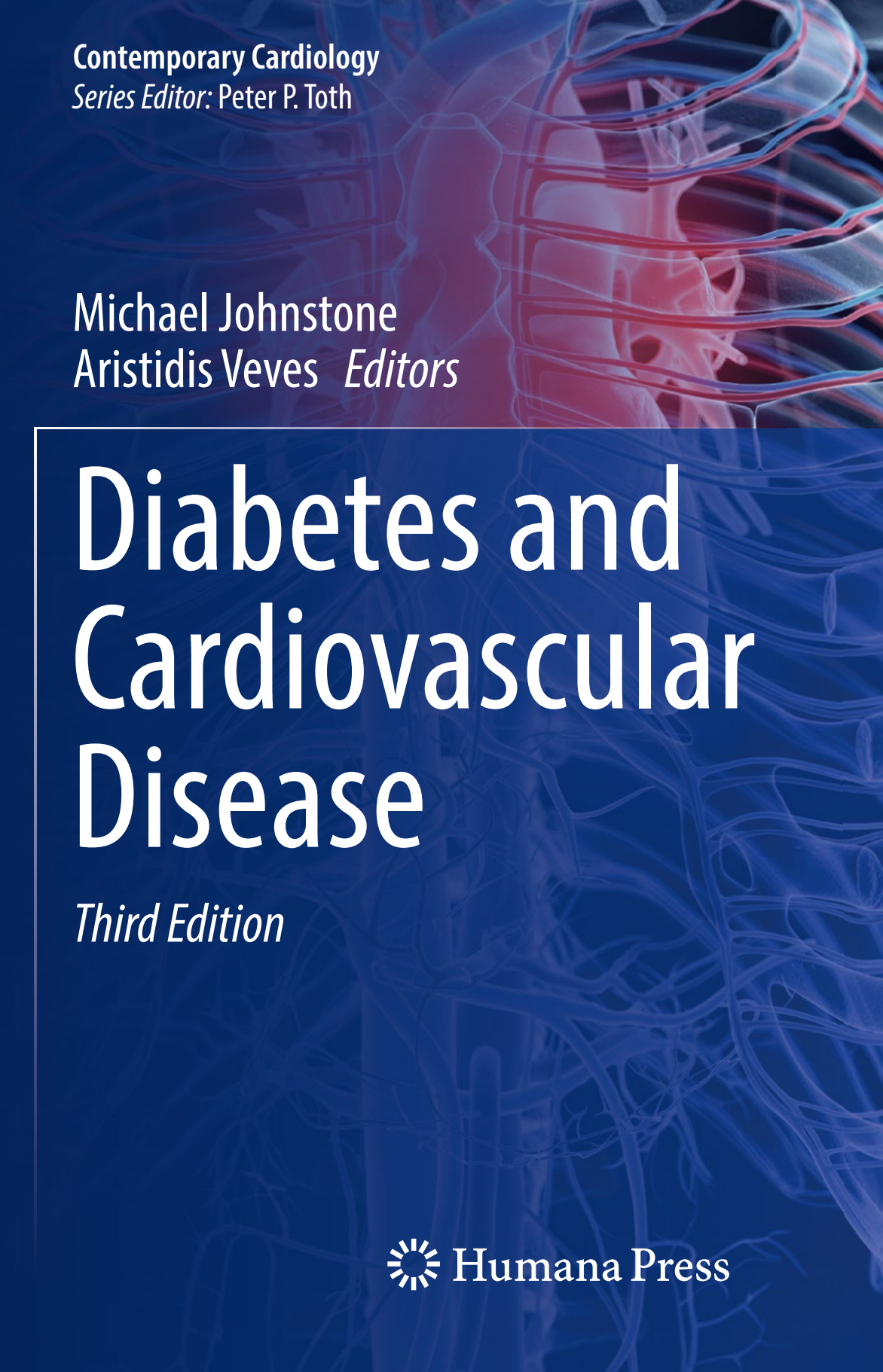
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Diabetes and Cardiovascular Disease
Third Edition
Humana Press

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Editors

Diabetes and Cardiovascular Disease

Third Edition

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***Dedication**: To my wife, Ellen, and my children, Lauren, Stephanie, Jessica, and Jason, for their inspiration, patience, love, and support. They are the light that guides me.*

Michael Johnstone, MD CM

***Dedication**: To my wife Maria and my son George for their constant and unconditional support, understanding, and love, which enable me to undertake such endeavors.*

Aristidis Veves, MD, DSc

Preface

Hard to believe it has been 17 years since the last edition of the text *Diabetes and Cardiovascular Disease*. Yet the need for such a text has only increased. We have limited our text to Type II Diabetes Mellitus since the number of patients with type II diabetes is particularly on the rise. Diabetes mellitus not only remains an important cause of morbidity and mortality in this United States and the Western nations, its prevalence is also increasing with rising obesity rates. In those ensuing 20 years new basic science breakthroughs and treatment options are present. We hope this book reflects these new discoveries.

The changes include a new publisher, Springer. We have included a general introduction to the topic of diabetes mellitus including its definition as well as the epidemiology of this growing disease.

Our basic research section has all updated chapters, but we have added new ones as well. Specifically, Drs. Moreiri and Doria have written a new chapter on the Genetics of Type II diabetes mellitus.

Other chapters transcend categorization. The chapter on Diabetes, Inflammation, and Cardiovascular Disease discusses both the basic science and clinical treatments as it relates to inflammation's role in diabetes mellitus and cardiovascular disease. Dr. Mantzoros' chapter on Adiponectin has a detailed review regarding GLP-1 which is now a new treatment modality. Dr. Plutsky's chapter on PPAR is another that contains both basic science and clinical studies as does the new chapter written by Dr. Balakrishna on Renin-Angiotensin and Aldosterone System.

In the section Associated Conditions, we have not only updated the chapters, we have a completely new chapter on the Metabolic Syndrome written by Drs. Via and Mechanick along with a new chapter on the Effects of Sleep Apnea on Cardiovascular Disease which may play a significant role in the etiology of not only hypertension but Type II Diabetes Mellitus itself. Both the chapters on Hypertension by Dr. McFarlane and colleagues and Dyslipidemia by Dr. Grunfeld et al. have been completely revamped and updated.

We have expanded the Microvascular System with new chapters on Diabetes Mellitus and Neurological Disease by Dr. Lioutas and colleagues while Drs. Holder and colleagues wrote a chapter on the Autonomic System and Diabetes Mellitus. We also added a chapter on erectile dysfunction, an important manifestation of diabetic vascular disease by Dr. Ko and colleagues.

We have also added a section on the treatment of obesity, in terms of lifestyle, medications, and surgical options. Dr. Sweeny's chapter and that of Dr. Hamdy's do overlap in terms of the treatment of lifestyle but Dr. Hamdy discusses more on medical treatments to lose weight while Dr. Sweeny's chapter limits itself to lifestyle modalities in the treatment of obesity. Dr. Pecqueux reviews the surgical options for the treatment of obesity.

The biggest difference in this text from both its predecessor and other similar texts is the section on treatment discussing the older medical treatments including Dr. Scheen's chapter on metformin and the sulfonylureas. Dr. Reaven discusses the use of insulin in the treatment of diabetic patients reviewing the clinical trials. Dr. Fitchett has done an extensive and scholarly review of the basic science and clinical use of GLP-1's and SGLT-2 inhibitors and the treatment of diabetes mellitus and cardiovascular disease.

There are several new chapters on the specific approach to treatment in 2022 of patients with diabetes mellitus and cardiovascular disease. One chapter is on the treatment of acute cardiovascular disease in diabetic patients as it pertains to glucose control, while another on the treatment of patients with stable cardiovascular disease. The chapter on Diabetes and Heart Failure has been completely rewritten to reflect the exciting changes in this area.

We have also added 2 special chapters on the treatment of DM in certain populations. The first is by Dr. Enrique Caballero on the treatment of diabetes mellitus in certain ethnic group. Also given that patients with diabetes mellitus were especially at risk to develop COVID pandemic, we have written a chapter on diabetes mellitus type II and COVID, reviewing the epidemiology of this ever-changing pandemic. The last chapter in the section is particularly salient given the burgeoning treatment options, written by Dr. Inzucchi and colleagues who do a superb job summarizing the proper approach to treating patients with type 2 diabetes mellitus.

Given the broad range in topics, we believe this book can be an important text for both the basic scientist and the clinician, specifically the endocrinologist, the cardiologist, or the internist. And given the increasing prevalence of diabetes mellitus, this text is an important addition to any medical library.

We would like to sincerely thank all the contributors of this edition as it is their hard effort that has resulted in this successful textbook. We would like also to thank Humana Press-Springer for their trust in our abilities and all their help in accomplishing this project. In particular, we want to thank Swathiga Karthikeyan, the Project Coordinator, as well as Michelle Tam, Associate Editor, Clinical Medicine

at Springer. We also want to thank my (MJ) administrative assistant, Nane Solakhyan, for her help in preparing the Table of Contents and my (MJ) daughter, Jessica Johnstone, for editing several of the chapters. Lastly, I want to thank my wife (MJ), Ellen, who is always my biggest supporter and toughest critic, and whose enduring love is never in question.

Brighton, MA, USA — Michael Johnstone
Boston, MA, USA — Aristidis Veves

Contents

Part I
Pathophysiology

Chapter 1
Introduction: Epidemiology, Definitions, and Pathophysiology

Gregory P. Westcott and Richard S. Beaser

Introduction

Today, it is almost impossible to think about diabetes without the potential comorbidity of cardiovascular disease. Their pathophysiology and clinical courses are so often intertwined that many think of them as varying manifestations of the same disease process. However, recognition of the interrelationship between these two conditions is a relatively recent event that underscores a significant progression in our understanding of their pathophysiology and treatment implications. Much of this evolution in our perceptions of these conditions has occurred in the lifetime of the senior author of this chapter (RSB). Thus, by way of introduction to this chapter and, as well, to this book will be a brief historic perspective to underscore how far we have come in the relationship between diabetes and cardiovascular disease.

Diabetes was functionally a glucocentric condition until about 50 years ago. The story of the discovery of insulin was all about glucose control [1] and the hope for survival that it afforded. Years later, physiologic control was demonstrated to impact the development of microvascular complications for people with type 1 diabetes [2]. However, for those with type 2 diabetes, while glycemic control did also impact the risk of developing microvascular complications, it had become clear that its impact on macrovascular disease was shared with other risk factors such as dyslipidemia and hypertension [3]. Therefore, the relationship of diabetes to macrovascular endpoints was not quite so clear.

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I (R.S.B.) will indulge the reader briefly with some personal perspectives on the evolution of thought regarding this relationship. My father was Dr. Samuel Beaser, a leading diabetes specialist at Boston's Beth Israel Hospital starting in the late 1940s. In the 1950s and 1960s, he worked closely with Dr. Leo Krall from the Joslin Clinic on a number of the early trials of the sulfonylureas. The investigative process was crude by today's standards of clinical trials. I have vivid childhood memories of him talking about these trials and storing boxes of the sample tablets in our basement. This is well before the era of double-locked storage and meticulous inventory. When these drugs were approved I recall him telling of many people with diabetes suddenly appearing in doctors' offices for treatment, having sought to avoid "the needle" and now enthused by an option in tablet form.

The enthusiastic reception for these medications was dampened with the publication of the University Group Diabetes Program (UGDP) [4, 5], which seemed to show an excess of cardiovascular deaths in people treated with tolbutamide vs. placebo. Much controversy followed [6], swirling with multiple points and counterpoints in the literature for many years thereafter. I can easily resist rehashing that decades-long controversy focused on randomization issues and study methods, as well as the issue of the cardiovascular safety of sulfonylureas! However, it is imperative to underscore the importance of these events in evolution of our thinking about the relationship between diabetes and cardiovascular disease [7]. Many physicians, including my father, may have been skeptical about the results of the UGPD, but did, early on, acknowledge the relationship between diabetes and cardiovascular disease in practice as they increasingly shared care of these people with cardiologists. I recall many dinner table conversations (pre-HIPAA, of course) about how his patients were discovered to have coronary disease "just in time" before they had a serious cardiovascular event.

It still took a while for that recognition to be widespread. At that time, the mid-to-late 1970s, diabetologists and cardiologists were different breeds. As a fourth-year medical student heading for a career in diabetes, I spent a month on a cardiology elective at an esteemed academic hospital. Still, my focus, even then, was on the chronic condition, diabetes. I enjoyed adjusting insulin, waiting a few weeks, seeing how it worked based on urine test results and a few blood glucose tests, and then adjusting the doses a bit more. If these people complained of chest pain, I would send them to the nearest cardiologist or an emergency room as fast as I could. However, for that elective month I was surrounded by those very cardiologists. They admitted people, immediately evaluated them for what could be acute coronary disease, rushed them into the cath lab and then to the OR for their CABG, all within a couple of days, not weeks or months. No two mindsets could be further apart! Their problem lists were headed by coronary disease and included items like peripheral vascular disease, cerebrovascular disease, and renal failure. Diabetes, usually at the bottom of that list, was invariably described as being "stable," meaning not acutely or dangerously hyper- or hypoglycemic on the day of admission.

From that time to now we have seen the convergence of these once divergent specialties. Studies such as the UKPDS [3] further elucidated the presence of multiple cardiovascular risk components that increased the risk of developing

cardiovascular disease. This constellation of risk factors and potential pathophysiologic mechanisms leading to cardiovascular disease was described by Dr. Gerald Reaven [8] as what eventually came to be called "the Cardiometabolic Syndrome" [9]. Cardiovascular outcomes trials, in various evolving forms, have now become a requirement for approval of all antidiabetes medications [10]. We have come to a point today when the diabetologist and cardiologist are brethren, working together in concert to address the metabolic and cardiovascular healthcare challenges that are still, all too frequently, the cause of death for our patients.

We are now following a common pathway for diabetes and cardiovascular disease, with research and clinical coordination seeking to optimize outcomes for our patients. This chapter gives an overview of the conjoining of these once-disparate specialties, and the rest of this book provides the further detail. We got to this point via two distinct pathways, both filled with controversy and uncertainty. Yet, these pathways have now converged, and today we travel together with more unified visions of the evolution of care for our patients.

The Diabetes Epidemic

Diabetes is a pressing health concern in the United States and worldwide. According to the CDC, 10.5% of the U.S. population (or 13% of adults) had been diagnosed with diabetes as of 2016, and an additional 4.5% were estimated to have undiagnosed diabetes [11]. These figures represent a steady increase in the prevalence compared to 2012 (8.9% diagnosed and 4.4% undiagnosed), 2002 (6.6% and 4.2%), and 1994 (4.9% and 3.4%) (see Fig. 1.1a for trend since 2000). Globally, the trend is similarly daunting. In 2004, the worldwide prevalence of diabetes in the year 2000 was estimated to be 2.8%, or 171 million people, and was projected to rise to 4.4% and 366 million people by 2030, assuming the same prevalence of obesity [12]. The authors rightly commented that this would likely be an

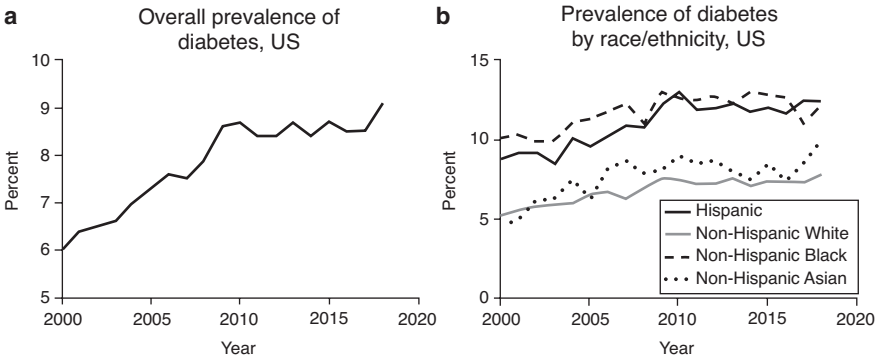

Fig. 1.1 Prevalence of diabetes in US adults, 2000–2018. And the source of the data should be attributed as: Adapted from Centers for Disease Control and Prevention. US Diabetes Surveillance System. Available at https://www.cdc.gov/diabetes/data

underestimate—indeed, according to the International Diabetes Federation, the worldwide prevalence of diabetes had already reached 9.3% in 2019, or 463 million adults, a figure projected to balloon to 10.2% and 578 million adults by 2030, an increase of 25% in just 11 years [13].

Diabetes has significant health sequelae, including cardiovascular, renal, ophthalmologic, and neuromuscular complications, and represents the seventh leading cause of death in the U.S [14]. The medical care required to treat diabetes and its complications is associated with substantial cost in terms of both medical expenditures as well as lost productivity. An analysis directed by the American Diabetes Association estimated the total cost of diabetes in 2017 was $327 billion, including $237 billion in direct medical costs and $90 billion in reduced productivity due to absenteeism, reduced productivity while at work, inability to work as a result of disability, and premature death [15].

The burden of diabetes is not shared equally among all demographic categories. According to the 2020 National Diabetes Statistics Report compiled by the CDC [16], there is a male-predominance (14% vs. 12% of females). In terms of race and ethnicity, the prevalence of diagnosed diabetes is highest among American Indians (14.7%), Hispanics (12.5%), and non-Hispanic blacks (11.7%), followed by Asians (9.2%) and non-Hispanic whites (7.5%) (Fig. 1.1b). However, these broad categories do not capture significant heterogeneity within racial categories; for example, only 5.6% of U.S. adults who report Chinese ethnicity have diabetes, while 12.6% of Asian Indian Americans are diabetic. Similarly, 6.5% of Cuban Americans have diabetes, while 14.4% of Mexican Americans are diabetic. Though confounding factors are likely, a higher level of educational attainment is associated with a lower prevalence of diabetes—13.3% of adults with less than a high school education have diabetes, while 7.5% of those with more than a high school education do.

As a chronic disease, the prevalence of diabetes grows with increasing age; total diabetes prevalence rises from 4.2% in adults 18–44 years old to 26.8% in those 65 years or older. But a particularly concerning trend is the rise of diabetes among youths. The incidence of type 2 diabetes in those aged 19 years and younger increased by 7.1% annually from 2002 to 2012, from a rate of 9 per 100,000 to 12.5 per 100,000, despite no overall increase in the prevalence of obesity in this age group over the study period [17].

A Heterogeneous Disease with Diverse Etiologies

As diabetes has increased in prevalence over the decades, so too has our understanding of the underlying pathophysiology. Common to all types of diabetes mellitus is elevated blood glucose as a result of inadequate insulin-mediated glucose disposal, though this may result from distinct mechanisms. Originally categorized into juvenile- or adult-onset based on the typical age of diagnosis, we now favor classifications that reflect the underlying etiology, as diabetes of any cause can present as a child or adult. Likewise, the terms “insulin-dependent” or “insulin-independent” are

now less-commonly used for classification, as patients with multiple types of diabetes use insulin as part of their therapy.

Autoimmune causes of diabetes result from autoimmunity directed against the insulin-producing pancreatic beta cell. This predominantly consists of classic type 1 diabetes, which presents rapidly during childhood, adolescence, or early adulthood, but also includes a more gradually progressive form that presents in mid-to-late adulthood, commonly called latent autoimmune diabetes in adults (LADA) or type 1.5 diabetes. There has been the suggestion that a more latent type of autoimmune diabetes may exist in the young as well [18], though this is not well-established. Patients with type 1 diabetes universally require exogenous insulin therapy to survive, as destruction of the beta cell mass results in the absolute lack of insulin production. Patients with LADA also eventually require insulin, though this may take several years from the time of diagnosis [19].

Monogenic forms of diabetes have also been identified, in which a single gene mutation results in decreased pancreatic insulin production due to defective glucose sensing or impaired insulin secretion response. Multiple genetic mutations have been identified in neonatal diabetes mellitus (NDM) and mature-onset diabetes of the young (MODY), the most common of which include HNF1A, in which hyperglycemia is responsive to sulfonylureas, and GCK, which produces mild fasting hyperglycemia and can be managed with lifestyle [20]. Monogenic diabetes often present in young adulthood and is associated with a strong family history of diabetes but no evidence of insulin resistance, and negative beta cell antibodies. These patients are often misdiagnosed as having type 1 or type 2 diabetes, but when you see this type of person, genetic testing for known mutations is available in situations where a monogenic cause is suspected. There is variability in clinical manifestations, even between individuals with the same mutation. While patients with GCK MODY rarely need treatment or develop diabetic complications, in other MODY types, complications arise at a similar frequency as in T2DM and depend on the degree of hyperglycemia [21].

An additional class of diabetes occurs as a result of loss of pancreatic tissue, either from surgical pancreatectomy or damage to islets, most commonly from chronic pancreatitis but also as a complication of cystic fibrosis or hemochromatosis [22]. Termed pancreatogenic or type 3c diabetes, this disease differs from type 1 diabetes due to loss of the entire islet rather than beta cells alone, resulting in the lack of glucagon counter-regulation, which may lead to more glycemic instability and hypoglycemia [23].

Although type 1, monogenic, and pancreatogenic diabetes are important causes of diabetes, 95% of adults with diabetes in the US are classified as having type 2 diabetes (T2DM) [16], which is a complex, polygenic disease that arises principally from impaired insulin sensitivity and action, rather than as a primary disorder of the beta cell, though beta cell dysfunction is also present. It was known within several years of insulin's discovery that patients could be classified as insulin-sensitive or insulin-insensitive [24], and later that insulin insensitivity, more commonly termed insulin resistance, was phenomenon that was later linked to visceral fat accumulation [25]. It was also noted that insulin resistance had to be coupled with inadequate

insulin production in order to produce overt hyperglycemia and type 2 diabetes [26]. In the early 1990s, an atypical form of type 2 diabetes originally termed Flatbush diabetes, and now referred to as ketosis-prone diabetes (KPD), was identified in African-American patients who developed diabetic ketoacidosis despite negative islet cell autoantibodies and lack of insulin dependence [27]. Patients from a wide range of ethnic backgrounds have subsequently been diagnosed with KPD, and it is now recognized that KPD itself has a number of subtypes distinguished based on beta cell function and autoimmunity [28]. It is important to recognize this entity, as sodium-glucose cotransporter-2 (SGLT2) inhibitors, which are increasingly used in patients with cardiovascular disease, are known to increase plasma ketones [29].

