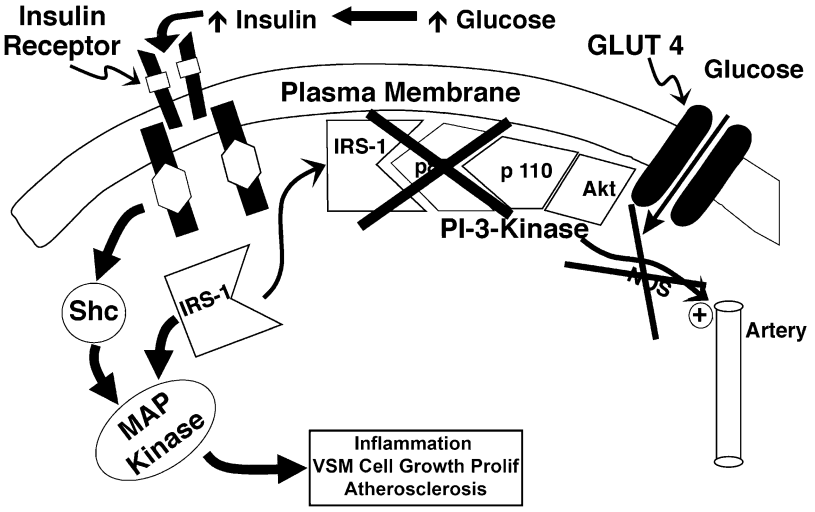

Fig. 11 Consequences of impaired insulin signaling in individuals with type 2 diabetes mellitus. See text for more detailed description

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.

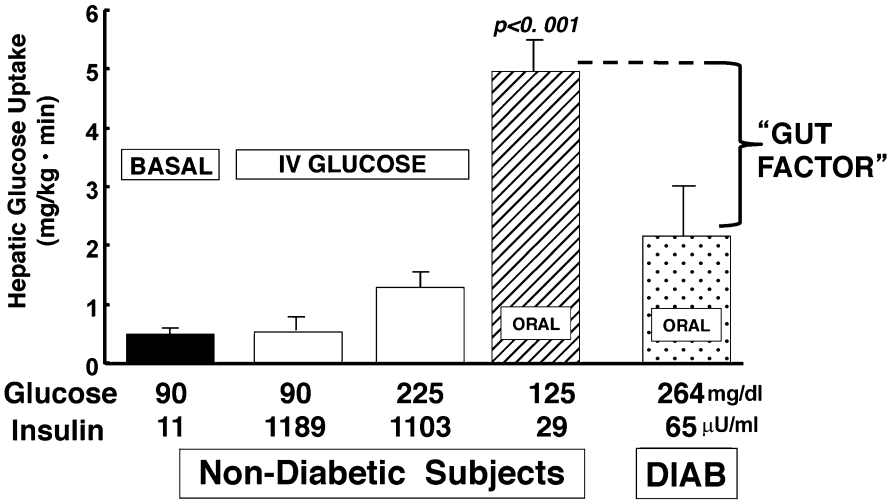

Fig. 12 Hepatic glucose uptake in nondiabetic and diabetic (DIAB) subjects as a function of plasma glucose and insulin concentration and route of glucose administration. Constructed from the results of *PNAS* 75:5173–77, 1978; *Diabetes* 32:35–45, 1983; *Metabolism* 37:79–85, 1988

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).

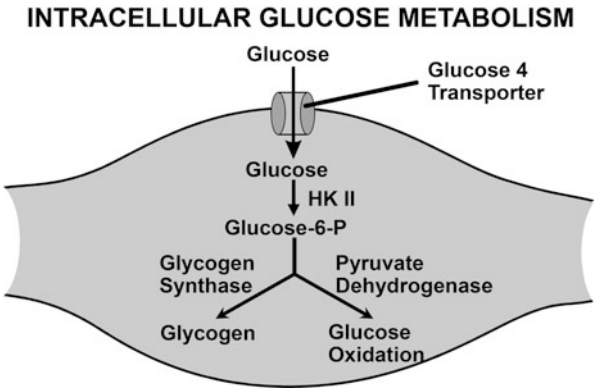

Fig. 13 Intramyocellular glucose metabolism. Type 2 diabetic individuals have defects in insulin-stimulated glucose transport, glucose phosphorylation, glucose oxidation, and glycogen synthesis. See text for detailed description

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

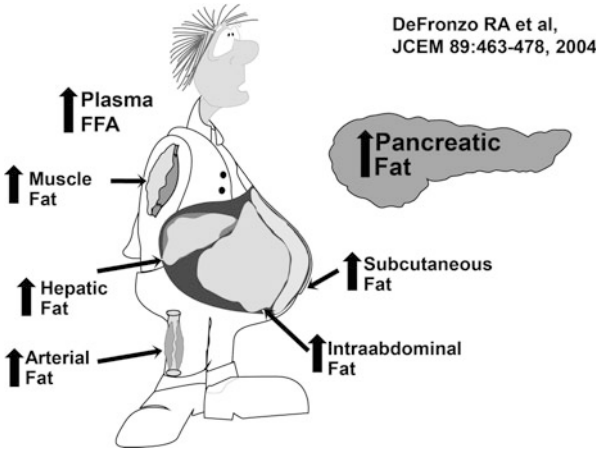

Fig. 14 Altered fat topography (visceral fat excess) is strongly associated with insulin resistance, while ectopic fat deposition in muscle and liver causes insulin resistance in these tissues. Ectopic fat deposition in the beta cell and arteries cause beta cell dysfunction and promotes atherogenesis

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.