Over time, there has been an increasing understanding that T2DM is not simply a function of lifestyle or body habitus, and a deeper appreciation for the role of genetic determinants of the disease. The genetics of T2DM is quite complex and has attracted a considerable amount of investigation. An early attempt to quantify genetic contribution to the disease based on the Framingham Offspring Study demonstrated that adults with one diabetic parent had 3.5 times the risk of having T2DM, and with two diabetic parents a 6.1 times higher risk, as compared with people with two non-diabetic parents [30]. Twin studies have generally demonstrated a significantly increased concordance of type 2 diabetes in monozygotic twins [31], though not all studies have shown a difference in concordance in monozygotic as compared to dizygotic twins [32, 33]. More recently, monozygotic twins discordant for T2DM have been demonstrated to have epigenetic differences in their adipose tissue, which may be a result of environmental factors and impact risk for disease [34].

Many genetic associations have been reported for type 2 diabetes starting in the early 90s with candidate gene approaches, as was the case, for example, with polymorphisms in the insulin receptor substrate-1 gene [35], though in the past 20 years, the increasing availability and depth of human sequencing data have improved our ability to robustly probe and confirm associations of genetic alterations with risk for type 2 diabetes. Since genetic risk of T2DM is conferred by the combination of a large number of genetic loci with individually small effect sizes [36], meta-analyses of genome-wide association studies (GWAS) incorporate tens of thousands of individuals' data [37] to identify genes of interest. More recent GWAS meta-analyses have additionally integrated epigenetic and gene expression data to identify additional loci which may be involved [38].

As might be expected given the genetic complexity, there is clinical heterogeneity within T2DM, and there has been increasing interest in exploring and defining subtypes of the disease. In 2015, an unsupervised subject similarity network approach was applied to patients with T2DM across multiple clinical dimensions, ultimately identifying three clusters with unique characteristics [39]. Some clinical insights could be drawn from the data, for example, while subtype 1 were the youngest and had the best renal function, they were also the most likely to have ICD codes associated with blindness and vision defects. A number of genetic polymorphisms were also found to be associated with certain clusters; for instance, *PLXDC2* and *HS6ST3*, genes associated with diabetic retinopathy [40], were enriched in subtype 1.

A Swedish analysis published in 2018 also used clinical factors including BMI, age of DM onset, HOMA2-B/IR, and C-peptide to define five separate clusters of diabetes (including T1DM); autoimmune diabetes composed one cluster, and the other four were defined as severe insulin-deficient, severe insulin-resistant, mild obesity-related, and mild age-related [41]. In addition to noting possible implications for different pathophysiological mechanisms between clusters, the authors also found differences in severity and complications between the groups, with the severe insulin-resistant cluster having a particularly high risk of chronic kidney disease, while the severe insulin-deficient had the highest risk of retinopathy. There was no significant difference in age- and sex-adjusted risk for coronary events and stroke between clusters.

While these two aforementioned studies categorized patients based on clinical characteristics and then analyzed genetic features of each cluster, a primary clustering based on germline genetic variants has recently been performed to attempt to identify underlying causal mechanisms [42]. Using a more flexible "soft clustering" approach, five clusters were identified based on genetic variants and associated traits. Defining traits for clusters 1 ("Beta Cell") and 2 ("Proinsulin") related to beta cell insulin production and processing, albeit by two different pathways. The traits most strongly associated with cluster 3 ("Obesity") were related to BMI, while cluster 4 ("Lipodystrophy") traits related to "lipodystrophy-like" insulin resistance, and cluster 5 ("Liver/Lipid") included traits associated with lipid metabolism and non-alcoholic fatty liver disease. Of note, the strongest weighted loci from the Beta Cell and Lipodystrophy clusters were most significantly associated with increased risk of coronary artery disease and ischemic stroke, including small vessel and large vessel subtypes, but not cardioembolic strokes. The Lipodystrophy cluster was also associated with increases in both systolic and diastolic blood pressure. Interestingly, the Lipid cluster did not significantly associate with CAD or stroke risk, underlying the complexity of determining unifying mechanisms and risk factors for these diseases.

It is important to emphasize that insulin resistance is not binary, but rather manifests with a spectrum of severity, and may also be evident in populations who do not meet criteria for a diagnosis of diabetes. For example, 34.5% of adults in the U.S. are estimated to have prediabetes [43]. A diagnosis of prediabetes is made based on an elevated HbA1c and/or elevated fasting glucose that does not meet the threshold for type 2 diabetes, and, unless the patient has MODY or early autoimmune diabetes, this is evidence of underlying insulin resistance. Similarly, during pregnancy, a state in which insulin resistance is physiologic and mediated by placental hormones such as human placental growth hormone as well as cortisol, estrogen, and progesterone [44], some patients without pre-existing diabetes may be unable to mount an adequate insulin secretory response to prevent hyperglycemia, and gestational diabetes results. Gestational diabetes often resolves after delivery, though these patients are at high risk of developing T2DM within 5 years after their pregnancy, likely due to the underlying genetic predisposition that was unmasked by the insulin resistance inherent in pregnancy [45].

The Twin Threat of Diabetes and Cardiovascular Disease

As described at the outset of this chapter, cardiovascular disease and diabetes are now understood to be closely related. Cardiovascular disease is the leading cause of death in people with diabetes [46]. The understanding of this relationship has evolved over several decades as diabetes and insulin resistance were linked to hypertension and atherosclerosis under a variety of different names and definitions [47], including, for example, the hypertension–hyperglycemia–hyperuricemia syndrome, the deadly quartet, the syndrome of affluence, the insulin resistance syndrome, and, as it is predominantly known today, the metabolic syndrome. The modern definition of the metabolic syndrome is attributed to Gerald Reaven, who in 1988 grouped insulin resistance/hyperinsulinemia, hyperlipidemia, and hypertension into Syndrome X [48]. The features of the metabolic syndrome are now generally accepted to include abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance and glucose intolerance, and a prothrombotic and proinflammatory state. Clinically, different definitions are used; the National Cholesterol Education Program introduced the most commonly used criteria which use waist circumference (which is more highly correlated with metabolic risk compared to BMI), triglyceride and HDL concentrations, blood pressure, and fasting glucose. The World Health Organization [49] and American Association of Clinical Endocrinologists [50] offered similar definitions, but included oral glucose tolerance testing in their criteria and are therefore more sensitive in detecting glucose intolerance [51].

The metabolic syndrome is clearly associated with an increased risk of overall and cardiovascular mortality [52, 53], though studies have disagreed on whether having multiple components of the syndrome confer more risk than the individual components [54] or not [55, 56]. There has been controversy as to whether the concept of a metabolic syndrome is useful, particularly as a unifying pathophysiology has remained elusive, and treatment consists of treating the individual components [57], but is generally thought to be helpful in encouraging clinicians and researchers to consider the connection between these diseases. Research into this complex relationship continues, including investigations into direct effects of hyperinsulinemia and hyperglycemia on inflammation, endothelial cells, liver metabolism, smooth and skeletal muscle, thrombosis and fibrinolysis, and more, as discussed in detail throughout this book.

Diabetes Treatments and Emerging Applications

Diabetes medications have traditionally been evaluated only with attention to their glucose-lowering capability. Given the baseline increased risk for cardiovascular disease in patients with diabetes, studies have explored the effect of older medical therapies on cardiovascular complications, beginning with the UGDP which is described at the beginning of this chapter. Observational studies and meta-analyses

of sulfonylureas have reported conflicting results, likely due to bias and choice of comparator drug [58], though typically show increased risk for cardiovascular events compared to metformin and no difference in risk compared to the dipeptidyl peptidase-4 inhibitor linagliptin [59].

The availability of adequately powered, prospective clinical trial data on diabetes drugs' modulation of cardiovascular risk has recently been addressed by a change in FDA policy. Due to concerns about the impact of the thiazolidinedione rosiglitazone on cardiovascular risk [60], FDA issued guidance [10] that drug makers must demonstrate a new diabetes therapy must not be associated with an increase in cardiovascular risk. While all trials performed for this purpose to date have demonstrated at least non-inferiority with respect to cardiovascular risk compared to placebo, several have unexpectedly demonstrated cardiovascular protection, most notably multiple members of the SGLT2inhibitor and glucagon-like peptide 1 (GLP-1) receptor agonist classes [61]. Intriguingly, even in patients without diabetes, the SGLT2 inhibitor dapagliflozin has been demonstrated to prevent worsening heart failure or death from cardiovascular causes [62] and progression of chronic kidney disease [63]. Though the exact mechanism by which these medications influence cardiovascular disease is not well understood, these advances have the potential to be transformative in the way we treat diabetes and understand its relationship to cardiovascular health.

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Chapter 2
Effects of Insulin on the Vascular System

Anthony S. Sallar and Helmut O. Steinberg

Introduction

The function of the vascular system is to allow the delivery of blood (oxygen and nutrients) to the tissues according to their unique metabolic needs, and the function of insulin is to enhance the storage of nutrients and to support tissue growth. To accomplish this task for the ever-changing tissue requirements for oxygen and nutrients and without compromising the blood supply of vital organs, the vascular system responds in a variety of ways. It responds at the local tissue level via the release of short-acting vasoactive hormones, which redirect blood flow from less active to more active tissue units. The vascular system reroutes blood flow from organs with (relatively) lesser needs to organ systems, which require higher rates of blood flow, for example, by activation of the sympathetic nervous system (SNS). Finally, if tissue requirements cannot be met by the above mechanisms, cardiac output will increase to meet all requirements and to avoid dangerous reductions in blood pressure.

When this chapter was first published in 2005, more than 20 years after insulin's action on the vasculature had been demonstrated in the dog [1], most groups in the field had come to agree that insulin, in the human, in addition to its actions on glucose, protein, and fatty acid metabolism also exhibited distinct effects on the vascular system. Equally important, elevated circulating insulin levels had been found to be an independent risk factor for cardiovascular disease (CVD). These observations raised the question whether elevated insulin levels per se might cause macrovascular disease or whether the insulin levels were elevated to compensate for the insulin resistance seen in obesity, hypertension, and type 2 diabetes. Thus, the question to be answered was whether insulin itself possessed direct vascular effects, which might accelerate atherosclerosis or cause hypertension. Overall, in the last 15 years,

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most of what we knew in 2005 has been confirmed; insulin elicits a coordinated response at the level of the skeletal muscle vasculature, the heart, and the SNS, and possibly even in the larger conduit vessels. Equally importantly, no studies have shown that insulin's actions contribute to CVD.

Insulin, in lean insulin-sensitive subjects, increases skeletal muscle and adipose tissue blood flow at physiological concentrations. Simultaneously, cardiac output and SNS activity increase. The majority of the increment in cardiac output is directed toward skeletal muscle, suggesting that the blood-flow elevation as a result of insulin's vascular action may be instrumental in augmenting skeletal muscle glucose uptake. Over the last 15 years, due to the increased use of contrast-enhanced ultrasonography (CEU) in skeletal muscle, it has been shown that insulin increases skeletal muscle and adipose tissue perfusion and is also highly likely to increase capillary recruitment and perfusion, even before cardiac output increases. However, the accuracy of these observations has been questioned by results from animal studies that applied newer technologies [2, 3].

The role of the rise in sympathetic nervous system activity (SNSA) in response to insulin is less well understood. It has been proposed that the increase in SNSA may counteract insulin's vasodilator effect to avoid a decrease in blood-pressure levels; there are more recent studies that support this notion. The insulin-induced change in SNSA may also be important for blood-flow regulation in adipose tissue. Furthermore, insulin may also exert part of its cardiovascular effects indirectly via modulation of renal sodium and volume handling.

It has been demonstrated that insulin's effect on skeletal muscle blood flow is mediated through the release of endothelium-derived nitric oxide (NO), the most potent endogenous vasodilator. Importantly, NO is not only a vasodilator but also exhibits a host of anti-atherosclerotic properties. In addition to its effect on NO release, insulin also modulates the response to other vasoactive hormones such as angiotensin II or norepinephrine (NE) at the level of the vascular endothelium and the vascular smooth muscle cell. Therefore, insulin's effect on the vasculature of normal subjects appears to be beneficial in that it may counteract blood-pressure elevation and inhibit the atherosclerotic process.

Assessment of insulin's effect on human microcirculation has flourished over the last 15 years mostly due to increased availability of CEU technology; progress of this technology and other technologies combined with enhanced computational capabilities are likely to lead to interesting findings in the study of single arterioles or capillaries in the future [4, 5]. Results obtained with CEU and with various techniques, such as tracer, positron emission tomography (PET) scanning and magnetic resonance demonstrate, with few exceptions, that insulin increased microvascular perfusion through capillary recruitment.

Insulin's vasodilator effect on skeletal muscle and adipose tissue vasculature is blunted in states of insulin resistance such as obesity, hypertension, and type 2 diabetes mellitus; and more evidence for impaired endothelial function and decreased NO production in obesity, hypertension, and type 2 diabetes has been developed over the last 15 years. Additional findings suggest that the size of adipocytes and adipose tissue depot may constrain blood supply and, therefore, affect perfusion. The mechanism(s) by which obesity and type 2 diabetes impair endothelial function

are not fully elucidated and are likely multifactorial; a combination of factors such as elevated free fatty acid (FFA) levels, increased endothelin-dependent vascular tone, increased levels of asymmetric dimethyl-arginine (ADMA), or endothelin as observed in these insulin-resistant subjects may account, at least in part, for the vascular dysfunction.

The following review will focus mainly on data obtained from human studies, but data from animal or in vitro studies will be used when providing mechanistic insight into insulin's effects on the vasculature.

Technical Considerations

Before exploring insulin's vascular actions, several technical considerations should be made. In vivo studies of insulin's effect on the vascular system require, in most cases, systemic administration of glucose (euglycemic hyperinsulinemic clamp technique) to maintain stable glucose concentrations. Using the euglycemic hyperinsulinemic clamp technique [6] avoids hypoglycemia and the release of hormones such as epinephrine, NE, or cortisol, which can blunt the metabolic and vascular action of insulin. However, glucose metabolism will be increased by insulin administration, and therefore, it may be difficult to dissociate insulin's vascular and metabolic effects. Furthermore, even small amounts of insulin may result in a decrease of systemic FFA levels or in an increase in SNSA, which may alter vascular responses to different stimuli.

The experimental conditions under which the data are obtained may influence the vascular response to insulin and other vasoactive substances. For example, the cardiovascular response in part may depend on whether the study is performed with the subject in the supine or upright-sitting position [7], whether the forearm or the leg is studied and so on. Finally, in regard to the assessment of skeletal muscle perfusion and blood pressure, all methods (strain gauge plethysmography vs thermodilution or PET scanning or CEU) have different sensitivities, which may explain part of the divergent observations in the literature. Similarly, results of vascular function studies may differ according to the methods. Interestingly, flow-mediated vasodilation (FMD), the change in brachial artery diameter in response to ischemia, did not correlate with insulin sensitivity in a larger Canadian study.

Physiology

Insulin's Effects on Skeletal Muscle Blood Flow

Insulin increases skeletal muscle blood flow in lean insulin-sensitive subjects. This insulin effect is observed in the leg [8, 9] and the forearm [10]. Insulin's vasodilator action occurs at physiological concentrations and in dose-dependent fashion (Fig. 2.1). Limb blood-flow rates nearly double at insulin levels in the high

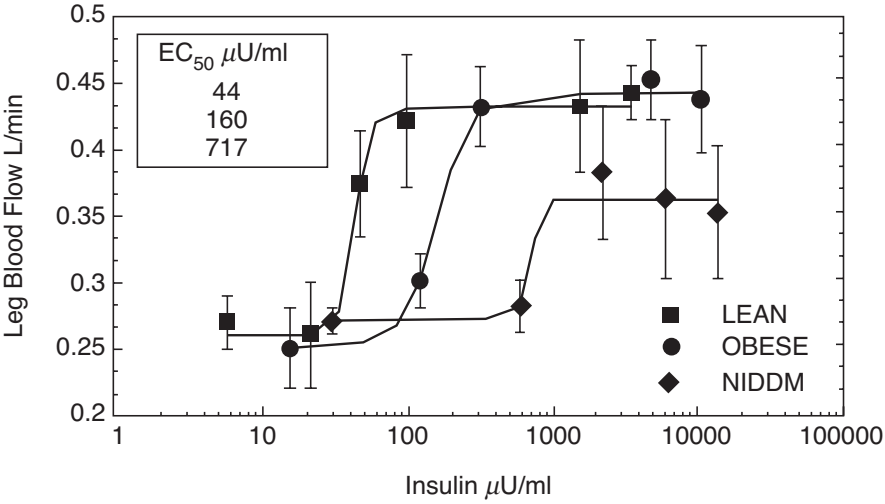

Fig. 2.1 Rates of leg blood flow in response to a wide range of steady-state insulin concentrations during euglycemic clamp studies in lean (filled square), obese (filled circle) and obese type 2 diabetic (filled diamond) subjects. The insert shows the insulin concentration required to achieve half-maximal increments in leg blood flow (EC50) in the different groups. (From ref. 11)

physiological range (~70–90 μU/mL). However, not all researchers have been able to observe the vasodilatory effects of insulin [12], except after a prolonged infusion, or at very high (~3000 μU/mL) systemic insulin levels [13]. The reasons for these divergent findings are not clear but are likely a result of differences in sensitivity and reproducibility of the methods used to determine blood flow.

In lean, insulin-sensitive subjects, the onset of insulin-mediated increments in skeletal muscle blood flow occurs early during a euglycemic/hyperinsulinemic clamp with a half-life of approximately 30 min, nearly identical to that of insulin's effect to increase glucose extraction [14]. A similar time course for insulin's vascular effect has also been described by Westerbacka and associates [15], who studied the effect of euglycemic hyperinsulinemia on pulse wave reflection in the aorta. In this study, the authors measured the pressure difference (central aortic augmentation) between the early and late systolic pressure peaks using applanation tonometry. They found that pressure augmentation and augmentation index decreased already after 30 min of hyperinsulinemia becoming statistically significant after 1 h. Because wave reflection is determined by compliance and vascular resistance, and because an early rise in skeletal muscle blood flow was not detected, which indicates a fall in peripheral vascular resistance, the authors concluded that insulin at physiological concentrations (~60 μU/mL) affects the caliber or distensibility (compliance) of large arteries. Studies in rats indicate [16] that insulin-mediated vasodilation may occur prior to an increase in cardiac output. Taken together, these studies provide evidence that insulin's effect on the vasculature occurs early in the course of hyperinsulinemia and parallels its effect on glucose metabolism.

Insulin does not only increase skeletal muscle blood flow at physiological concentrations but also augments the response to the endothelium-dependent vasodilator methacholine chloride (MCh). We have demonstrated [17] nearly a 50% augmentation of endothelium-dependent vasodilation at insulin levels of about 25 μU/mL. However, insulin did not augment the leg blood-flow response to the endothelium-independent vasodilator sodium nitroprusside (SNP). In support of our observation, insulin augmenter endothelium-dependent relaxation in response to the endothelium-dependent vasodilator acetylcholine in the isolated rat aorta but did not affect the response to SNP [18]. Taken together, these data indicate that insulin augments the production of but not the response to NO. In contrast to the above findings, euglycemic hyperinsulinemia was found to decrease FMD independent of insulin sensitivity or plasma lipid concentrations [19]; however, these results are difficult to interpret because this study used a less well-defined model to estimate endothelial function [20].

Insulin augmented the endothelial response to MCh, and therefore, we hypothesized that insulin causes skeletal muscle vasodilation via the release of NO. Using NG-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, we found that insulin's vasodilatory effects could be nearly completely annulled. In addition, the increment in leg blood flow was prevented by administration of L-NMMA into the femoral artery prior to initiating the systemic insulin infusion [14]. Moreover, leg blood flow, which nearly doubled in response to 4 h of euglycemic hyperinsulinemia returned to baseline (Fig. 2.2) levels within 5 min of an infusion of L-NMMA

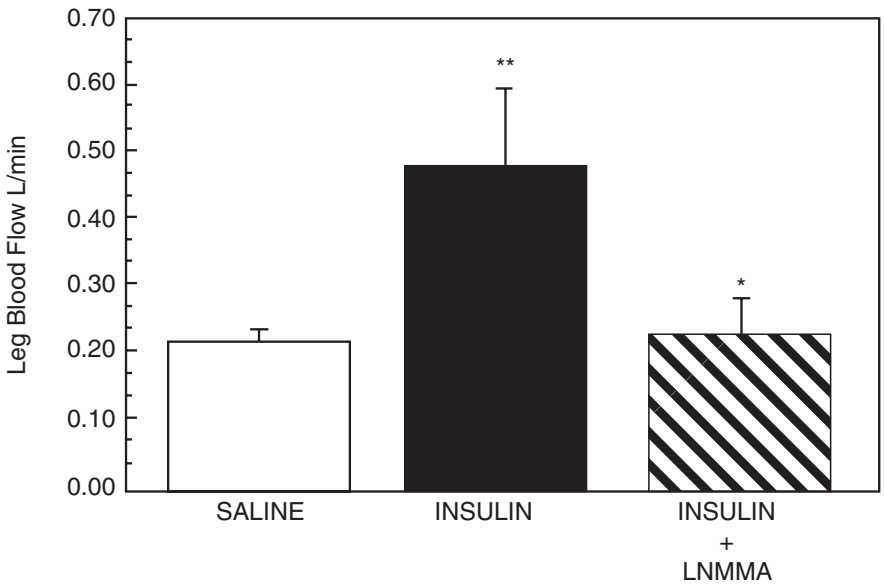

Fig. 2.2 Leg blood flow under basal conditions (saline), in response to 4 h of euglycemic hyperinsulinemia alone (insulin) and with superimposed intrafemoral artery infusion of L-NMMA (insulin + L-NMMA). (From ref. 17)

into the femoral artery [17]. Our findings have been confirmed by others in humans [21] and in animals [22]. The notion that insulin acts via release of NO from endothelial cell is supported by the observation that insulin directly releases NO from human umbilical vein endothelial cells [23]. This insulin-mediated NO release occurred in a dose-dependent fashion and could be completely abolished by N(omega)-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase. These results establish that insulin increases skeletal muscle blood flow, at least in part, via release of endothelial-derived NO.

Further investigation of the signaling pathway involved in insulin-mediated NO release revealed that genistein (an inhibitor of tyrosine kinase) nearly completely prevented the release of NO. Importantly, application of wortmannin, which inhibits phosphatidylinositol 3-kinase (PI3K), a signaling molecule required for insulin's effect to increase glucose uptake, caused about a 50% decrease in NO production. These in vitro results indicate that insulin-induced release of NO is mediated through signaling pathways involving tyrosine kinase, PI3K, and Akt downstream from the insulin receptor [24]. Importantly, Akt has recently been shown to phosphorylate endothelial NO synthase (eNOS), which results in increased activity of eNOS [25, 26]. Since insulin also increases the transport of amino acids into cells, the increased NO production may represent the complementary effects of eNOS phosphorylation and increased intracellular availability of arginine, the precursor of NO. Together, these findings suggest that insulin's metabolic and vascular actions share common signaling pathways which might explain the similar time course of skeletal muscle vasodilation and glucose uptake in response to insulin. Moreover, impairment of a common signaling pathway in obesity, hypertension, or diabetes could lead to both blunting of insulin-mediated blood-flow increments and decreased rates of skeletal muscle glucose uptake. In this regard, it is important to note that mice deficient of eNOS were insulin resistant and mildly hypertensive [27], but mice deficient of endothelial insulin receptors [28] exhibited normal glucose metabolism. Results from a recent study in primates [29] suggest that epoxyeicosatrienoic acids also mediate insulin-mediated augmentation in skeletal muscle perfusion.

Insulin's Effects on the Heart

Our lab [30] investigated the effect of different insulin infusion rates on stroke volume in groups of lean normotensive volunteers (Fig. 2.3a). Hyperinsulinemia in the low physiological range (35 ± 4 μU/mL) and in the high physiological range (78 ± 6 μ/mL) increased stroke volume by about 7%. A nearly 15% augmentation of stroke volume was observed with supraphysiological insulin concentrations (2145 ± 324 μU/mL). A similar effect of insulin on stroke volume was reported by Ter Maaten and associates [31], who observed a nearly 13% rise at insulin levels of about 30 μU/mL. The increase in stroke volume could be a result of either a decrease in peripheral resistance (see section "Insulin's Effect on Blood Pressure and Vascular Resistance") or as a result of an increase in inotropy of the heart muscle. Experiments in the isolated beating heart or with heart muscle preparation indicate that insulin

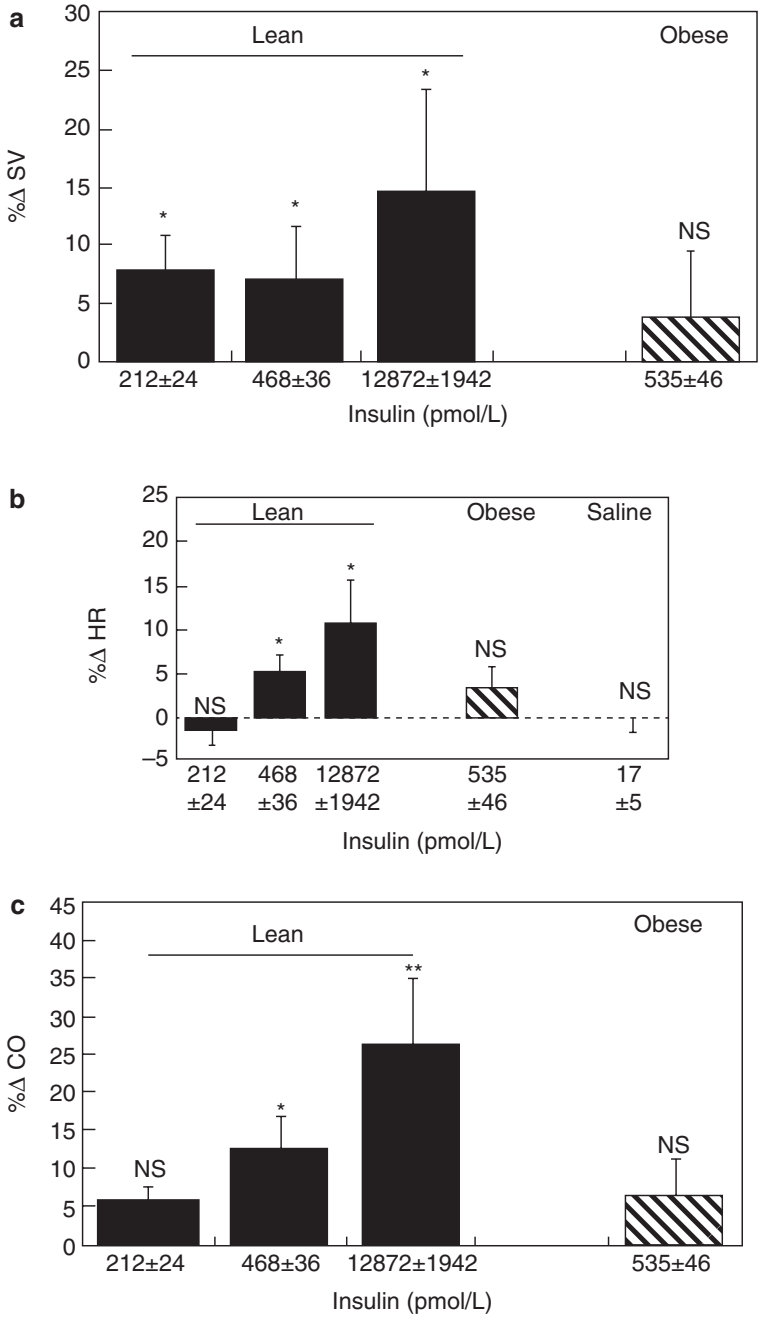

Fig. 2.3 Percent change (%Δ) from baseline (**a**) in stroke volume (SV), (**b**) in heart rate (HR), (**c**) in cardiac output (CO), (**d**) in mean arterial blood pressure (MAP), and (**e**) in total peripheral resistance (closed bar) and leg vascular resistance (hatched bar) during systemic hyperinsulinemic euglycemia and saline (control) infusion studies in lean and obese subjects. *$p < 0.05$, **$p < 0.01$ and not significant (NS) vs baseline. (From ref. 30)

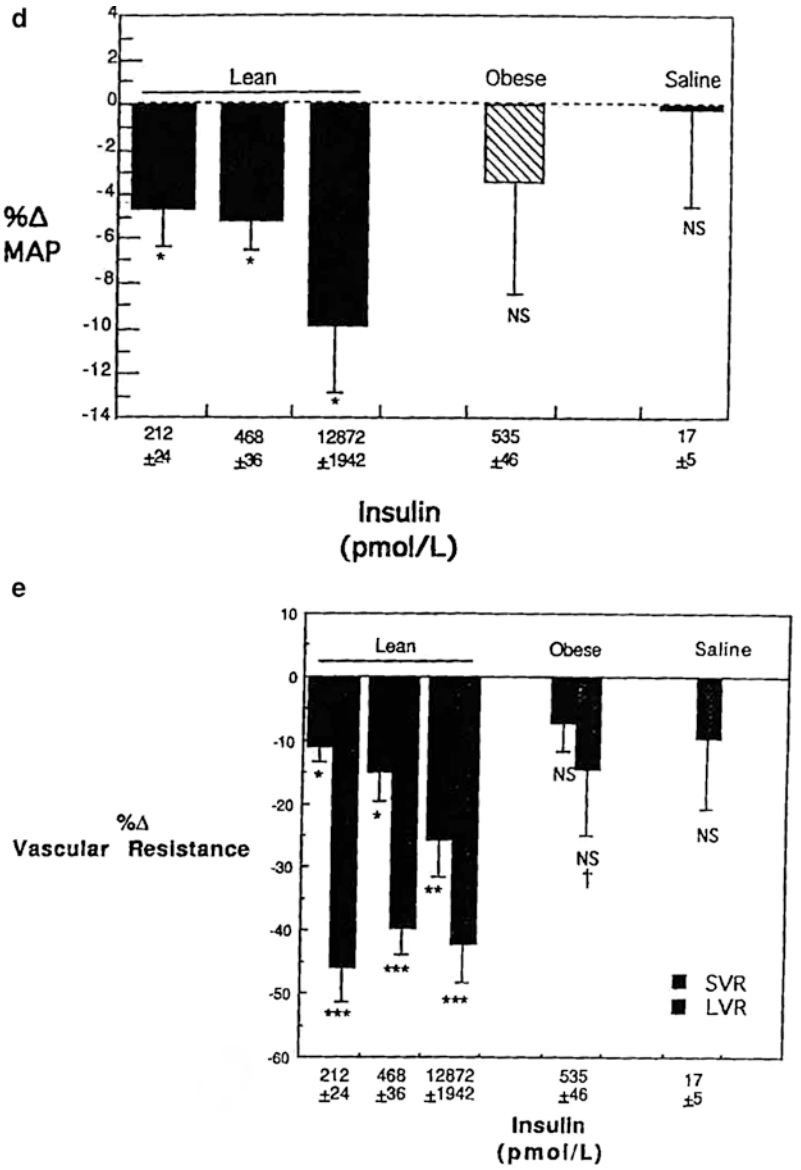

Fig. 2.3 (continued)

increases contractility of heart muscle. Taken together, these data indicate that insulin has a direct effect on the heart to increase cardiac stroke volume.

In addition to augmenting stroke volume, insulin increases heart rate. In our groups, heart rate did not change at low (~35 μU/mL) levels but increased by 5% and 10% at insulin concentrations of about 80 and about 2100 μU/mL,

respectively (Fig. 2.3b). Thus, our data indicate that insulin increases heart rate in a dose-dependent fashion. Increments in heart rate in response to hyperinsulinemia were also found by others [9, 10, 32] but not by all [31]. The reason for the discrepancy is not clear, but differences in volume status or position during the study may explain in part the different observations. Whether the increase in heart rate is a direct insulin effect or whether it is mediated by activation of the SNS is not known. Nevertheless, the increase in SNS activity likely represents normal physiology to maintain blood pressure [33, 34] and secure delivery of nutrients to the tissue.

As a result of the rise in heart rate and stroke volume in response to insulin, cardiac output upsurges. In our study groups, cardiac output increased by about 6%, 12%, and 26% in response to insulin concentrations of about 35, 80, and 2100 μU/mL (Fig. 2.3c). In support of our data, Ter Maaten and colleagues found about a 9% increase in cardiac output with insulin concentrations of about 50 μU/mL [31]. Moreover, Fugman and associates' study replicated most of the above findings in a more recent study [35], demonstrating increased cardiac output in response to high physiological levels of insulin. These insulin effects are not only of academic interest but also may have implications under conditions in which cardiac output needs to be augmented. For example, insulin's effect of increasing cardiac output has been used to improve severe heart failure in patients undergoing cardiac surgery who were unresponsive to catecholamines and vasodilators [36].

Over the last 15 years, there were no relevant new studies in the literature regarding insulin's hemodynamic effects on the heart, but there is current interest in insulin receptor signaling in the maintenance cardiomyocyte health and in models of cardiomyopathy.

Insulin's Effects on the Sympathetic/Parasympathetic Nervous System

Insulin had been shown to increase SNSA years before its vasodilator action was appreciated [37]. Systemic insulin infusion causes a dose-dependent rise in NE levels. In one study [6], NE levels in response to insulin increased from 199 ± 19 pg/mL under basal conditions to 258 ± 25 and 285 ± 95 pg/mL at insulin concentrations of 72 ± 8 μU/mL and 144 ± 13 μU/mL, respectively. In the same study, skeletal muscle SNSA measured by microneurography exhibited an even more impressive rise in response to insulin. Microneurography allows to measure frequency and amplitude of electric activity directly at the level of sympathetic nerve fibers. Determined by microneurography, SNSA increased from baseline of about 380 U to about 600 U and about 750 U in response to euglycemic hyperinsulinemia. Similar differences between the methods to assess changes in SNSA have been found by others [38], suggesting that plasma NE levels may underestimate the true effect of insulin to stimulate SNSA.

Interestingly, insulin modulates SNSA in a non-uniform manner. Van De Borne and colleagues [39] studied the effect of insulin on skeletal muscle SNSA with microneurography. The effect of hyperinsulinemia on cardiac SNSA and parasympathetic tone was assessed by power spectral analysis of the decrease in R–R interval. Power spectral analysis allows one to distinguish between low-frequency and high-frequency components of the changes in R–R intervals. The high-frequency component is thought to reflect parasympathetic nervous system activity (PNSA; vagal tone) whereas the low-frequency component reflects SNSA. Additionally, systemic infusion of the B-blocker propranolol allows to distinguish the contribution of the PNS and the SNS on the R–R interval variability.

In response to hyperinsulinemia (84 ± 5 U/mL), skeletal muscle SNSA increased more than twofold. In contrast, the SNSA effect on the reduction in R–R interval and variability in response to hyperinsulinemia was relatively small. This observation suggests that insulin's effect on the SNSA may be targeted specifically toward skeletal muscle, the place of insulin's metabolic action. Interestingly, the increase in skeletal muscle SNSA may delay insulin's vasodilator action [40].

The mechanism(s) for the increments in SNSA during hyperinsulinemia are not well understood. It may be mediated via the baroreceptor reflex to counteract insulin's vasodilator action or may represent a direct insulin effect on the central nervous system. Moreover, coupling of insulin's effects on the SNS and its effect to increase glucose uptake/metabolism cannot be excluded. Although activation of the baroreceptor reflex in response to a decrease in blood pressure causes activation of the SNS, it cannot explain all of the observed changes. First, time course of blood-pressure decline and SNSA were different [10] and second, the increments in SNSA in response to insulin were nearly two times those in response to blood pressure fall achieved by nitroglycerin infusion [41]. In support of a direct role of insulin on SNSA at the level of the brain, injection of insulin directly into the third ventricle has been shown to increase SNSA in rats [42]. This increase in SNSA activity could be abolished by generating a lesion in the surrounding the lateroventral portion of the third ventricle, a region implicated in sympathetic neural control. Overall, the results suggest a direct effect of insulin on the brain to increase SNSA, but other mechanisms cannot be excluded.

It has also been demonstrated that insulin modulates PNSA. Unfortunately, no biochemical markers of PNSA exist, which can be easily measured in vivo. As mentioned above, PNSA is studied by measuring the changes in R–R intervals using power spectral analysis. The PNSA (vagal component of heart rate control) is represented in the high-frequency part of the spectrum.

In 1996, Bellavere and associates [43] reported a decrease in high-frequency variability of R–R intervals in response to hyperinsulinemia indicating that PNSA decreased. Similar results were obtained by Van De Borne and associates [39] in which euglycemic hyperinsulinemia decreased both R–R interval and the high-frequency variability of the R–R intervals. Moreover, this insulin-induced reduction of both R–R interval and high-frequency variability could not be suppressed by the B-blocker propranolol. These data indicate that the reduction in PNSA and not increments in SNS were likely responsible for the changes in R–R interval and

variability. Furthermore, these data suggest that the effect of hyperinsulinemia on cardiac SNSA may be less than originally thought. Taken together, these data suggest that insulin's effect to stimulate SNSA may be mediated at least in part via a direct insulin effect on the brain. Furthermore, hyperinsulinemia appears to reduce parasympathetic tone at the level of the heart, which may contribute to the increments in heart rate.

Insulin's Effects on the Kidneys

The effect of euglycemic hyperinsulinemia on renal hemodynamics has not been studied by many groups. In one study [44], insulin at levels of about 100 U/mL has been reported to increase renal plasma flow by 10 ± 5%. A similar rise in renal plasma flow has been reported in response to L-arginine-induced insulin secretion.

Insulin's effect on electrolyte handling is well established. Insulin has been found to cause antinatriuresis [45, 46], antikaliuresis, and antiuricosuria in healthy volunteers. The antinatriuresis is achieved via a decrease in fractional sodium excretion. Fractional sodium excretion fell by 20–30% in response to euglycemic hyperinsulinemia with insulin levels of 50–60 μU/mL, well in the physiological range. Reductions in potassium and uric acid excretion in response to insulin were of similar magnitude [42]. Based on animal studies [47], it was thought that insulin exerts the antinatriuretic effect at the level of the distal tubule in which the highest density of insulin receptors is found, but it may be that the proximal tubule is the more likely site of insulin's antinatriuretic action in humans [48]. The mechanism of the antikaliuretic and antiuricoretic effects of insulin is less well elucidated.

Insulin's Effect on Blood Pressure and Vascular Resistance

Insulin's effect on skeletal muscle vasculature, stroke volume, heart rate, cardiac output, SNS, and renal sodium handling can affect blood pressure. Blood pressure is determined by cardiac output and total peripheral resistance (TPR). In other words, blood pressure in response to insulin may increase, stay unchanged, or decrease dependent on the changes in cardiac output and resistance. In lean, insulin-sensitive subjects, insulin causes a small but significant fall in blood pressure. In our study [30], hyperinsulinemia in the low (35 ± 4 μU/mL) and high (72 ± 6 μU/mL) physiological range caused about a 5% drop in mean arterial pressure (MAP), and supraphysiological insulin concentrations (2100 ± 325 μU/mL) were associated with about a 10% fall in MAP (Fig. 2.3d). However, although a drop in MAP has been reported by many groups, it has not been observed in all studies; MAP remained unchanged in a study reported by Scherrer [12] and even increased by nearly 7 mmHg in another study [31]. The reasons for the different effects of euglycemic hyperinsulinemia on blood pressure are not clear.

The decrease in MAP in light of increased cardiac output indicates [29] a fall in TPR. In fact, TPR decreased in a dose-dependent fashion by 11.1 ± 2.2, 15.0 ± 4.7, and 26.0 ± 6.0% at insulin concentrations of 35 ± 4, 72 ± 6, and 2100 ± 325 μU/mL, respectively (Fig. 2.3e). A similar decrease in TPR with comparable levels of hyperinsulinemia was also observed by Fugman and associates [35]. Even more impressive than the fall in TPR was the drop in leg vascular resistance (LVR) LVR decreased by nearly 45% at an insulin concentration of 35 ± 4 μU/mL (Fig. 2.3e). Higher prevailing insulin levels did not result in further decrements in LVR. Similar decrements in resistance have been observed by Anderson in the forearm [10, 49] and by Vollenweider in the calf [38]. However, in one study [31] in which both blood pressure and forearm blood flow increased, no changes in vascular resistance were detected.

Metabolic Implications of Insulin's Vascular Effects

Our lab has long championed the idea that insulin's vascular effects may contribute to the rate at which glucose is taken up by skeletal muscle, which represents the majority of insulin-sensitive tissues. In other words, insulin's vascular effects may determine, at least in part, insulin sensitivity and impairment of insulin's vascular effects may result in insulin resistance.

In support of this idea, we found that insulin's effect to increase skeletal muscle blood flow and cardiac output is positively and strongly associated with the rates of glucose uptake achieved in response to euglycemic hyperinsulinemia. In two studies [30, 50] performed nearly 5 years apart, the correlation coefficients between leg blood-flow increments and whole-body glucose uptake were 0.63 and 0.56, indicating that blood flow achieved during euglycemic hyperinsulinemia explains one-quarter to one-third of the variation in insulin sensitivity. Similarly, Ter Maaten and associates [31] found that the correlation coefficient between percent increments in leg blood flow and insulin sensitivity index was 0.88, again suggesting that insulin's effect to augment blood flow contributes to rates of glucose uptake. Furthermore, cardiac output or changes in cardiac output in response euglycemic hyperinsulinemia also correlated significantly albeit not as strongly as leg blood flow with rates of whole-body glucose uptake [30, 31]. Finally, the similar time courses [9] of insulin-mediated vasodilation and insulin-mediated glucose uptake suggest that metabolic and vascular actions of insulin might be coupled.