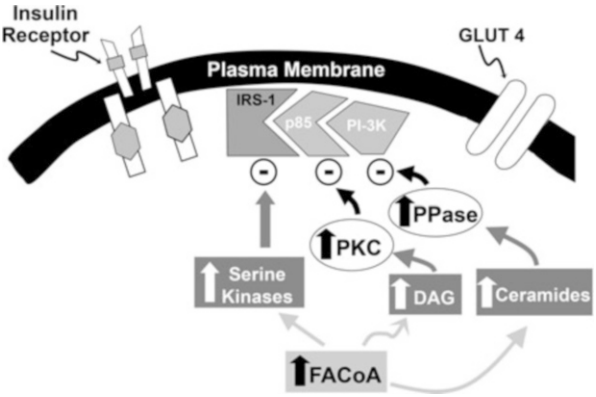

Fig. 15 Elevated intracellular levels of fatty acyl CoAs (FACoA) and diacylglycerol (DAG) inhibit insulin signaling by activating serine kinases and PKC isoforms, causing serine phosphorylation of the insulin receptor and insulin receptor substrate-1. Increased intracellular ceramide levels inhibit insulin 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

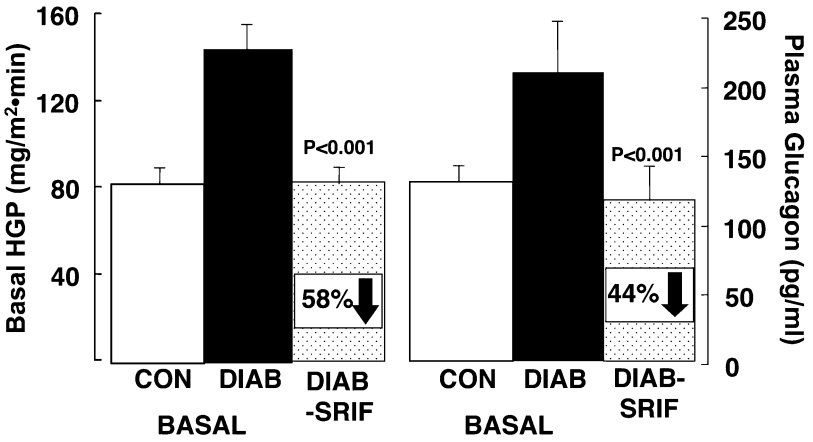

Fig. 16 Effect of somatostatin (SRIF) infusion with basal insulin replacement on basal (fasting) hepatic glucose production (HGP) (left) and plasma glucagon concentration (right) in normal glucose-tolerant control (CON) and type 2 diabetic (DIAB) subjects. Normalization of the plasma glucagon concentration 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).

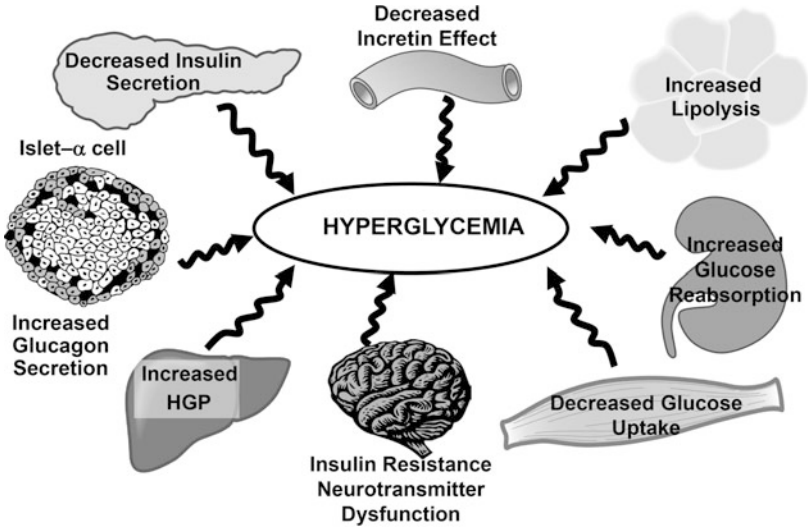

Fig. 17 The Ominous Octet describing the major pathophysiologic defects which involve multiple organs in type 2 diabetes. See text for a more detailed explanation. (Source: DeFronzo RA. *Diabetes* 2009;**58**:773–795)

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.