Taken together, these data suggest but do not prove that insulin's effects on metabolism and the vascular system are coupled. To test our hypothesis more rigidly, we assessed the effect of leg blood flow changes on leg glucose uptake. In one set of studies [50], we increased leg blood flow from 0.32 ± 0.12 L/min during euglycemic hyperinsulinemia to 0.60 ± 0.12 L/min ($p < 0.05$) by administering an intrafemoral artery infusion of the endothelium-dependent vasodilator MCh. As a result of the blood-flow increments, leg glucose uptake increased from 87.6 ± 13.4 to 129.4 ± 21.8 mg/min ($p < 0.05$). In a second set of studies [51], we decreased leg blood flow during euglycemic hyperinsulinemia by nearly 50% via an intrafemoral artery infusion of the NO synthase inhibitor L-NMMA. The fall in leg blood flow

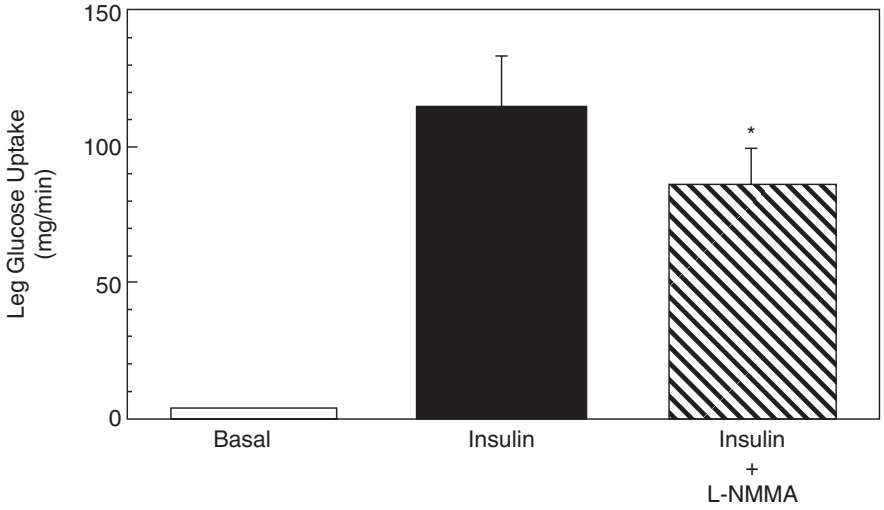

Fig. 2.4 Leg glucose uptake under basal conditions (basal), in response to 4 h of euglycemic hyperinsulinemia alone (insulin) and with superimposed intrafemoral artery infusion of L-NMMA (insulin + L-NMMA). (From ref. 51)

induced by L-NMMA caused leg glucose uptake to decrease from 114 ± 18 to 85 ± 13 mg/min ($p < 0.05$) representing about a 25% reduction of glucose uptake (Fig. 2.4), well in line with what had been predicted according to the experimentally defined correlation coefficients. In a third series of studies, we examined whether rates of skeletal muscle glucose uptake in response to changes in leg blood flow followed a noncapillary recruitment model as proposed by Renkin or whether changes in glucose uptake were dependent on capillary recruitment. The results of this study revealed that leg glucose uptake in response to pharmacological manipulation of blood flow was different than predicted by the Renkin model indicating that capillary recruitment is important for insulin's metabolic actions [52]. These findings are supported by studies of Bonadonna and associates [53] who looked at forearm glucose uptake using multiple tracer technique and Rattigan and associates [54] who measured glucose uptake in the isolated rat hindlimb. And Coggins and associates [55], using CEU, provided more direct evidence for insulin's effect to recruit skeletal muscle capillaries in men. Together, these data provide strong evidence that insulin's vascular effects relate to its metabolic effects and that this metabolic effect is mediated by capillary recruitment.

The above-discussed effects of insulin on the vascular system are also observed in response to meals [56]. Depending on the amount of carbohydrate or fat ingested and the circulating insulin levels achieved, heart rate, stroke volume, skeletal muscle blood flow, and SNSA increase substantially, indicating that this coordinated cardiovascular response occurs under physiological conditions and may be necessary to maintain both metabolic and hemodynamic homeostasis. Postprandial hypotension, which is frequently observed in the elderly, may be a result of insufficient increments in heart rate and/or stroke volume to compensate for insulin's vasodilator effect.

Interactions Between Insulin and Norepinephrine and Angiotensin II

Because elevated insulin levels were associated with higher rates of hypertension, it was hypothesized that insulin might augment the action of vasoconstrictor hormones such as NE or angiotensin II. Indeed, earlier studies [32, 37] reported that exogenous insulin enhanced the blood-pressure response to NE. About a 20% and 40% reduction of the NE concentrations required to rise diastolic blood pressure by 20 mmHg was reported after 1 and 6 h of euglycemic hyperinsulinemia. In contrast to this finding, we [57] observed that euglycemic hyperinsulinemia caused a right shift in the response to graded systemic infusions of NE. The reason(s) for the discrepant findings are not clear but are likely a result of differences in study protocol and the method by which blood pressure was determined (intra-arterial vs. cuff). Nevertheless, our data suggest that insulin attenuates vascular responsiveness to NE. In support of this notion, Sakai and associates [58] reported that an intra-arterial infusion of insulin attenuated the vasoconstrictor response to NE by nearly 50%. Moreover, Lembo and coworkers also demonstrated that that insulin augmented beta-adrenergic vasodilation in response to isoproterenol and attenuated a-adrenergic vasoconstriction [49]. Furthermore, this insulin action was blocked by L-NMMA and inhibitor of NO synthase. These results indicate that insulin's modulatory effect on adrenergic response is mediated via the release of NO.

The effect of hyperinsulinemia on blood-pressure response to angiotensin II has been studied by a number of groups [32, 59, 60]. Insulin does not augment nor attenuate the blood pressure response to systemic angiotensin II infusion. However, Sakai and associates [48] demonstrated that insulin, when directly infused into a vessel, may modulate the vasoconstrictor response to angiotensin II. In their study, the direct intrabrachial artery infusion of insulin caused a more than 50% attenuation of the forearm blood-flow response to angiotensin II.

Insulin modulates the response to vasopressor hormones such as NE, vasopressin, and angiotensin II not only at the level of the vascular endothelium but also directly at the level of the vascular smooth muscle cell independent of the endothelium. Insulin attenuates agonist-evoked calcium transients [61] resulting in decreased vascular smooth muscle contractions. Whether this insulin effect at the level of the vascular smooth muscle can be explained by its effect on shared signaling pathways as described with angiotensin-1 [62] or by a different mechanism remains to be clarified. It is clear, however that an imbalance between insulin's vasorelaxant effects and other vasoconstrictor hormones may result in the accelerated development of blood-pressure elevation and macrovascular disease. Interestingly, blood-pressure elevation by systemic administration of NE [57] or angiotensin II [59, 63, 64] failed to decrease rates of insulin-mediated glucose uptake and induce insulin resistance. To the contrary and somewhat unexpectedly, the blood-pressure elevation increased rates of insulin-mediated glucose uptake. The reason for this unexpected finding was most likely that limb blood flow increased which allowed for the higher delivery rates of substrate, glucose, and insulin and, thus, augmented skeletal muscle glucose uptake.

Interactions Between Insulin and Adipocytokines

Adipose tissue has been shown to release a number of hormones that may interact with the vasculature. Leptin, a hormone secreted from the adipocyte, causes not only the release of NO from endothelial cells and but also augments insulin's effect to release NO [65]. Furthermore, adiponectin, another adipocyte-derived hormone, has been shown to cause the release of NO from endothelial cells [66]. Finally, interleukin-6, released from intra-abdominal fat cells may decrease in endothelial NO production via increasing C-reactive protein [67] or via decreasing adiponectin secretion [68].

Pathophysiology: The Metabolic Syndrome

The metabolic syndrome which is also called "syndrome X" describes the clustering of a number of metabolic and hemodynamic abnormalities commonly seen in obesity and diabetes. More important, the metabolic syndrome is an independent risk factor for CVD. Syndrome X [69, 70] is associated with resistance to insulin-mediated glucose uptake, glucose intolerance, hyperinsulinemia, increased very low-density lipoprotein triglyceride, decreased high-density lipoprotein cholesterol, increased plasminogen activator inhibitor-1, and hypertension. Because classic risk factors account for only about 50% of the increased rates of cardiovascular morbidity and mortality associated with obesity and type 2 diabetes [71], other factors must play a role. One way to probe for potential candidates that might contribute to the higher rate of hypertension and the accelerated atherosclerotic process in insulin resistance is to evaluate the effect of obesity, hypertension, and type 2 diabetes on insulin's vascular effects.

The Metabolic Syndrome and Insulin's Effects on Skeletal Muscle Blood Flow

The effect of obesity, hypertension, and diabetes on insulin's vascular effects has been studied by a number of groups including our own. We [72] have demonstrated that obesity causes a left shift in the response to insulin's vasodilatory effect (Fig. 2.1); the dose that achieves half-maximal effect (ED) 50 for insulin's effect to increase skeletal muscle blood flow in the obese was nearly four times (~160 μU/mL) that of the lean (~45 μU/mL). Impaired insulin-mediated vasodilation in the obese was confirmed by Vollenweider and associates [73] who report about an 8% increment in calf blood flow in response to 2 h of euglycemic hyperinsulinemia in obese subjects, which is in stark contrast to the 30% increment achieved in the lean subjects.

Arterial stiffness is decreased in type 2 DM [74] and the effect of insulin to reduce arterial stiffness is impaired in obesity; Westerbacka [11] and colleagues demonstrated that in contrast to lean controls, arterial stiffness did not change in response to hyperinsulinemia with insulin levels of about 70 μU/mL and decreased only slightly in response to insulin levels of about 160 μU/mL.

Type 2 DM was associated with even more pronounced impairment of insulin-mediated vasodilation. In our study [72], only supraphysiological hyperinsulinemia (~2000 μU/mL) achieved about a 33% rise in blood flow and the limitation in flow increments could not be overcome by higher insulin concentrations (Fig. 2.1).

Because insulin-mediated vasodilation depends on NO and is impaired in obesity and type 2 DM, we studied whether this impairment results from defective endothelial function or whether or defective NO activity. To this end, we generated dose–response curves for the leg blood-flow response to the endothelium-dependent vasodilator MCh and to the endothelium-independent vasodilator SNP. Leg blood flow in response to methacholine increased threefold in the lean but only twofold in both obese and type 2 diabetics (Fig. 2.5). In contrast, the leg blood-flow response to SNP did not differ between lean, obese and type 2 diabetics. Resistance to leg blood-flow increments in response to the endothelium-dependent vasodilator bradykinin has also been reported in obesity [76], thus, supporting our data that NO production is impaired.

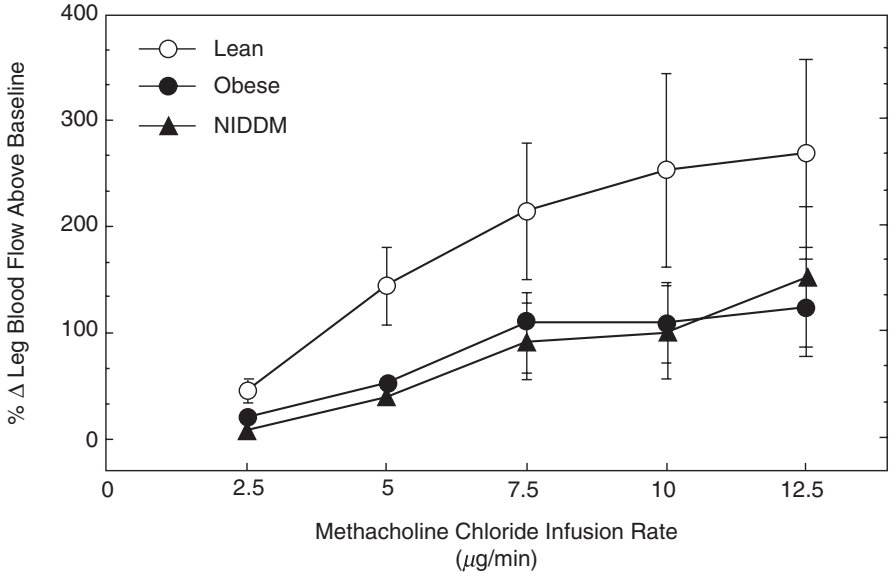

Fig. 2.5 Percent change (%Δ) from baseline in leg blood flow (LBF) in response to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride in groups of lean (open circle), obese (filled circle), and obese type 2 diabetic (filled triangle) subjects. (From ref. 75)

In addition to obesity and type 2 diabetes, elevated blood-pressure levels are associated with impaired insulin-mediated vasodilation [77]. Laine and associates [76] demonstrated that insulin-stimulated leg blood flow increased by 91% in the control subjects but only by 33% in the hypertensive subjects. This is important because hypertension has been shown by Forte and associates [78] to be associated with significantly decreased rates of NO production. Therefore, it is likely that in hypertension, impaired NO production is responsible for the blunted vasodilation in response to hyperinsulinemia.

Direct measurements of NO production in the skeletal muscle vasculature of obese and type 2 DM subjects, however, have yielded conflicting data. In one preliminary study [75], we measured insulin-induced changes in NO flux rates in subjects exhibiting a wide range of insulin sensitivity. NO flux was calculated by multiplying the concentration of nitrite and nitrate times leg blood-flow rates before and after 4 h of euglycemic hyperinsulinemia. In this study, NO flux rates more than doubled in athletes who exhibited high insulin sensitivity but did not change in diabetics who were insulin resistant. However, Avogarro and associates [79], who measured NO flux rates in the forearm in obese and type 2 diabetic subjects, were unable to detect a difference in NO flux between the two groups. The reason for the discrepant observations is not clear, but further research will help to clarify this issue. Measurements of whole-body NO production using labeled L-arginine, the precursor of NO, revealed lower NO production rates in type 2 diabetics as compared to normal subjects [80] provides strong evidence for impaired NO production in type 2 diabetes.

Taking the data together, basal whole-body NO production is decreased in hypertensive and in type 2 diabetic patients, and it is likely that obesity, hypertension, and type 2 diabetes exhibit impaired NO production in response to insulin. Because NO is not only a potent vasodilator but also possesses a number of antiatherogenic properties, this defect in NO production could theoretically contribute to the increased rate of CVD in insulin-resistant states such as obesity, hypertension, or type 2 diabetes.

The mechanism(s) of impaired insulin-mediated vasodilation in obesity or type 2 DM are not known. One of the metabolic abnormalities consistently observed in insulin resistance is elevated FFA levels. Elevation of FFA levels also induces insulin resistance, which may be mediated, in part, via impairment of insulin-mediated vasodilation. Therefore, we studied the effect of FFA elevation on endothelial function in lean, insulin-sensitive subjects. The results of this study indicated that moderate two- or threefold elevation of FFA levels sustained for 2 h, achieved by systemic infusion of Intralipid plus heparin, blunted the response to the endothelium-dependent vasodilator MCh (Fig. 2.6) but not to the endothelium-independent vasodilator SNP [81]. Similar results were reported by de Kreutzenberg and colleagues, who measured forearm vascular responses to before and after elevation of FFA [82]. Interestingly, the postischemic flow response was also impaired by FFA elevation [74]. Importantly, elevation of triglyceride levels without inducing insulin resistance may not impair vascular function which is suggested by studies of patients with low lipoprotein lipase activity who exhibit normal endothelial function [83] despite markedly elevated triglyceride levels.

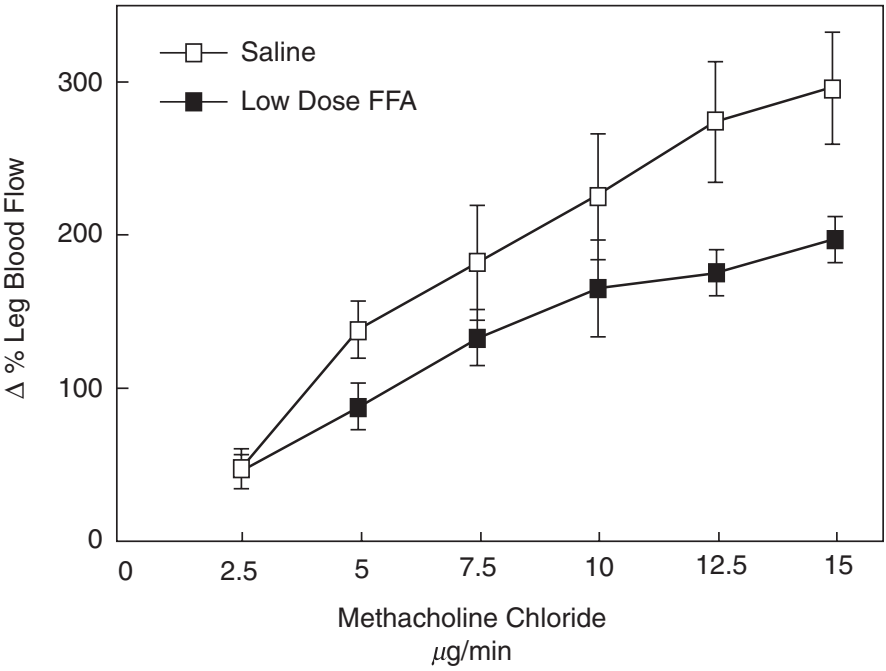

Fig. 2.6 Leg blood flow increments from baseline (%Δ) in response to graded intrafemoral artery infusion of methacholine chloride during infusion of saline (open squares) or during 20% fat intralipd emulsion (closed squares) combined with heparin designed to increase systemic circulating free fatty acid levels two- or threefold. (From ref. 81)

To further investigate the relation among elevated FFA levels, insulin sensitivity, and insulin-induced vasodilation, we investigated the time-course effect of FFA elevation on insulin-mediated increments in blood flow. Between 4 and 8 h, but not as few as 2 h of FFA elevation reduced insulin-mediated vasodilation [84]. Furthermore, increments in NO flux in response to euglycemic hyperinsulinemia were nearly completely abrogated by superimposed FFA elevation. This effect on insulin-induced vasodilation was only observed when FFA elevation also caused insulin resistance. These data indicate that insulin-mediated vasodilation is coupled with insulin's effect on glucose uptake. In contrast, muscarinergic-agonist-induced endothelium-dependent vasodilation appears to be regulated by other mechanisms as this signaling pathway can be disrupted by FFA elevations as short as 2 h [81]. Indirect evidence for this proposed effect of FFA elevation on insulin-mediated vasodilation comes from muscle biopsy studies in response to hyperinsulinemic euglycemia with and without superimposed FFA elevation [85]. Dresner and colleagues [85] demonstrated that insulin resistance induced by FFA elevation was associated with decreased PI3K activity in skeletal muscle. Therefore, if insulin-signaling pathways are shared in endothelial cells and skeletal muscle, one may expect impaired insulin signaling in the endothelial cells in response to euglycemic

hyperinsulinemia with superimposed FFA elevation. Other support of the negative effect of elevated FFA levels on endothelial NO production comes from in vitro studies that demonstrated a dose-dependent effect of oleic acid to impair NO release from cultured endothelial cells [86] and an attenuated the aortic strip relaxation in response to acetylcholine [87].

Additional mechanisms by which FFA may impair endothelial function include increased plasma levels of asymmetric dimethyl-L-arginine (ADMA) and/or increased endothelin action [88]. Lundman and associates [89] demonstrated that acute elevation of triglyceride (and likely elevated FFA) levels achieved by systemic infusion of a triglyceride emulsion was associated with elevation of ADMA levels and decreased flow-mediated vasodilation. Similarly, Fard and associates [90] showed that a high fat meal given to diabetic subjects resulted in increased plasma ADMA levels and impaired flow-mediated vasodilation.

Endothelin levels have been shown to increase in response to FFA elevation. Because elevated FFA levels are a hallmark of obesity and type 2 diabetes mellitus, Cardillo and associates [91] and Mather and associates [92] infused an inhibitor of endothelin, BQ 123 (a specific inhibitor of the endothelin-1-A receptor) directly into the brachial and femoral artery, respectively. Both studies revealed more pronounced vasodilation in response to BQ123 in the obese and diabetic subjects, indicating a higher endothelin-dependent tone in the insulin-resistant subjects. In addition, one study looking at vastus lateralis muscle biopsies showed reduced eNOS content and activity in type 2 diabetic subjects while endothelin-1 peptide and mRNA were higher [93]. Additionally, endothelin secretion may increase in response hyperinsulinemia and contribute to the impaired vasodilation observed in insulin-resistant states [94]. Results from studies in rats [95] have demonstrated that myeloperoxidase may also impaired vascular function in insulin resistance.

Taken together, these findings from in vivo and in vitro studies strongly suggest role of elevated FFA levels to impair endothelial function and decrease the rates of NO release, increase endothelin action, and increase vascular response to adrenergic stimulation.

The Metabolic Syndrome and Insulin's Effects on the Heart

Before discussing the effect of insulin on heart rate in insulin-resistant obese and diabetic subjects, two points should be made: first, basal heart rate and cardiac output [96] in obese and diabetic subjects is almost always increased as compared to lean subjects; second, heart function in diabetes may be abnormal as a result of autonomic neuropathy and third, since the heart is an on-demand pump, lesser increments in insulin's metabolic actions may be associated with a reduced need to supply tissues with additional oxygen and nutrients. Thus, the data have to be interpreted with caution especially when comparing relative changes between insulin-sensitive and insulin-resistant groups.

The effect of insulin resistance on insulin-induced change in stroke volume has received little attention. Stroke volume did not change in our group of obese subjects (Fig. 2.3a) exposed to insulin concentrations of about 90 μU/mL. However, we may have failed to detect a less than 5% increase in stroke volume because of small group size. Muscelli and associates [97], however, report a near 10% rise in stroke volume at insulin concentrations of about 120 μU/mL. The reason for the different results is not clear. Groups were comparable regarding body mass index or blood pressure. However, Muscelli and associates [97] used two-dimensional echocardiography, whereas we used dye dilution technique to determine stroke volume. Thus, the discrepant results may be explained, at least in part, by different sensitivities of the methods by which cardiac output was determined.

We did not observe a change in heart rate in response to hyperinsulinemia about 90 μU/mL in our obese subjects (Fig. 2.3b). In contrast to our findings, Vollenweider and associates detected about a 10% increase in heart rate in obese subjects with insulin levels comparable to our study (~100 μU/mL). Heart rate was also found to rise in a dose-dependent fashion in response to hyperinsulinemia [98] in type 2 diabetics.

Because stroke volume and heart rate did not change in our obese group (Fig. 2.3c), cardiac output did not change either. However, other studies report a significant 15% increment in cardiac output in obese subjects [97]. In type 2 diabetes, data on changes in cardiac output in response to hyperinsulinemia are not available. Nevertheless, because heart rate has been reported to increase in diabetics in response to hyperinsulinemia, it is reasonable to assume that cardiac output may increase as well. Taken together, the observations suggest that insulin's stimulatory effect on stroke volume, heart rate, and cardiac output may be intact in obese and type 2 diabetic subjects.

Insulin's action on the heart may extend well beyond modulation of hemodynamics. Cardiomyocytes possess insulin receptors which are important in postnatal development of the heart [99]. It is not known whether impaired insulin receptor signaling in the cardiomyocyte plays a role in the increased incidence of left ventricular hypertrophy and congestive heart failure which is often observed in obesity and diabetes. It may be of interest, however, that obesity and insulin resistance appear to be associated with a higher incidence of heart failure with preserved ejection fraction as compared to lean and more insulin-sensitive subjects in population studies [100].

The Metabolic Syndrome and Insulin's Effects on the Sympathetic/Parasympathetic Nervous System

When assessing the SNSA by measuring NE, no differences were detected between lean and obese subjects [38, 101, 102]. Tack and colleagues used tritiated NE combined with forearm blood-flow measurements to assess the effect of hyperinsulinemia on SNSA in the forearm of lean type 2 diabetic and controls; in response to

insulin, arterial and venous NE concentrations increased in both groups. For example, 45 min of hyperinsulinemia caused arterial NE levels to increase by 63.8 ± 14.1% and 41.3 ± 9.1% in diabetic and control subjects, respectively. In both groups, the rise in NE concentration was as a result of higher rates of total body and forearm NE spillover which were comparable between the diabetic and controls. Unfortunately, no obese subjects were studied, which would have allowed to distinguish the effects of diabetes (hyperglycemia) from those of obesity.

When measured by microneurography, basal skeletal muscle SNSA was found to be elevated more than twofold in obesity [101–103]. In response to euglycemic hyperinsulinemia, SNSA increased significantly [38]. Although the relative rise in SNSA was blunted in the obese subjects, the absolute levels of SNSA achieved during hyperinsulinemia were comparable between lean and obese subjects. These data suggest that SNSA is nearly maximally stimulated in obese insulin-resistant subjects and that added hyperinsulinemia is unable to increase SNSA above levels achieved in lean controls. SNSA appears to be abnormal in the states of metabolic syndrome, the prediabetic state [104, 105], and diabetes [106]. For example, Dell'Oro and colleagues [104] report 30–40% greater MSNA values in middle-aged prediabetic subjects when compared to matched control irrespective of being expressed as burst incidence over time or when corrected for heart rate. In addition, this neurogenic abnormality was associated with a 30–40% reduced spontaneous baroreflex MSNA sensitivity. Furthermore, in a multivariate analysis, MSNA values were directly and significantly related to HOMA index and inversely and significantly to baroreflex–MSNA sensitivity in the prediabetic group (Fig. 2.7).

Only two groups have thus far studied the effect of the metabolic syndrome on PNSA. Unfortunately, the results are somewhat contradictory. Muscelli and associates [107] report an increase in the low-frequency/high-frequency (LF/HF) ratio in

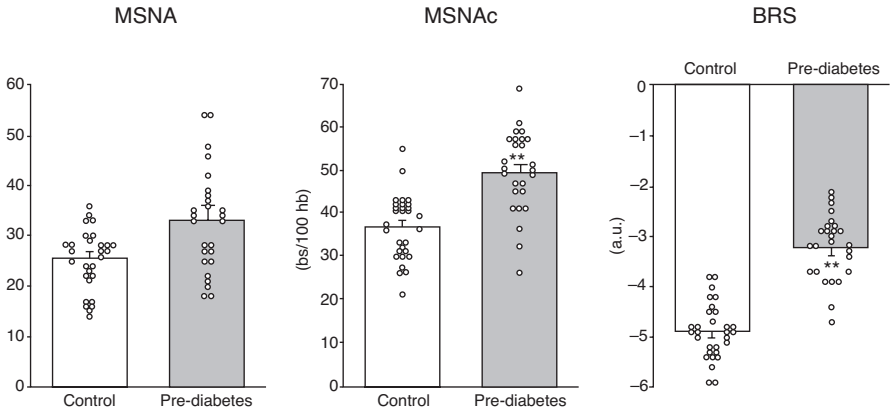

Fig. 2.7 Bar graphs refer to individual and mean (standard errors) values of muscle sympathetic nerve traffic, expressed as bursts incidence over time (MSNA, left panel) and bursts incidence corrected for heart rate values (MSNAc, central panel), and spontaneous baroreflex–MSNA (BRS, right panel) in 30 healthy controls (open bars) and in 26 patients with prediabetes (gray bars). Asterisks ($p < 0.01$) refer to the statistical significance between groups. (From ref. 104)

response to euglycemic hyperinsulinemia in lean normal subjects but not in obese insulin-resistant subjects. The authors conclude that insulin alters cardiac control by enhancing sympathetic outflow and withdrawal of parasympathetic tone. On the other hand, Laitinen and associates [108] demonstrate the opposite, an increase in the LF/HF in obese insulin-resistant subjects but not in the normal controls.

The Metabolic Syndrome and Insulin's Effect on the Kidney

The effect of euglycemic hyperinsulinemia on renal hemodynamics in obesity has not been studied. In one study assessing the effect of euglycemic hyperinsulinemia on renal function in type 2 diabetes, no differences in estimated renal plasma flow were observed. Thus, the scarce data suggest that insulin's effect on renal blood flow is intact in obesity and type 2 diabetes.

Insulin's effect on electrolyte handling has been well studied in type 2 diabetes but data on obesity are not available. The antinatriuretic effect of insulin is well preserved in type 2 diabetes. Gans and associates [98] report a fall in fractional sodium excretion fell by 43 ± 6% and 57 ± 9% in response to euglycemic hyperinsulinemia with insulin levels of 64 ± 12 μU/mL and 1113 ± 218 μU/mL, respectively. Because no control group was available in this study, it is not possible to determine whether the antinatriuretic response was normal or exaggerated in type 2 diabetes. Exaggerated antinatriuresis could lead to volume retention and contribute to the development of hypertension.

The Metabolic Syndrome and Insulin's Effect on Blood Pressure

Insulin's effect on the heart, the SNS, and the kidneys appear to be intact in subjects with the metabolic syndrome. This is in contrast to the impairment of insulin's effect to vasodilate skeletal muscle vasculature, which contributes to the decrease in peripheral vascular resistance during euglycemic hyperinsulinemia. Therefore, because the product of cardiac output and vascular resistance determine blood pressure, one might expect euglycemic hyperinsulinemia to result in blood-pressure elevation. In our study, acute euglycemic hyperinsulinemia did not alter blood pressure in the obese subjects (Fig. 2.3d). Other groups have reported that blood pressure in response to euglycemic hyperinsulinemia increased [38], decreased [109], or remained unchanged [110] in obese and diabetic subjects. Thus, the current data do not support the idea that hyperinsulinemia per se is causally related to the blood-pressure elevation associated with the metabolic syndrome. Since the publication of this chapter in 2005, no dedicated studies to study the effect of insulin on blood pressure have been published, but there were a few studies looking at the effect of a fatty meal and lipid infusion on blood pressure and endothelial function [111]

showing that orally or intravenously administered lipids increased blood pressure and impaired endothelium-medicated vasodilation, determined by FMD in the forearm.

The Metabolic Syndrome and Interactions Between Insulin and Norepinephrine

Although there is a great interest in the effect of the metabolic syndrome on the vascular responses to vasopressors such as NE or angiotensin II, few data are available in humans. We have demonstrated that the pressure response to systemic infusion of NE is augmented in obesity [57]. At similar NE concentrations, the obese subjects exhibited a nearly 50% more pronounced blood pressure rise than the lean controls. Furthermore, insulin's effect to attenuate the pressure response to NE was abolished by obesity. In another study (unpublished data), we found that elevation of FFA enhanced the blood-pressure response to intra-arterial as well as systemic infusion of a selective alpha-one adrenergic agonist while blunting baroreceptor-medicated vasodilation in the leg.

The effect of insulin resistance on the pressure response to angiotensin II was evaluated by Gaboury and associates [112] in normotensive and hypertensive subjects. In normotensive subjects, no relationship between insulin sensitivity and the blood-pressure response to angiotensin II was detected. However, insulin sensitivity correlated inversely with the blood-pressure response to angiotensin II in the hypertensive subjects.

Taken together, these data suggest that vascular responses to pressors may be increased in insulin resistance, which could contribute to the development of hypertension. The data also indicate that the relationship between insulin resistance and pressure responsiveness is not linear and may be modulated by additional factors that are poorly understood.

Interventions to Ameliorate the Effects of the Metabolic Syndrome on the Vascular System

If the increased rate of CVD associated with metabolic syndrome is partially mediated via the effects of insulin resistance on the vascular system, amelioration of insulin resistance should improve the abnormalities of the vascular system, which have been described above. In other words, maneuvers that improve insulin sensitivity should result in lower blood pressure, decreased heart rate, reduced SNSA, and improved endothelial function. Over the last 15 years, additional studies have been conducted to assess the effect of improved insulin sensitivity on insulin-mediated vasodilation and endothelial function.

It has been known for a long time that weight loss improves insulin sensitivity and lowers blood pressure [113]. Weight loss also decreases heart rate and reduces the heightened SNSA [114–116] and improves blunted SNS responsiveness to glucose ingestion [117]. Weight loss has been shown to improve blood flow in adipose tissue in some but not all studies [118, 119].

Troglitazone, a thiazolidinedione derivative, has been described to improve insulin sensitivity [120] and lower blood pressure in obese subjects. Furthermore, troglitazone decreased peripheral vascular resistance in diabetics [121], and pioglitazone decreased blood pressure in diabetic subjects [122]. Rosiglitazone which is also an insulin sensitizer was shown to improve insulin sensitivity, increase blood flow and glucose uptake in subjects with newly diagnosed type 2 diabetes [123]; in the same study, metformin improved neither insulin sensitivity nor blood flow. These data suggest that improvement of insulin sensitivity without changes in body fat content ameliorates cardiovascular abnormalities observed with the metabolic syndrome.

Our own findings [124] using 600 mg of troglitazone per day for 3 months in obese females suffering from polycystic ovary syndrome suggest a beneficial effect of troglitazone on both insulin-mediated vasodilation and the blood-flow responses to the endothelium-dependent vasodilator MCh. In contrast to our study, Tack and coworkers [125] found no effect of troglitazone (400 mg/day for 8 weeks) on insulin-induced blood-flow increments in obese insulin-resistant subjects despite a 20% improvement in insulin sensitivity. While the above studies represented longer-term interventions, an acute infusion of autonomous nervous system blockade with trimethaphan [126] improved insulin action in insulin-resistant but not in insulin-sensitive subjects. Thus, given the sparse and somewhat contradictory literature about the effect of increased insulin sensitivity on insulin-mediated increments in blood flow and endothelial function, further studies are required. Nevertheless, reduction of insulin resistance leading to improved endothelial and vascular system function may result in decreased cardiovascular morbidity and mortality in obese, hypertensive, and diabetic subjects.

Conclusion

Over the last 25 years, it has been established that insulin is a vascular hormone. Insulin's vascular actions extend beyond its effect to increase skeletal muscle blood flow and glucose uptake. Current data suggest that insulin modulates vascular tone and vascular smooth muscle cell proliferation and migration via the release of NO and other yet unidentified mechanisms (Fig. 2.8). Thus, insulin's effects on the vascular system may be important to prevent or delay the progression of CVD. The metabolic syndrome affects the vascular system at multiple levels. Resistance to the vascular actions of insulin may explain, at least in part, the abnormalities associated with the metabolic syndrome. The altered state of the vascular system in metabolic syndrome may contribute to higher rates of hypertension and macrovascular disease. States of insulin resistance that occur naturally or due to an intervention are

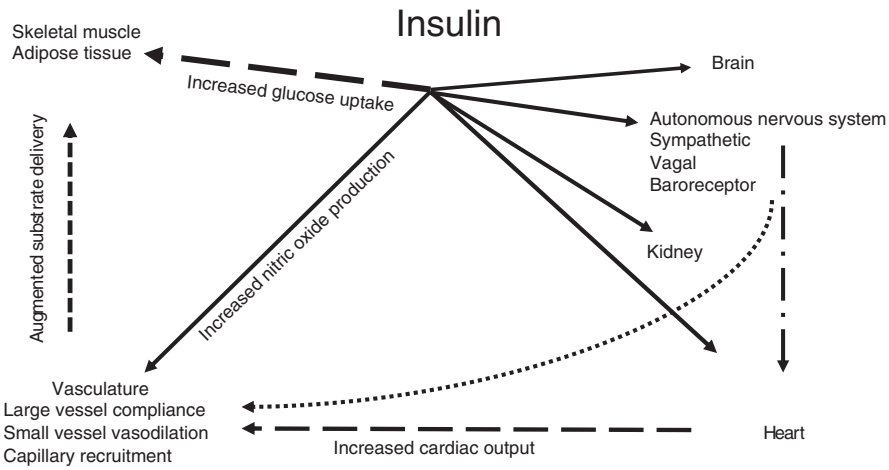

Fig. 2.8 Schema of classic and non-classic insulin action on different targets to enhance insulin delivery and glucose uptake

almost always associated with impaired endothelium-dependent vasodilation. However, the opposite that impaired endothelial function induces insulin resistance, is not the case [127]. Future research assessing the interaction between insulin's effect on the vasculature and newly discovered adipocytokines and other vasoactive hormones will better define the pathophysiological abnormalities underlying insulin-resistant states and help design therapies to improve endothelial function and reverse the accelerated atherosclerotic process.

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Chapter 3
Effects of Diabetes and Insulin Resistance on Endothelial Functions

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Introduction

Cardiovascular (CV) complications are the primary cause of mortality and morbidity in patients with Type 1 (T1D) and Type 2 diabetes (T2D), which affect a variety of tissues and organs including the retina, myocardium, nerves, skin, and kidney [1–4]. Complications including coronary artery disease (CAD), hyperglycemic crises, stroke, amputations, and end-stage renal disease (ESRD) have all declined substantially in the past two decades in the United States, with the improvement of diabetes care, risk factor control, and medical treatment. Recently, in the National Health and Nutrition Examination Survey (NHANES), CV mortality was not found to be significantly different in diabetic and nondiabetic patients from 2005 to 2010, whereas there was a significantly higher rate of CV death in the former group during previous years [5]. However, despite this recent remarkable decline in CV morbidity and mortality rates, the prevalence of diabetes continues to increase over the years, remaining a large public health burden, and thus studies on CV complications remain necessary as ever [6, 7]. The risk of CAD increases with diabetes duration, reflecting an effect of the aging process, and more than half of overall mortality in diabetic patients is related to cardiovascular disease (CVD). Additionally, the incidence of CVD is two to four times higher in diabetic patients than in the general population [8, 9]. In T2D patients, CV complications develop, on average, 14.6 years earlier than in individuals without diabetes, with more severe clinical outcomes [10, 11]. Many studies have shown that this is particularly true in women [2, 12]. Furthermore, several studies have shown that insulin-treated T2D patients have a higher CAD risk than those who were not on insulin, suggesting that disease

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severity, loss of islet cell function, or exogenous insulin treatment may all have an impact on CAD. On the other hand, for patients with T1D who were followed for 20–40 years, CAD mortality between the ages of 30 and 55 years was 33%, compared to only 8% of men and 4% of women in the nondiabetic population [13]. Similarly, unlike the general population, the CAD risks in T1D patients are similar in men and women and increase at the same rate after the age of 30.

The influence of diabetes on CVD is synergistic, with many contributory factors such as age, hyperglycemia, hyperlipidemia, hypertension, nephropathy (DN), obesity or sedentary lifestyle, altered coagulation, hyperinsulinemia and insulin resistance, and smoking. Additionally, diabetes itself is also an independent risk factor [2, 14–16]. Although the increase in CV mortality probably has several causes, one of the most important and most widely studied is hyperglycemia. Glycemic control has been applied for many years in diabetes care and has contributed to the reduction of diabetes complications. This is well supported by both the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS). The DCCT has clearly established that intensive glycemic therapy in T1D patients reduced both macrovascular [17, 18] and microvascular complications such as retinopathy (DR), DN, and neuropathy [19, 20]. In the UKPDS [4, 21], intensive glucose control in T2D was associated with a 12% reduction in the risk of pooled macrovascular and microvascular events. However, in other T2D studies including ACCORD (Action to Control Cardiovascular Risk in Diabetes) [22], ADVANCE (Action in Diabetes and Vascular Disease), VADT (Veterans Administration Diabetes Trial), BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial), and ORIGIN (Outcome Reduction with an Initial Glargine Intervention Trial) [23], intensive glucose control did not reduce macrovascular complications in older patients with long-standing T2D and either CVD or risk factors for CVD, while the progression of microvascular complications such as DN and DR were significantly ameliorated by glycemic control. These results suggest that while glycemic control can increase the survival of diabetic patients, longitudinal studies, especially in T2D, have not strongly supported its role in decreasing CVD events [22, 24]. These studies suggest other mechanisms and risk factors, aside than hyperglycemia, that could be associated with the acceleration of CV pathologies in people with diabetes, including insulin resistance, dyslipidemia, inflammation, reactive oxygen species, endothelial dysfunction, hypercoagulability, and vascular calcification.

The second major CV risk factor for patients with diabetes or glucose intolerance is hyperinsulinemia, which is related to insulin resistance in many tissues including the vascular tissues [25]. There has been a great deal of discussion about the effects of hyperinsulinemia and insulin resistance on the development of CVD [26, 27]. However, the recent ORIGIN and DEVOTE (A Trial Comparing Cardiovascular Safety of Insulin Degludec Versus Insulin Glargine in Patients With Type 2 Diabetes at High Risk of Cardiovascular Events) trials did not report any increases in CVD risk and mortality with exogenous hyperinsulinemia [23, 28]. These studies suggest that endogenous, rather than exogenous, hyperinsulinemia may be related to increased CVD risk in diabetes [29]. In addition, other cohorts have reported that

Table 3.1 Alterations of cell numbers observed in various vascular tissues in diabetes

	Retina	Glomeruli	Macrovessels	Myocardium
Endothelial cells	↑	↓	↓	↓
Contractile cells	↓	↓	↑	↓
Epithelial cells		↓		

proinsulin and C-peptide may contribute to CVD risk, which are mechanisms difficult to separate from insulin resistance [30–33]. A substantial body of evidence suggests that the relationship between insulin resistance and CVD may be associated not only with insulin sensitivity but also with hypertension and endothelial function [27, 34–37]. In this chapter, we will first review the role of insulin resistance, hyperglycemia, and hyperlipidemia in the vasculature, and then describe cellular and functional abnormalities in endothelial cells in the presence of insulin resistance and diabetes.

In summary, all complications of diabetes are the final results of systemic dysmetabolites such as hyperglycemia, genetic/epigenetic factors, and local/tissue responses to systemic changes. Most studies have focused on the effects of systemic factors and genetic contributions. However, specific tissue responses or local factors to systemic changes, such as dyslipidemia, pathologic oxidation and glycation, and inflammation are also contributors to CVD in diabetic patients [19, 38]. The importance of tissue-specific responses is clearly demonstrated by differences in vascular cell changes in the retina, renal glomeruli, and arteries with diabetes (Table 3.1). In the retina, the number of endothelial cells appears to be increased, as exemplified by the formation of microaneurysms and neovascularization [39]. In contrast, endothelial cells in macrovessels are injured, as shown by pathological studies leading to the initiation and acceleration of the atherosclerotic process [40, 41]. Furthermore, poor collateral circulation in areas of ischemia in the myocardium and lower extremities may be responsible for the high prevalence of myocardial ischemia and loss of lower limbs in diabetes. Finally, the tissue-discordant response to diabetes is also exemplified by the finding of hypertrophy or proliferation of arterial smooth muscle cells (contractile cells), as noted in the high rates of restenosis and the dramatic increases of pericyte apoptosis in the capillaries—a dramatic hallmark of DR.

Insulin Resistance

Hyperinsulinemia and insulin resistance have been shown to increase atherosclerosis and CVD risk in diabetes. Likewise, they are important risk factors in the development of hypertension, not only in diabetes but also in the general population. The mechanism by which hyperinsulinemia or insulin resistance increases atherosclerosis risk is still unclear. It has been suggested that insulin has direct effects on arterial walls and accelerates the progression of atherosclerosis [42–45]. However, In apolipoprotein E knockout ($ApoE^{-/-}$) mice, exogenously induced hyperinsulinemia

with implanted insulin pellets, which mimicked exogenous insulin treatment in diabetic patients, decreased atherosclerosis not only by improving lipid profile through its actions on the liver, but also by significantly lowering inflammatory cytokines, inhibiting vascular cell adhesion protein 1 (VCAM1), activating endothelial NO synthase (eNOS) in endothelial cells, and decreasing monocyte recruitment in the arterial wall [44]. However, atherosclerosis was not altered in single allele insulin receptor ablation ($IR^{+/-}$) $ApoE^{-/-}$ mice with endogenously induced hyperinsulinemia in the absence of systemic insulin resistance. This model exhibited increased plasma insulin levels but comparable insulin sensitivity and atherosclerosis o control $ApoE^{-/-}$ even after 52 weeks of hyperinsulinemia [43]. Furthermore, as mentioned, the ORIGIN and DEVOTE trials [6, 10] have both clinically shown that exogenous hyperinsulinemia did not increase the risk of CVD. Thus, these studies suggest that the acceleration of atherosclerosis is related to insulin's direct action on vascular cells in insulin resistance and diabetic conditions.

Structure and Signaling of Insulin Receptors (IR) on the Vascular System

We have characterized insulin receptors (IR) on the vascular cells and reported that they are identical to those in the nonvascular cells with respect to binding, structure, and tyrosine phosphorylation activity [46]. IR is a member of the tyrosine kinase family, which consists of an α chain that binds insulin and a β chain containing tyrosine kinase, and the activation of the receptor by insulin binding results in autophosphorylation of the receptor and activation of tyrosine kinase (Fig. 3.1). As in other cells, IR in vascular cells can activate two main signal transduction pathways: the IR substrate (IRS) 1/2 and phosphoinositide (PI)-3-kinase (PI3K)/Akt cascade (IRS/PI3K/Akt); and the Src/mitogen-activated protein (MAP) kinase cascade. These signaling processes mediate many actions of insulin in vascular cells, including regulation of metabolism and endothelial cell function. For instance, even though insulin does not affect glucose uptake (as endothelial cells predominantly express GLUT1 as glucose transporter), it can still affect glucose and fatty acid metabolism through mitochondrial fluxes, and is indirectly involved in many cellular functions such as cell growth, gene expression, protein synthesis, and glycogen incorporation [47, 48]. However, IR can mediate unusual and different functions in endothelial cells. We have demonstrated that endothelial cells can internalize insulin via a receptor-mediated process and thereby transport insulin without degradation [49, 50]. In contrast, hepatocytes, adipocytes, and vascular smooth muscle cells (VSMC) will heavily degrade insulin when internalized [49–51]. This lack of internalized insulin degradation in endothelial cells is due to receptor-mediated transcytosis such as the caveolin cycling process, which transports insulin from the apical to the luminal surfaces of endothelial cells [49, 52–56]. This mechanism may be important for tissues with tight endothelial

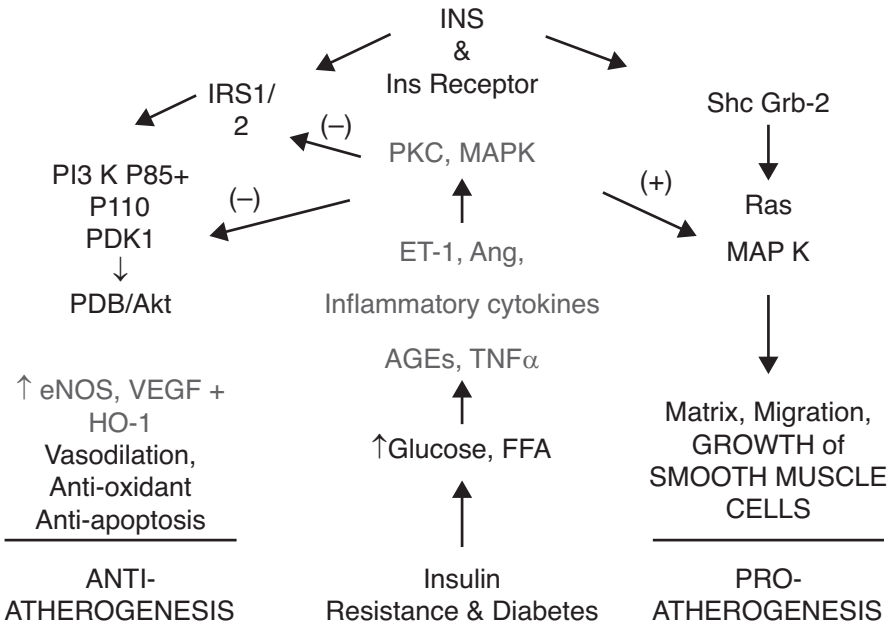

Fig. 3.1 Schematic diagram of the signaling pathways of insulin in vascular endothelial cells. Activation of either PI3K/Akt or Ras/MAP kinase pathways can mediate most actions of insulin, with the former stimulating mainly anti-atherogenic effects, and the latter stimulating atherogenic actions. In diabetic or insulin-resistant states, metabolic derangements or activation of PKC have been suggested to selectively inhibit insulin receptor-mediated activation of PI3K/Akt pathway, but spares the Ras/MEK/MAP pro-atherogenic arm of insulin's signaling cascade. This may in turn contribute to atherogenic lesion formation. *IRS* insulin receptor substrate, *PI3K* phosphatidylinositol 3-kinase, *MAPK* mitogen-activated protein kinase, *AGE* advanced glycation end-products, *FFA* free fatty acid, *ET-1* endothelin-1, *Ang* Angiotensin

barriers and limited paracellular capillary permeability, such as the retina and brain [57]. In addition, when endothelial cells are exposed to high levels of insulin, IR on the cell surface will be decreased and will further contribute to systemic insulin resistance by indirectly affecting blood flow or permeability or cytokine levels [48, 54, 57].

Another vascular-specific action of insulin is the activation or increased expression of nitric oxide (NO), resulting in localized vasodilation [58–61]. Mice null for the insulin receptor specifically in endothelial cells (VENIRKO mice) were recently established [62]. Although less than 5% of IR mRNA expression was left in endothelial cells, these mice developed normally and did not show major differences in vasculature when compared to their control littermates, save for a mild reduction of gene expression for eNOS and endothelin-1 (ET-1) in endothelial cells [62]. However, when challenged with hypoxia, VENIRKO mice developed more than 50% reduction in retinal neovascularization [63]. These results suggest that the alteration of insulin signaling may affect the expression of vascular regulators in

endothelial cells and may further affect vascular biology such as neovascularization. In addition, eNOS knockout mice have insulin resistance but they do not exhibit diabetes [64, 65].

Physiologic Actions of Insulin on the Vascular System

Insulin has been reported possess many physiological actions on vascular cells. It is believed that hyperinsulinemia or insulin resistance can contribute to the acceleration of atherosclerosis by increasing the proliferation of aortic smooth muscle cells and the synthesis of extracellular matrix (ECM) proteins in the arterial wall (Fig. 3.2). Previously, the mitogenic actions of insulin on cells have not been viewed as being significant in physiological conditions, because insulin can only stimulate the growth of vascular cells at concentrations greater than 10 nmol/L. However, it is likely that in severe insulin-resistant or hyperinsulinemic states, insulin can exert its growth-promoting actions in smooth muscle cells (SMCs) by enhancing the mitogenic action of homozygous insulin receptors, possibly additively with other potent growth factors such as platelet-derived growth factor and insulin-like growth factors [66].

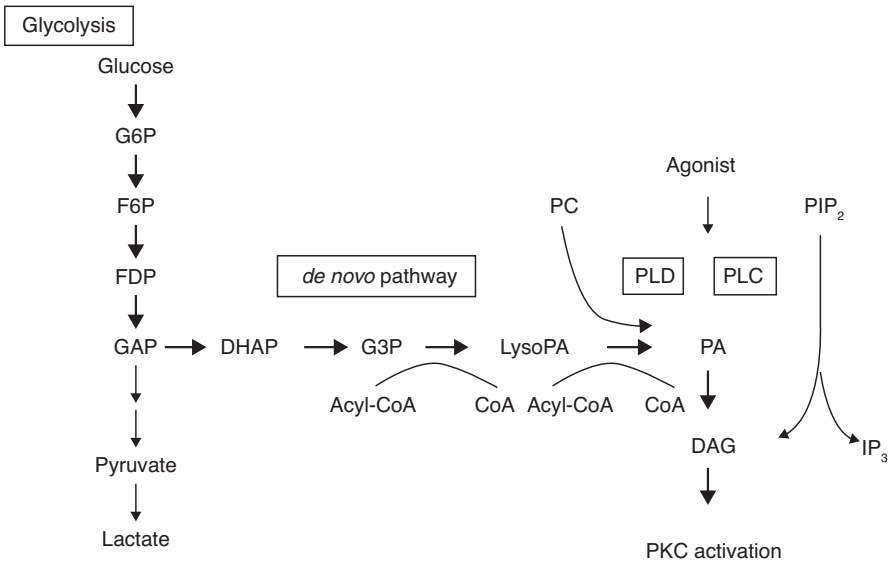

Fig. 3.2 Mechanism of DAG synthesis and PKC activation in diabetes mellitus. Hyperglycemia activates the de novo synthesis of DAG and leads to PKC activation. *Acyl-CoA*, acetyl-coenzyme A, *CoA* coenzyme A, *DAG* diacylglycerol, *DHAP* dihydroxyacetone phosphate, *FDP* fructose 1,6-diphosphate, *F6P* fructose 6-phosphate, *GAP* glyceraldehyde 3-phosphate, *G3P* glycerol 3-phosphate, *G6P* glucose 6-phosphate, *IP3* inositol 1,4,5-triphosphate, *LysoPA* lysophosphatidic acid, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PIP2* phosphatidylinositol 4,5-bisphosphate, *PKC* protein kinase C, *PLC* phospholipase C, *PLD* phospholipase D

Moreover, recent studies from our laboratory have shown that the enhancement of intimal hyperplasia in insulin resistance or diabetes is due to insulin signaling by homodimers of IR. Mice with deletion of IR in VSMCs (SMIRKO) exhibited decreased insulin-stimulated VSMC proliferation and reduced wire injury-induced intimal hyperplasia in a rodent model of restenosis. In contrast, deletion of IGF1R (SMIGF1RKO) with increased homodimers of IR enhanced insulin-stimulated VSMC proliferation and exacerbated injury-induced intimal hyperplasia by enhancing insulin signaling and upregulation of hyaluronan synthase2 (Has2), which has important biological effects on VSMC migration and proliferation [67].

Physiologically, insulin can be transported and can regulate peripheral organs with continuous vascular connections, such as the central nervous system, adipose tissue, and skeletal muscle but not in organs with fenestrated capillaries, such as the liver and renal glomeruli [49, 68]. One of the best-characterized vascular effects of insulin is its vasodilatory action, which is mainly mediated by the production of NO in endothelial cells [59]. Baron [58] reported that blood flow to the leg increased by twofold after 4 h of hyperinsulinemia during a euglycemic–hyperinsulinemic clamp. Increased skeletal muscle blood flow was observed in a time-, dose-, and NO-dependent manner, and was affected earlier than insulin's action on its downstream pathways [69–72]. With superimposed infusion of NG-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, into the femoral artery, the vasodilation was completely abrogated. It has also been reported that insulin-mediated vasodilation is impaired in states of insulin resistance [73], and that insulin treatment can improve forearm blood flow in diabetic patients [74]. Additionally, activation of the renin–angiotensin–aldosterone system (RAAS) or inflammatory cytokines such as tumor necrosis factor (TNF)-α can inhibit insulin-induced NO production and vasodilation and result in endothelial dysfunction, insulin resistance, hypertension, and CVD in diabetes [70, 71, 75–78]. Consistent with this observation, obese nondiabetic subjects often have impaired endothelium-dependent vasodilation, especially relative to T2D patients [60]. These findings suggest that endothelial cell dysfunction may have a genetic basis and may be involved in atherosclerosis risk in subjects with insulin resistance, regardless of whether they have diabetes or not [60].

The effect of insulin on NO production in endothelial cells may be biphasic, with rapid and delayed components. Relative to other stimulants of NO production, insulin is rather weak, with 10–100 times less maximum effect than acetylcholine. However, it is possible that the delayed positive effect of insulin on eNOS expression has an important consequence in sustaining the level of eNOS expression, which will have a general effect on all stimulators of NO production. The mechanism of insulin's effect on NO production appears to be mediated by the activation of PI3K pathway [61]. The acute effect appears to be an eNOS activation, whereas the delayed effects are a result of the upregulation of eNOS gene expression.

Thus, in the vascular tissues, insulin has a variety of effects, which can be mediated by at least two signaling pathways involving PI3K and Ras–MAP kinase. At physiological concentrations, insulin mediates its effects through the activation of the PI3K pathway, causing actions such as NO production. This effect can be

interpreted as anti-atherogenic. In contrast, the effects mediated through the Ras–MAP kinase pathway include stimulation of extracellular matrix production; induction of plasminogen activator inhibitor-1 (PAI-1) and ET-1 expression; and cell proliferation and migration, which all appear to be pro-atherogenic. The latter pathway requires the presence of relatively high concentrations of insulin that may be observed in insulin-resistant states. We have proposed that the increased risk of atherosclerosis in insulin-resistant states is caused by the loss of insulin action on PI3K/Akt pathway activation and the subsequent production of NO, whereas activation of the Ras–MAP kinase pathway remains intact. In support of this theory, we have documented that the activation of PI3K/Akt and eNOS expression by insulin are significantly reduced in microvessels from insulin-resistant Zucker obese rats, as compared to that of healthy lean control rats, whereas the activation of the Ras–MAP kinase pathway was not affected or was even increased [61, 79–81]. These results have provided a molecular explanation for the clinical findings that both insulin deficiency (as in T1D) and insulin-resistant states (as in patients with metabolic syndrome and T2D) can lead to acceleration of CVD through insulin actions on blood flow, angiogenesis, thrombosis, inflammation, and cytokine and oxidant production [48, 82] (Fig. 3.1).

Selective Insulin Resistance

Insulin was discovered in 1921 and has been reported ever since to exert actions on almost every type of cell and tissue, and has been proven to have both direct and indirect actions on vascular cells, including endothelial cells, VSMC and capillary pericytes. Our lab initially reported in 1999 that selective insulin resistance occurred in the aorta of obese Zucker rats [79], with a decrease in the anti-atherogenic PI3K/Akt pathway, but no change in the pro-atherogenic Ras–MAP kinase pathway [48]. This selective insulin resistance has also been observed in other tissues, such as the myocardium, liver, renal glomeruli, wound fibroblasts, gingivae, and angioblasts. Since then, as mentioned, we and others have reported that insulin actions on endothelial cells are mainly regulated by these two signaling pathways. The PI3K/Akt pathway leads to increased expression of eNOS, endothelin-B receptor (ETBR)-mediated NO production [48, 61], vascular endothelial growth factor (VEGF) [83] and heme oxygenase (HO)-1 [84]; at the same time it decreases the expression of VCAM1 [85, 86]. As also mentioned, this pathway is considered anti-atherogenic with increased NO production and vasodilation, enhanced angiogenesis by VEGF, and decreased inflammation and oxidation via HO-1 and VCAM1 [42, 45, 79, 84, 87] (Fig. 3.1). In addition, insulin has also been reported to suppress the expression of transcription factor FoxO and the pro-apoptotic molecule caspase-9, further protecting against atherosclerosis [88, 89]. On the other hand, insulin actions on the pro-atherogenic Ras/MAP kinase pathway elevate vasoconstriction through ET-1, inflammation through PAI-1, and induce the proliferation and migration of SMC [85–87]. In insulin-resistant states, the former pathway is selectively lost and the

latter pathway enhanced in endothelial cells, resulting in the loss of insulin's action anti-oxidative and anti-inflammatory actions and further causing endothelial dysfunction. This selective suppression of insulin action is termed as selective insulin resistance.

The importance of endothelial insulin action on atherosclerosis was demonstrated in VENIRKO mice. Loss of insulin signaling in endothelial cells in these mice increased aortic atherosclerosis development and progression by more than twofold compared to apoE$^{-/-}$ mice, without altering systemic metabolic parameters. Using a genetically engineered PI3K/Akt pathway in apoE$^{-/-}$ mice, multiple groups have confirmed that the mechanism of insulin in inhibiting atherosclerosis is indeed mediated by the PI3K/Akt pathway [42, 90–92]. On the contrary, the severity and development of atherosclerosis subjected to selective insulin resistance in endothelial cells was significantly reduced by overexpressing IRS1 specifically targeted to the endothelial cells in apoE$^{-/-}$ mice (IRS1/ApoE$^{-/-}$), with enhanced NO production through increasing ETBR expression and eNOS activity [45], and deletion of ETRB in the endothelial cells of IRS1/Ldlr$^{-/-}$ mice decreased NO production and accelerated atherosclerosis [45]. In addition, overexpression of IRS1 in endothelial cells can also improve angiogenesis and wound healing in diabetic mice [93], with increased NO production and VEGF expression. These results all strongly suggest that insulin actions, especially via the PI3K/Akt pathway, are important for maintaining endothelial function.

Recently, we also reported the mechanism of insulin action in VSMCs on restenosis. Intimal hyperplasia is attenuated in SMIRKO mice, but SMIGF1RKO mice exhibited enhanced proliferation and exacerbated hyperplasia. This selective and enhanced action of insulin on the proliferation of VSMC with IGF1R deletion was related to the increased binding of insulin to the homodimers of IR (homo-IR). By using chimeras of IR and IGF1R, we found that the α-binding subunit of homo-IR contributed mainly to cellular actions during restenosis, especially through the mitogenic effects of insulin on VSMC to increase Has2 expression, which appeared to be mediated by both the PI3K/Akt and Ras/MAP kinase pathways [67].

Hyperglycemia

Hyperglycemia has been shown to be the main cause of microvascular complications in the DCCT [17–19] and UKPDS studies [21]. For CV complications, the contribution of hyperglycemia is probably also significant, but this is likely to be in combination with other risk factors, such as dyslipidemia or insulin resistance. Several biochemical mechanisms appear to explain the adverse effects of hyperglycemia on vascular cells (Table 3.2). This is not surprising because the metabolism of glucose and its metabolites can affect multiple cellular pathways. Glucose is transported into the vascular cells mostly by GLUT1 transporters, which can be regulated by extracellular glucose concentration and other physiological stimulators, such as hypoxia [94]. Once glucose is transported, it is metabolized to alter

Table 3.2 Proposed mechanisms of the adverse effects of hyperglycemia

Activation of the polyol pathway
Increases in nonenzymatic glycation products
Activation of DAG–PKC stress kinases cascade
Increases in oxidative glycated stress
Enhanced flux via hexosamine metabolism
Vascular inflammation
Altered expression and actions of growth factors and cytokines

signal transduction pathways such as the activation of diacylglycerol (DAG) and protein kinase C (PKC), or to increase mitochondrial flux thus changing the redox potential [95–98]. Lastly, another normally inactive metabolic pathway (such as that of aldose reductase) can be used. The elevation of intracellular glucose and its metabolites can also interact with amines to form adducts and glycated products, reducing protein functions unless neutralized by glyoxalases. In this review, we describe these theories and suggest that the common pathways for most of the adverse effects of hyperglycemia are mediated by alterations in the signal transduction of such substances as DAG–PKC or other kinases and phosphatases.

Advanced Glycation End-Products

Extended exposure of proteins to hyperglycemia can result in nonenzymatic reactions, in which the condensation of glucose with primary amines forms Schiff bases. These products can rearrange to form Amadori products and advanced glycation end-products (AGE). The glycation process occurs both intracellularly and extracellularly. It has been reported that glycation modification targets intracellular signaling molecules and extracellular structure proteins alike, and furthermore, alters cellular functions. Multiple forms of proteins subjected to glycation have been identified with Nε-(carboxymethyl)lysine (CML), pentosidine, and pyralline being the major forms of AGEs in diabetes.

A significant role for AGE in diabetic vascular complications is supported by their increased serum concentration in patients with diabetes [99, 100]. As AGEs are not easily metabolized, they accumulate even after a long period of strict glucose control, and can still increase CV risk in diabetic patients—a phenomenon called metabolic memory. Intracellular enzymes such as glyoxalases can reduce AGE levels. Recent studies in renal glomeruli of people with diabetes but protected from DN have shown high levels of glyoxalase [101, 102]. Furthermore, while infusion of AGE into animals without diabetes reproduces some pathological vascular abnormalities similar to that in diabetes, inhibition of AGE formation in diabetic animals can partly prevent these same pathological changes [103]. Treatment of diabetic rats with aminoguanidine, an inhibitor of AGE formation and inducible NOS, can prevent the progression of both DN [103] and DR [104], as evidenced by the reduction

of albuminuria, mesangial expansion, endothelial cell proliferation, pericyte loss, and even the formation of microaneurysms. Other inhibitors of protein glycation, such as OPB-9195 [105] or ALT-711 [106] have yielded similar results in animals with diabetes.

Recently, receptor for advanced glycation end-products (RAGE) has received substantial attention on its role in endothelial cell dysfunction in diabetes, especially in the development of atherosclerosis [107]. RAGE belongs to the immunoglobulin superfamily and has been reported to be expressed in vascular cells including endothelial cells and SMCs [108], and RAGE accumulation in the vasculature has been reported in diabetic states [100, 103]. Infusion of RAGE is associated with vascular hyperpermeability similar to that in diabetes, and these changes can be neutralized in the presence of soluble RAGE (sRAGE) [103], the extracellular domain of RAGE that disrupts AGE–RAGE interaction. Additionally, when mice deficient for apolipoprotein (apo)E (apoE$^{-/-}$) were induced to develop T1D by streptozotocin injection, they developed much more advanced atherosclerotic lesions in their aortae as compared to apoE$^{-/-}$ mice without diabetes [100], and the progression of atherosclerotic lesions can be reversed by intraperitoneal injection of sRAGE [100]. Although the molecular and cellular mechanisms underlying RAGE-induced vascular permeability change are still not fully understood, it is postulated that the induction of extracellular protein glycation [109], vascular oxidative stress [110], activation of PKC and other intracellular signaling events [111], and inflammation [112, 113] are contributors.

These results provide supportive evidence suggesting an important role for AGE formation and RAGE activation in the development of diabetic vascular complications. The AGE–RAGE axis could therefore be a potential target for clinical interventions. Indeed, aminoguanidine has been evaluated in a clinical trial for its effect on the progression of DN in 599 T2D patients across the United States and Canada, which showed significant side effects [114]. Aside from this, however, the majority of our current understanding still relies on animal studies and an affirmative role for AGE in the pathogenesis of diabetic vascular complications requires further clinical evaluation.

Activation of the Polyol Pathway

Increased activity of the polyol pathway has been documented in culture studies using vascular cells exposed to levels of D-glucose in humans and animals with diabetes [115, 116]. In these studies, hyperglycemia has been shown to increase the activity of aldose reductase (AR) and enhance the reduction of glucose to sorbitol, which is then further oxidized to fructose by sorbitol dehydrogenase (SORD). Abnormalities in the polyol pathway have been suggested to cause vascular damage in the following ways: (a) osmotic damage by the accumulation of sorbitol [115]; (b) induction of oxidative stress by increasing nicotinamide adenine dinucleotide phosphate (NADP)/NAD$^+$ ratio and the activation of Na^+/K^+ adenosine triphosphate

(ATP)ase [116]; and (c) reduction of NO in the vasculature by decreasing cellular NADPH, a cofactor used by AR to reduce glucose to sorbitol [117]. Multiple rodent studies have shown that inhibition of AR, the key enzyme in the polyol pathway, could prevent some pathological abnormalities in DR, DN, and neuropathy [116]. Overexpression of AR with activation of the polyol pathway in diabetic apoE-/- mice was also shown to accelerate atherosclerosis, while AR inhibitors may potentially reduce atherosclerosis [118]. However, these results were not supported by data from clinical trials on AR inhibitors. A 3-year follow-up of diabetic patients treated with an AR inhibitor, Sorbinil (250 mg/day), failed to detect differences in DR [119], although another AR inhibitor, Zenarestat, may have improved nerve conduction in diabetic peripheral polyneuropathy in the early phase [120]. Based on the largely negative clinical data, a significant role for the activation of the polyol pathway in the pathogenesis of diabetic microvascular complications is not promising. Furthermore, we recently reported that while activation of the polyol pathway in the glomeruli was associated with DN protection, the DN-protected patients showed the elevation of glycolytic enzymes instead [101]. Thus, these results suggest that AR inhibitors may not be a good target for diabetic microvascular complications.

Alteration in Oxidative Stress

Increased oxidative stress from metabolic derangements has been reported in diabetes and has been proposed to cause vascular complications [98, 116, 121, 122]. In diabetes, oxidative stress may result from increased production of superoxide via the induction of NADPH oxidase and the mitochondrial pathway; decreased superoxide clearance; lipid and protein modification; and the reduction of endogenous antioxidants such as ascorbic acid, vitamin E, and glutathione [123, 124]. In addition, increased oxidant levels can react with NO to yield various oxidized nitric derivatives, especially in the vascular tissues [125, 126].

Several lines of evidence support the role of increased oxidative stress in the pathogenesis of diabetic vascular complications. Reactive oxygen species, an index of oxidative stress, have been reported to be increased in patients with DR [127] and CVD in the Framingham Heart Study [128] and correlated with the severity of these complications. Furthermore, these results have been replicated in diabetic animals or even in vascular cells cultured in media with high levels of D-glucose [116, 122].

Induction of oxidative stress has been suggested to cause vascular dysfunction via multiple mechanisms including cellular DNA damage, which activates the poly (ADP-ribose) polymerase [116, 129, 130]; reduction of NO bioavailability [116]; and activation of other mechanisms known to induce vascular cell damage, such as AGE formation, PKC activation, and induction of the polyol pathway [131]. AGE, in turn, may break down NO in redox processes or further induce oxidative stress through receptor-mediated activation of nuclear factor κB [132]. In mice overexpressing catalase in macrophages (mCAT), which suppressed mitochondrial

oxidative stress, atherosclerosis was significantly reduced with less macrophages in the plaques [133]. Additionally, evidence has shown that reactive oxygen species can cause severe disturbances in the regulation of coronary blood flow and cellular homeostasis, leading to the severe macrovascular lesions typically observed in diabetic patients after more than 10 years of disease [131, 134]. Conversely, inhibition of reactive oxygen species can prevent the generation of AGE and the activation of PKC in cultured endothelial cells [130]. The partial reduction of ROS production in the aortic wall of both animals and humans treated with PKC inhibitors further suggest that the auto-oxidative process plays an important role in the complex reaction cascade leading to AGE formation [135–137].

Several pathways in diabetes, such as PKC activation (especially the β2 isoform) [134, 138], AGE formation [110], lipid oxidation [122, 128], and altered polyol activity [116] can lead to the activation of NADPH oxidase or flux through the mitochondrial respiratory chain [131, 139], thus generating reactive oxygen species that further increase tissue oxidative stress. On the other hand, oxidative stress can precede formation of some AGE, such as pentosidine and CML, as well as activation of the DAG–PKC pathway [140].

Although multiple studies on either cultured or animal vascular cells have all supported the role of oxidative stress in vascular complications of diabetes, clinical trials on antioxidants—in particular antioxidant vitamins—have not shown a beneficial effect on microvascular or CV events. These may be explained by insufficient concentrations of antioxidants in the relevant intracellular compartments [141]. The Heart Outcomes Prevention Evaluation Study (HOPE) has shown that treatment with vitamin E (400 IU/day; mean: 4.5 years) had no apparent effect on CV outcomes in patients with CVD or diabetes plus one other risk factor [142]. Similarly, the MICRO-HOPE study also revealed that the same dose of vitamin E failed to show differences in CV outcomes and DN [143]. However, we have reported that oral vitamin E treatment at a dose as high as 1800 IU/day appeared to be effective in normalizing retinal hemodynamic abnormalities and improving renal function in short-duration T1D patients, without inducing significant changes in glycemic control [144]. In all, these largely inconclusive clinical results suggest that oxidative stress probably plays a supportive, rather than central, role in the pathogenesis of diabetic vascular complications.

Activation of the DAG–PKC Pathway

A major advancement in the understanding of diabetic vascular disease is the unraveling of changes in signal transduction pathways. One of the best-characterized is the activation of the DAG–PKC pathway, which appears to be related to the elevation of DAG, a physiological activator of PKC. Increases in total DAG have been demonstrated in tissues associated with diabetic vascular complications, including the retina [145], capillaries, aorta, heart [146], renal glomeruli [147], chronic wounds, gingiva, and liver [94, 95, 145, 146, 148–153], but not in the brain or peripheral

Table 3.3 Summary of DAG levels and PKC activity in cultured cells exposed to high glucose conditions and tissue isolated from diabetic animals

	Diacylglycerol	Protein kinase C
Cultured cells		
Retinal endothelial cells	↑	↑
Retinal pericytes	↑	↑
Aortic endothelial cells	↑	↑
Aortic smooth muscle cells	↑	↑
Renal mesangial cells/podocytes	↑	↑
Tissues		
Retina	↑	↑
Heart	↑	↑
Aorta	↑	↑
Renal glomeruli	↑	↑
Liver/skeletal muscle	↑	↑
Monocytes	ND	↑
Granulation tissue	↑	ND
Brain	–	–
Peripheral nerve	–, ↓	↑, ↓, –

ND = not determined in references cited. ↑ = increased, ↓ = decreased, – = unchanged

nerves (Table 3.3). Increasing glucose levels from 5 to 22 mol/L in the media elevated cellular DAG levels in aortic endothelial cells and SMCs [146], retinal endothelial cells [145], renal mesangial cells [154, 155], and circulating monocytes [156]. The increase in DAG–PKC reaches maximum levels in 3–5 days after the elevation of glucose levels and remains chronically elevated for many years. In fact, we have already shown that euglycemic control via islet cell transplant after 3 weeks was not able to reverse the increases in DAG or PKC levels in the aorta of diabetic rats [146]. This suggests that the activation of DAG–PKC could be sustained chronically but is difficult to reverse, similar to pathways of diabetic complications.

DAG can be generated from multiple pathways. Agonist-induced formation of DAG depends mainly on hydrolysis of phosphatidylinositol by phospholipase C [157]. However, this mechanism is most likely minimally involved in diabetes, because inositol phosphate products were not found to be increased by hyperglycemia in aortic cells and glomerular mesangial cells [158, 159]. When fatty acids in DAG were analyzed [160], DAG induced by high glucose conditions had a predominantly palmitate- and oleic acid-enriched composition, whereas DAG generated from hydrolysis of phosphatidylinositol had the composition of 1-stearoly-2-arachidonyl-SN-glycerol [161]. In labeling studies using [6–3H] or [U-14C] glucose, we have shown that elevated glucose increases its incorporation into the glycerol backbone of DAG in aortic endothelial cells [162] and SMCs [158], as well as renal glomeruli [150]. These facts indicate that the increased DAG levels in high glucose conditions are mainly derived from the de novo pathway (Fig. 3.2).

It is also possible that DAG is produced through the metabolism of phosphatidylcholine as a result of the activation of phospholipase D [157]. One potential

pathway for DAG increase is through glyco-oxidation, since oxidants such as H_2O_2 are known to activate the DAG–PKC pathway (Fig. 3.2) [158]. We have reported that vitamin E, in addition to being an antioxidant, further inhibited DAG–PKC activation in vascular cells exposed to high glucose levels [140]. This occurred at very high vitamin E doses and probably decreased DAG levels rather than PKC, because the direct addition of vitamin E to purified PKC-α or -β isoforms in vitro had no inhibitory effect [159].

PKC belongs to a family of serine–threonine kinases and plays a key role in intracellular signal transduction for hormones and cytokines. There are at least 11 isoforms of PKC and are classified as conventional PKCs (α, β1, β2, γ); novel PKCs (δ, ε, η, θ, μ); and atypical PKCs (ζ, λ) [160, 161]. Multiple isoforms of PKC, including α, β1, β2, δ, ε, and ζ, are all expressed in endothelial cells [146, 163]. Activation of classical and/or novel PKC isoforms has been suggested to play key roles in the development of diabetic microvascular and CV complications [80, 164–170]. PKC activation by hyperglycemia appears to be tissue-selective, because it has been noted in the retina, aorta, heart, and glomeruli, but not in the brain and peripheral nerves of diabetic animals (Table 3.3). Among the various PKC isoforms, PKC-β and PKC-δ appear to be preferentially activated in the aorta and heart of diabetic rats [146] and in cultured aortic SMCs exposed to high levels of glucose [140]. However, increases in multiple PKC isoforms were observed in some vascular tissues, such as PKC-α, -β2, and -ε in the retina and PKC-α, β1, and δ in the glomeruli of diabetic rats [171]. Additionally, characterization of macrophage cell lines showed that PKC-α and PKC-β1 and β2 were activated when cells were exposed to elevated glucose concentrations (5.5–16 mM) [156]. We and others have shown that a number of in vivo abnormalities, such as renal mesangial expansion, basement membrane thickening, blood flow, and monocyte activation in diabetic rats, can be prevented or normalized using an orally effective specific inhibitor for PKC-β, LY333531 (ruboxistaurin) [150]. One of the early vascular changes in diabetes is the reduced bioavailability of endothelium-derived NO, which further aggravates endothelial dysfunction. This process appears to be at least partly caused by the activation of PKC-β by hyperglycemia. To demonstrate, Beckman and colleagues applied forearm hyperglycemic clamps on 14 healthy subjects and observed that endothelium-dependent vasodilation in response to methacholine chloride was decreased in hyperglycemia as compared to that in euglycemic conditions [172]. The reduction of vasodilation was normalized by oral ruboxistaurin (32 mg/day) [172]. We found that activation of PKC-β in endothelial cells could induce selective insulin resistance by phosphorylation of threonine-86 of P85/PI3K and serine of IRS2 with enhanced pro-atherogenic actions, while the PI3K/Akt pathway and its anti-atherogenic actions were inhibited [164, 165]. In apoE$^{-/-}$ mice with endothelial cell-specific overexpression of PKC-β2 (Tg (Prkcb) ApoE$^{-/-}$ mice), endothelial dysfunction and atherosclerosis were accelerated by loss of the Akt/eNOS pathway and activation of angiotensin-induced ET-1 expression [168, 169], while atherosclerosis was reduced when PKC-β was deleted or inhibited by ruboxistaurin [80, 170]. Similar effects were found in myocardia with PKC-β activation in diabetes or insulin resistance [166, 167]. These data support that the role of PKC-β activation in the

development of some aspects of diabetic vascular complications. Moreover, another PKC isoform, PKC-δ, was also activated in many vascular tissues, such as the aorta, retina, heart, renal glomeruli, and chronic wounds. Inhibition of PKC-δ in fibroblasts may improve insulin signaling via the PI3K/Akt pathway and increase VEGF expression in the wound, further improving the rate of wound healing [173]. The mechanisms for selective activation of certain PKC isoforms in various tissues remain unclear, however, since the main cause for PKC activation is the increase in DAG levels. It is likely that PKC isoforms which redistribute to the intracellular compartments are more affected than those located in the plasma membrane, since glucose metabolites which lead to increased DAG are formed mostly in intracellular organelles [174]. These studies all suggested that PKC activation in diabetes is related to selective insulin resistance and loss of the PI3K/Akt pathway, and contribute to endothelial cell dysfunction. After selectively activating PI3K/Akt pathways in macrophages to decrease selective insulin resistance and generating macrophage-PKCδ knockout (MPKCδKO/apoE$^{-/-}$) mice, we surprisingly found opposite results compared to deletion of PKCβ in endothelial cells. MPKCδKO/apoE$^{-/-}$ mice exhibited accelerated aortic atherosclerotic lesions with decreased apoptosis and increased proliferation in macrophages, which were associated with elevated phosphorylation levels of the pro-survival cell-signaling proteins Akt and FoxO3a, and reduction of the pro-apoptotic protein Bim [175]. These data suggested that inhibition of PKCδ to improve insulin resistance in different cell types may have contradictory effects on the progression of atherosclerosis in diabetes and insulin resistance.

For a hyperglycemia-induced change to be credible as a causal factor of diabetic complications, it has to be chronically altered, difficult to reverse, able to cause similar vascular changes when activated without diabetes, and able to prevent complications when inhibited. So far, we have presented evidence on DAG–PKC activation that fulfills at least three of these criteria. However, phase III clinical studies using ruboxistaurin have only shown modest efficacy in DR [176], DN [177], and neuropathy [178, 179]. These results indicate that inhibition of several PKC isoforms will be necessary to achieve significant clinical results for the various vascular complications of diabetes.

Dyslipidemia

In most patients with diabetes, especially those with T2D and insulin resistance, hypertriglyceridemia and decreased high-density lipoprotein (HDL) cholesterol have been reported [180]. Increased low-density lipoprotein (LDL) cholesterol is also frequently observed, but more frequently occurs in those with poor glycemic control or in parallel with hypertriglyceridemia. Additionally, LDL can be modified in diabetes, leading to the formation of glycated or oxidized LDL [181, 182]. Recent findings have shown that small, dense LDLs and excess triglyceride-rich remnants, which are highly atherogenic, are increased in insulin resistance [183]. Hyperinsulinemia and central obesity, which are commonly accompanied by insulin resistance and T2D, can lead to overproduction of very low-density lipoproteins

(VLDL) [184], which contain a number of apolipoproteins and triglycerides. Increased free fatty acid and glucose levels can increase VLDL output from the liver, and elevated triglyceride levels can inhibit apoB degradation, resulting in increased secretion of VLDL. Lipoprotein lipase (LPL) activity is decreased in diabetic patients because insulin is a major regulator of LPL activity. As LPL is necessary for the breakdown of chylomicrons and triglycerides, decreased LPL activity leads to increased VLDL. A decrease in LDL levels results in more glyceride-rich particles, fewer HDL particles, and much smaller and denser LDL particles in T2D patients. Increased VLDL levels can accelerate atherosclerosis as they can be inherently toxic for the metabolism and growth of endothelial cells [185]. VLDL in diabetic animals may also deposit more lipids in macrophages, which are precursors of foam cells in the arterial walls [180]. On the other hand, HDLs, which are decreased in diabetes, reduce the inhibitory effect of LDL on endothelium-mediated vasodilation [186]. Hypercholesterolemia also increases the expression of endothelial adhesion molecules, enhances platelet aggregability and adhesion [187–190], and augments vasoconstriction.

Small, dense LDLs, which are known to be a potent risk factor for CAD, oxidize easily and are rapidly taken up by macrophages [191], subsequently interacting with endothelial cells, releasing vasoactive factors, and becoming foam cells. Experimental and clinical data suggest that elevated serum levels of total and LDL cholesterol are associated with impaired endothelial function [192–194]. Modified (mostly oxidized) LDLs impair endothelial function more than native LDLs at similar doses, based on in vitro vasodilator responses [194, 195]. The levels of oxidized LDLs correlate better with impairment in endothelial function than cholesterol levels. Modified/oxidized LDL can affect gene expression (i.e., decrease eNOS expression and increase endothelin-gene expression and production), thus promoting vasoconstriction and hypertension.

Several studies have suggested that a key detrimental effect of hypercholesterolemia is in decreasing NO availability [191]. Administration of the NO precursor L-arginine restores endothelial dysfunction induced by oxidized LDLs, suggesting an impairment in NO synthesis or a decrease in L-arginine availability [193, 194]. In clinical studies, infusion of L-arginine can improve impaired endothelium-dependent vasodilation, including that mediated by hypercholesterolemia [193, 196].

Cellular and Functional Alterations in Vascular Endothelial Cells Induced By Diabetes

Vascular Contractility and Blood Flow

Hemodynamic abnormalities in blood flow and vascular contractility have been reported in many organs of diabetic animals or patients, including the kidney, retina, peripheral and coronary arteries, and microvessels of peripheral nerves. In the retina of subjects with short-duration diabetes and no DR, blood flow has been shown to

be decreased. However, in severe proliferative DR (PDR), retinal blood flow is increased, likely due to elevated VEGF expression in response to retinal hypoxia [166, 197–202]. One possible explanation for the decreased retinal blood flow in the early stages of diabetes is the result of PKC-induced increased vascular resistance at the microcirculatory level. We have reported that this decreased retinal blood flow can be mimicked by intravitreous injection of phorbol esters, which are PKC activators [145]. Furthermore, decreases in retinal blood flow in diabetic rats have been reported to be normalized by PKC inhibitors [150]. In addition to the retina, decreases in blood flow have also been reported in the peripheral nerves of diabetic animals by most investigators; these were normalized by PKC inhibitors, AR inhibitors, and antioxidants, respectively.

One possible mechanism by which PKC activation can cause retinal vasoconstriction is by increased expression of ET-1, a potent vasoconstrictor. ET-1 is increased in the retina of diabetic rats, and intravitreous injection of the endothelin-A receptor antagonist BQ123 prevented the decrease in retinal blood flow in these rats [203]. The induction of ET-1 expression can also be normalized by ruboxistaurin [204]. As mentioned, the decrease in retinal blood flow can lead to local hypoxia, which is a potent inducer of VEGF, consequently leading to increased permeability, microaneurysms, and ultimately neovascularization in diabetic retina [205, 206]. It was also reported that IR knockout in endothelial cells could decrease VEGF, eNOS, and ET-1 expression and reduce ischemia-induced retinal neovascularization [63], but no changes were found with regards to either blood–brain permeability or systemic insulin sensitivity [207].

Meanwhile, the role of PKC activation in hemodynamic abnormalities leading to diabetic neuropathy is not clear. Hyperglycemia-induced ischemia has been suggested to play a role in the development of diabetic neuropathy since vasodilators which increase nerve blood flow appear to improve nerve function in diabetic rodents [178], although some reports have suggested that phosphatidylinositol turnover and DAG levels were reduced, causing a decrease in PKC activity [179]. NOS inhibitors blocked the benefits of WAY 151003, a nonisoform specific inhibitor, on blood flow and conduction velocity, which suggests that PKC contributes to diabetic neuropathy via a neurovascular mechanism [208]. However, clinical studies using ruboxistaurin for treatment of painful neuropathy did not achieve significant results [209].

Abnormalities in hemodynamics have also been documented to precede DN. Elevated renal glomerular filtration rate and modest increases in renal blood flow are characteristic findings in T1D patients and experimental diabetic animals with poor glycemic control [210–213]. Diabetic glomerular filtration is likely to be the result of hyperglycemia-induced decreases in arteriolar resistance, especially at the level of the afferent arteriole, resulting in an elevation of glomerular filtration pressure. This effect of hyperglycemia can be mimicked in vitro by incubating renal mesangial cells with elevated glucose levels that reduced cellular response to vasoconstriction. Several reports have suggested that PKC activation via prostaglandin induction may involve these adverse effects of hyperglycemia [214–216], with ruboxistaurin being able to normalize glomerular filtration rate and renal albumin excretion rate in parallel with inhibition of PKC activity [150].

Changes in NO can also alter vascular contractility and blood flow. NO, or endothelium-derived relaxing factor (EDRF), is produced by eNOS from L-arginine and oxygen, which regulates arterial vasomotor tone, suppresses VSMC proliferation, and protects the endothelium from leukocyte interaction and thrombosis. In endothelial cells, insulin has been reported to activate eNOS via phosphorylation at Ser1177 through the PI3K/pAkt pathway [217]. In resistance vessels isolated from diabetic patients and animals, the relaxation phase after acetylcholine stimulation appeared to be delayed [218–221]. This impaired vascular relaxation can be restored by PKC inhibitors and mimicked by phorbol esters in normal arteries [221], with PKC inhibitors increasing mRNA expression of eNOS in aortic endothelial cells [222]. We have observed reduced eNOS expression in the microvasculature of Zucker fatty rats, which are models of insulin resistance [61]. Additionally, activation of RAAS or inflammatory cytokines such as TNF-α can likewise inhibit insulin-induced NO production and vasodilation [70, 71, 75–78].

Clinically, hemodynamic changes in DN are also affected by RAAS-blocking agents such as angiotensin-converting enzyme (ACE) inhibitors (ACEi) and angiotensin receptor blockers (ARBs), both of which comprise current standard of care in DN [223, 224]. Among other mechanisms, these drugs act by causing efferent arteriole constriction, leading to increased perfusion pressure in the glomeruli. Early on, the Collaborative Study Group (CSG) Captopril Trial [225] already demonstrated the ability of the ACEi captopril in reducing creatinine doubling time compared to placebo, while subsequent ARB studies such as the Irbesartan Diabetic Nephropathy Trial (IDNT) [224] and the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) Trial both showed similar efficacy of ARBs in slowing DN progression [223]. Recently, a new class of glucose-lowering agents, sodium-glucose cotransporter-2 (SGLT2) inhibitors, have shown promise in ameliorating DN through their actions on the proximal tubules, thereby reducing glomerular hyperfiltration (EMPA-REG OUTCOME [226–228], CANVAS [229–232], DECLARE-TIMI 58 [233, 234]). Aside from their renal benefits, SGLT2 inhibitors also demonstrated benefit in reducing CV events, particularly heart failure, in T2D patients (EMPA-REG OUTCOME [226], CANVAS [229], DECLARE-TIMI 58 [233]).

Vascular Permeability and Neovascularization

Increased vascular permeability is another characteristic vascular abnormality in diabetic patients and animals, in which increased permeability can occur after as early as 4–6 weeks of diabetes, suggesting endothelial cell dysfunction [235]. Because the vascular barrier is formed by tight junctions between endothelial cells, the increase in permeability is a result of abnormalities in the endothelial cells. Deletion of IR in endothelial cells can reduce ischemia-induced retinal neovascularization via reduction of VEGF, eNOS, and ET-1 expression at regular diets [63]. The activation of PKC can directly increase the permeability of albumin and other

macromolecules through barriers formed by endothelial cells, probably by phosphorylating cytoskeletal proteins that form intercellular junctions [236–238]. Recently, PKC-β overexpression in human dermal microvascular endothelial cells has been reported to enhance phorbol ester-induced increases in permeability to albumin [239]. Thus, the actions of phorbol ester and hyperglycemia in endothelial barrier function are mediated in part through activation of PKC-β.

PKC activation can also regulate vascular permeability and neovascularization via the expression of growth factors, such as VEGF/vascular permeability factor (VPF), which is increased in ocular fluids from diabetic patients and has been implicated in the neovascularization process of PDR [240]. This is probably only applicable to the retina, since VEGF expression is decreased in the peripheral limbs and myocardium in diabetes, although these tissues also exhibited increases in vascular permeability. We have reported that both the mitogenic and permeability-induced actions of VEGF/VPF were partly a result of PKCβ activation via tyrosine phosphorylation of phospholipase-δ, and can be decreased by ruboxistaurin [241, 242]. Recently, clinical trials on anti-VEGF agents such as pegaptanib, bevacizumab, and ranibizumab have all shown efficacy in patients with PDR and diabetic macular edema (DME). Intravitreal injections of these novel treatments were associated with improved rates of vision loss, retinal capillary leakage, and reduced PDR progression, and have provided alternative therapeutic options to panretinal laser photocoagulation [243–245].

Na^+-K^+-ATPase

The Na^+-K^+-ATPase, an integral component of the sodium pump, is involved in several cellular functions such as maintenance of cellular integrity, contractility, growth, and differentiation [246]. It is well established that Na^+/K^+-ATPase activity is generally decreased in the vascular and neuronal tissues of diabetic patients and animals [95, 97, 246–248]. However, the mechanism by which hyperglycemia inhibits Na^+/K^+-ATPase activity has provided conflicting results, particularly with regard to the role of PKC. Phorbol esters have been shown to prevent the inhibitory effect of hyperglycemia on the Na^+/K^+-ATPase, suggesting that PKC activity might be decreased in diabetes. However, we have reported that elevated glucose levels increased PKC and cytosolic phospholipase A2 (cPLA2) activities, resulting in increased arachidonic acid release and prostaglandin E2 (PGE2) production and decreased Na^+-K^+ ATPase activity [249]. Inhibitors of PKC or cPLA2 prevented hyperglycemia-induced reduction in Na^+-K^+ ATPase activity in aortic SMC and mesangial cells. The apparent paradoxical effects of phorbol esters and hyperglycemia in the enzymes of this cascade are probably due to the quantitative and qualitative differences in PKC stimulation by these stimuli. Phorbol esters, which are not physiological PKC activators, probably activated many PKC isoforms and increased PKC activity by 5–10 times, whereas hyperglycemia can only increase PKC activity by twofold, a physiologically relevant change that affected selective PKC isoforms. Thus, results derived from studies using phorbol esters are difficult to interpret with respect to physiological significance.

Basement Membrane Thickening and Extracellular Matrix Expansion

Thickening of the capillary basement membrane is an early structural abnormality observed in almost all the tissues, including the vascular system, in diabetes. Because the basement membrane can affect numerous cellular functions, such as structure support, vascular permeability, cell adhesion, proliferation, differentiation, and gene expression, alterations in its components may cause vascular dysfunction. Histologically, increases in type IV and VI collagen, fibronectin and laminin, and decreases in proteoglycans are observed in the mesangium of diabetic patients with DN, and probably in the vascular endothelium as well [250, 251]. These effects can be replicated in mesangial cells incubated in high glucose levels that were prevented by general PKC inhibitors [252–254]. Additionally, increased expression of transforming growth factor (TGF)-β has been implicated in the development of mesangial expansion and basement membrane thickening in diabetes. We have shown recently that excessive expression of connective tissue growth factor (CTGF), TGF-β, and extracellular proteins induced by diabetes were significantly reduced in PKC-β knockout mice as compared to their wild-type controls, and were more protected from renal hypertrophy, glomerular enlargement, and hyperfiltration [255]. Similar findings regarding PKCβ's effects on CTGF expression were observed in the myocardium of diabetic mice and rats [256]. Because PKC activation can increase ECM and TGF-β production, it is not surprising that reports have shown PKC inhibitors being also able to prevent hyperglycemia- or diabetes-induced increases in ECM and TGF-β in mesangial cells or renal glomeruli [171].

Thrombosis

Abnormalities of coagulation and platelet biology in T2D patients are well documented [257]. The development of thrombosis within the vasculature depends on the balance between pro- and anti-thrombotic factors, which are shifted towards the former in T2D patients [258]. PAI-1 is produced by the liver and endothelial cells and binds to the active site of both tissue and urokinase plasminogen activator, neutralizing their activity [259]. Thus, increased expression of PAI-1 can lead to decreased fibrinolytic activity and predispose to thrombosis. Higher insulin concentration, similar to those seen in the plasma of diabetic patients, induced accumulation of PAI-1. It was also shown that performance of euglycemic–hyperinsulinemic or hyperproinsulinemic clamps on intact anesthetized rabbits increased PAI-1 accumulation. Insulin alone does not have a significant effect on PAI-I expression in normal subjects. However, elevated insulin levels together with increased glucose and triglycerides, as is typical of T2D patients, elicit an insulin-dependent increase in circulating PAI-1. The PAI-1 content in atherectomy specimens from T2D patients has also been shown to increase in normal subjects.

RAAS abnormalities in diabetic patients can also induce PAI-1 accumulation. The contribution of the RAAS to diabetic vascular complications has been attributed mainly to an increased vascular responsiveness to angiotensin II [260]. We observed that angiotensin II induced both PAI-1 and -2 expression levels in vascular endothelial cells and VSMCs, which is partially dependent on PKC [261]. These data suggest that therapies for insulin resistance and glycemic control can restore the fibrinolytic response.

Since studies have also demonstrated relationships between inflammation and thrombosis, clinical trials have sought to evaluate the effects of anti-inflammatory treatments on CVD in diabetic patients. Interleukin (IL)-1, in particular, has been reported as a therapeutic target for CVD, as it induces inflammatory functions including elevation of intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and monocyte chemoattractant protein (MCP)-1 in endothelial cells. Similarly, induction of IL-6 has been shown to promote thrombosis in VSMCs. The Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) showed that canakinumab, an IL-1β inhibitor, can reduce CV events in diabetic patients, with reductions in plasma levels of fibrinogen, IL-6, and high sensitivity C-reactive protein (CRP). However, treatment did not affect lipid levels or all-cause mortality, and was even associated with an increased incidence of fatal infection [262–265].

Conclusion

It is likely that insulin resistance and hyperglycemia are responsible, directly, indirectly or in combination, for many abnormalities of vascular endothelial function in diabetes. New studies on the adverse effects of hyperglycemia have suggested that alterations in the signal transduction pathways induced by AGE, reactive oxidative stress, and DAG–PKC are important mechanisms in endothelial and vascular cell function, because these pathways may affect both anti- and pro-atherogenic actions. Insulin actions are also important in the regulation of many vascular functions by transporting and communicating different nutrients, hormones, cytokines, and other signaling molecules. Selective impairment or enhancement of insulin signaling causes the blunting of insulin's anti-atherogenic actions, accelerating endothelial and myocardial dysfunction, atherosclerosis, restenosis, and impaired wound healing. Hyperinsulinemia, when present concomitantly with insulin resistance, may enhance insulin's pro-atherogenic actions. Agents that can target the abnormalities of hyperglycemia-induced vascular dysfunction and improve insulin resistance in the endothelium can ultimately prevent the microvascular and CV complications of diabetes.

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Chapter 4
PPARs and Their Emerging Role in Vascular Biology, Inflammation and Atherosclerosis

Javier Balda, Argyro Papafilippaki, Michael Johnstone, and Jorge Plutzky

Introduction

For many years, advances in understanding steroid hormone action typically proceeded through sequential stages that involved first identifying the role of a putative hormone, then isolating it—often from large quantities of body fluid, and ultimately identifying the nuclear receptor through which the cellular effects are being achieved. This stepwise progression has been reversed by modern molecular biology techniques allowing rapid identification of many genes as encoding nuclear receptors based on structural motifs, even without any information regarding the functional role of these so-called orphan receptors. This process has been termed reverse endocrinology [1]. Peroxisome proliferator-activated receptors (PPARs) are examples of such orphan receptors, although their status changed through the serendipitous discovery of synthetic ligands that could bind to PPARs [2]. The fact that these synthetic agonists are now in clinical use for treating diabetes mellitus (DM) and dyslipidemia has helped in drawing attention to this nuclear receptor subfamily and its potential as a therapeutic target [3]. The identification of a possible role for

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PPARs in inflammation and atherosclerosis only heightened this interest [4]. Only the therapeutic use of PPAR agonists has been complicated by a host of issues, as discussed further below, the fact remains that PPARs are powerful, central determinants of transcriptional programs that govern energy balance and related to pathways. As such, PPAR biology and past, current and future attempts at PPAR therapeutics remain of scientific and clinical relevance.

The Basic Science of Peroxisome Proliferator-Activated Receptor Biology

Like all steroid hormone nuclear receptors, PPARs, including its three isotypes PPAR-γ, PPAR-α and PPAR-β/δ, are ligand-activated transcription factors [1, 2]. Similar to other nuclear receptors, PPARs contain both ligand-binding and DNA-binding domains. In response to specific ligands, PPARs form a heterodimeric complex with another nuclear receptor, the retinoic X receptor (RXR), which is activated

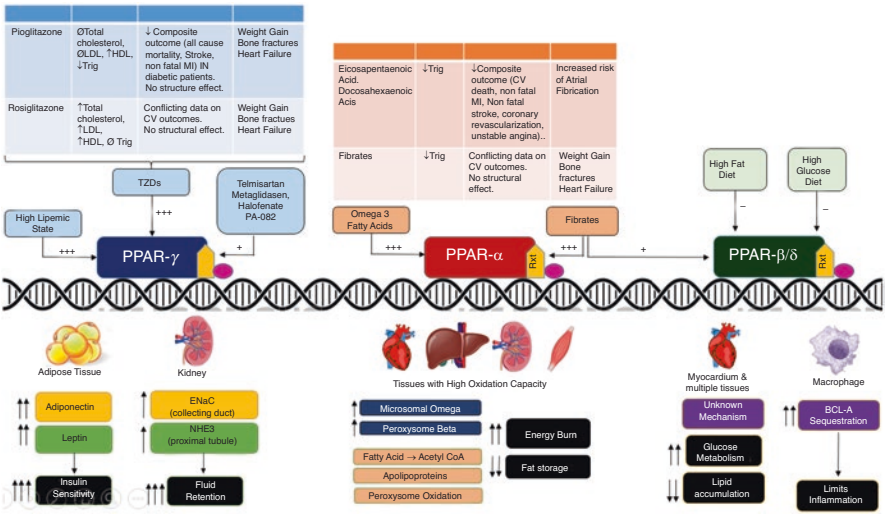


Fig. 4.1 General overview of peroxisome proliferator-activated receptor isotypes isoform PPAR-α, PPAR-γ, PPAR-β/δ. Major tissues liver fat ubiquitous heart muscle ligands fenofibrate pioglitazone prostacarbacyclin gemfibrozil rosiglitazone WY14643* biologic roles fatty acid metabolism adipogenesis wound healing in metabolism lipid metabolism insulin sensitivity lipid metabolism lipid metabolism. Although peroxisome proliferator-activated receptor (PPAR) isoforms have a number of common attributes, they can also be distinguished by a number of unique characteristics. Perhaps most central to their different roles in metabolism, each PPAR is activated by different ligands, leading to regulation of specific target genes. A general overview utilizing illustrative examples for each PPAR isoform is listed to provide a general characterization. The evidence for PPAR expression and function in vascular and inflammatory responses is discussed elsewhere. 96 Plutzky

by its own ligand-9 cis-retinoic acid [2]. This heterodimeric complex binds to defined PPAR-response elements in the promoters of specific target genes, determining their expression. Importantly, PPAR activation can either induce or repress the expression of different target genes. The mechanism through which PPAR repression occurs is not well understood but is thought to be indirect, for example, influencing the critical inflammatory regulator nuclear factor kappa B (NF-κB) or controlling the small co-regulatory molecules (co-activators, co-repressors) that are central to transcriptional responses. Extensive studies over many years have defined specific metabolic roles for each PPAR isoform (Fig. 4.1). These individual characteristics provide a context for considering the potential role of each PPAR isoform in atherosclerosis and vascular biology [5].

Peroxisome Proliferator-Activated Receptor-γ: Key Regulator of Adipogenesis and Insulin Sensitivity

PPAR-γ was first identified as a part of a transcriptional complex essential for the differentiation of adipocytes, a cell type in which PPAR-γ is highly expressed and critically involved in the regulation of adipogenesis, energy balance, lipid biosynthesis, lipoprotein metabolism and insulin sensitivity [6]. It acts mainly by transactivation and subsequent modulation of target gene expression [5]. PPAR-γ activation can both induce target genes as well as exert repressive effects, decreasing the expression of other genes. Homozygous PPAR-γ-deficient animals die at about day 10 in utero as a result of various abnormalities including cardiac malformations and absent white fat [7–9]. PPAR-γ2 is predominant and abundant in adipose tissue, promoting adipose tissue expansion in response to a high lipemic state (which in turns protects non-adipose tissues such as liver and skeletal muscle from excessive lipid overload). Moreover, it promotes the secretion of adiponectin and leptin (mediators of insulin action in peripheral tissue) resulting in increased insulin sensitivity [5]. Chemical screening and subsequent studies led to the serendipitous discovery of synthetic ligands of PPAR-γ, the thiazolidinediones (TZDs), which act in the same way. Used clinically as anti-diabetic agents, the TZD class includes Troglitazone (Rezulin)—withdrawn from the market because of idiosyncratic liver failure, Pioglitazone (Actos) and Rosiglitazone (formerly BRL49653, now Avandia) [10]. Despite a similar mechanism of action, the influence of TZDs on cardiovascular outcomes among patients with diabetes may differ. Pioglitazone has been shown to reduce cardiovascular (CV) complications in a prospective clinical trial by 16% (composite outcome of all-cause mortality, non-fatal myocardial infarction (MI) and stroke) as reported in PROACTIVE [11, 12]. However, the primary endpoint in PROACTIVE was negative, though related to the inclusion of peripheral artery disease outcomes, notoriously difficult clinical issues to reverse, in the multi-component primary endpoint. In keeping with the notion that a positive CV outcome with pioglitazone may have been missed in PROACTIVE, in 2016 Kernan et al. reported in the Insulin Resistance Intervention after Stroke (IRIS)

selected a cohort of 3876 non-diabetic patients with recent ischemic stroke or TIA who had insulin resistance by homeostasis model assessment (HOMA-IR) >3.0, and randomized them to pioglitazone or placebo. When compared to placebo, the pioglitazone group showed statistically significant reduction of the primary endpoint of fatal or non-fatal stroke or myocardial infarction, a major advance by showing a significant decrease in risk of stroke and doing so outside of a context of clinical diabetes [13]. Subjects in IRIS who received pioglitazone also had a significant decrease in the risk of developing diabetes (hazard ratio = 0.48; $p < 0.0001$) at 4.8 years of follow-up, mainly in those with fasting glucose levels >100 mg/dL and HbA1C > 5.7% [13]. From this cohort, 12% of the subjects had 225 acute coronary events during the 4.8 years of follow-up. A post hoc analysis of these events revealed that when compared to placebo, pioglitazone had a statistically significant risk reduction of acute coronary events (hazard ratio 0.71, $p = 0.02$), Type I MI (hazard ratio 0.62, $p = 0.03$) and not Type II MI (hazard ratio 1.05, $p = 0.87$), providing support for the concept that PPAR-g activation, at least with pioglitazone could decrease atherosclerotic events [14]. Interestingly, pioglitazone has been shown to decrease triglycerides while increasing high-density lipoprotein (HDL)—without affecting total cholesterol or low-density lipoprotein (LDL)—effects that align with PPAR-a activation. In keeping with this, in preclinical models, some of the pioglitazone effects in vitro and in vivo depend on the presence of PPAR-a, suggesting pioglitazone, and/or one of its biologically active metabolites, may be a dual PPAR-g/a activator [15].

Although rosiglitazone is also a PPAR-g agonist, it has distinct effects from pioglitazone. While rosiglitazone can increase HDL, it can also increase total cholesterol and LDL, with no effects on triglycerides. Early concerns arose regarding possible increases in MI and CV death after short-term exposure, perhaps related to its effects on lipid sub-fractions [5]. Whether rosiglitazone actually does increase cardiovascular remains controversial, which has been difficult to resolve in the absence of an appropriate, large, well-controlled, prospective clinical trial. In patients with diabetes mellitus, PPAR-g agonists including both pioglitazone and rosiglitazone can increase heart failure, most likely through effects on fluid retention. PPAR-g agonists do not cause a structural change in the myocardium nor do they increase all-cause or cardiovascular mortality.

The differences between pioglitazone and rosiglitazone align with the concept of selective PPAR-γ modulators (SPPAR-γMs). The particularly large ligand-binding domains of PPARs, and their mechanism of action that involves interaction with small accessory molecules, allow for the potential for different ligands to bind uniquely to PPARs, resulting in alternative gene expression and distinct biologic responses. The SPPARM concept was behind thus far unsuccessful efforts to subtract adverse effects of PPAR-g agonists while preserving their insulin-sensitizing effects. Another class of SPPARγMs has been proposed to include the angiotensin receptor antagonists (ARBs), metaglidasen–halofenate, and PA-082. Telmisartan may function as a partial PPAR-γ agonist more so than other ARBs. Telmisartan appears to bind to the PPAR-γ ligand-binding domain in a different way than the full PPAR-g agonist rosiglitazone does, resulting in approximately 70% less PPAR-γ activation and possible inhibition of adipogenesis [16].

In terms of adverse events, as noted, PPAR-γ has been associated with fluid retention and increased heart failure in some patients with diabetes, likely from activation of the renal collecting duct epithelium's sodium channel (ENaC) and sodium transporters in the proximal tubule (NHE3); other possible contributors may include increased vascular permeability via vascular endothelial growth factor secretion. Weight gain is a common adverse PPAR-g agonist effect, which can be significant in some cases and especially if combined with insulin therapy or secretagogues. While the increase in weight may seem to contradict the potent insulin-sensitizing effects of these agents, the increase in adiposity appears to be predominantly in subcutaneous fat. An important side effect of PPAR-g agonists includes a dose-related increase in the risk of bone fractures in pre- and postmenopausal women. Also of note is the association between TZDs and bladder cancer; more robust studies including a large trial in 2012 where nearly 200,000 were followed for 10 years suggested that these medications do not increase bladder cancer risk [17].

Of note, individuals born with dominant negative mutations in PPAR-γ have severe insulin resistance and hypertension, helping to establish through a genetic line of evidence the importance of these receptors in human biology [18, 19].

Peroxisome Proliferator-Activated Receptor-α: Key Regulator of Fatty Acid Oxidation

PPAR-α is expressed in tissues with high oxidation capacity such as heart, liver, kidney and skeletal muscle where it plays a central role in the regulation of lipid, and especially fatty acid, metabolism [20]. PPAR-α activates expression of genes in the major—microsomal omega and peroxisome beta—oxidizing systems, which in turn promote energy burning and reduced fat storage [5, 21]. Target genes participate in the conversion of fatty acids to acyl-coenzyme A derivatives, peroxisome G-oxidation, and apolipoprotein expression (A1, AII and CIII) [22, 23]. Reminiscent of PPAR-γ, fibrates, namely gemfibrozil (Lopid) and fenofibrate (TriCor), in clinical use for lowering triglycerides, raising high-density lipoprotein (HDL), were first identified before they were found to act by binding to PPAR-α agonists [24]. Early insights into PPAR-α come from the study of PPAR-α-deficient mice, which lack peroxisome proliferation in response to fibrates, confirming the connection between PPAR-α and peroxisome proliferation, a phenomenon that does not occur in humans [25, 26]. PPAR-α-deficient mice also manifest abnormal lipid profiles with increased total cholesterol, elevated apo-AI and mildly increased total HDL levels as a result of apparently decreased HDL catabolism [27]. PPAR-α activators do not lower triglycerides in PPAR-α null mice, thus implicating PPAR-α in the clinical effects of these drugs and their use to lower hypertriglyceridemia. Gemfibrozil was initially found to decrease CV events in patients with average LDL levels and prior MIs, as seen in VA-HIT [28]. Evidence that this benefit was driven by those patients with diabetes prompted a subsequent study in patients with diabetes, who failed to meet its primary CV endpoint, even if some secondary CV endpoints were positive.

Disproportionately higher drop-in rates of statin use in the placebo group may have been a factor in outcomes. An additional study investigating adding fenofibrate to a statin versus statin alone failed to show a reduction in the primary CV endpoint fatal when compared to a statin alone (Varga et al. [5]). In several fibrate trials, more definitive evidence for benefit is seen among the subgroup with elevated triglycerides and lower HDL levels. In the near future, the PROMINENT trial will report outcomes in 10,000 participants with type 2 diabetes, non-severe hypertriglyceridemia and low HDL cholesterol levels on moderate to high-intensity statin or meet specified LDL-C criteria randomly assigned to either the selective PPAR-a modulator pemafibrate or placebo. Patients will be followed for a period of 3.75 years and primary endpoints of composite non-fatal MI, non-fatal stroke, hospitalization for unstable angina requiring urgent revascularization and cardiovascular death determined. Secondary and tertiary endpoints include hospitalization for heart failure and new or worsening peripheral artery disease, retinopathy and nephropathy, and change in biomarkers (i.e., Hb A1c, lipid panel) [29, 30]. A positive outcome in PROMINENT may help increase the use of fibrates, whose use now more often involves patients with severe hypertriglyceridemia or persistent hypertriglyceridemia despite statin use and significant CV risk.

In the context of hypertriglyceridemia, it should be noted that omega-3 fatty acids, more specifically, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are also used to lower triglycerides and are thought to be natural ligands for PPAR-α. Omega-3 fatty acids, when oxidized, activate PPAR-α. While omega-3 fatty acids do not improve insulin resistance, a pure form of EPA alone, icosapent ethyl, has been shown to reduce acute CV events, as reported in REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention) Trial [31]. In REDUCE-IT, patients receiving 2gr icosapent ethyl twice daily had a statistically significant 25% reduction in composite CV death, non-fatal MI, non-fatal stroke, coronary revascularization and unstable angina (4.8% absolute reduction). Icosapent ethyl also increased the incidence of atrial fibrillation and showed a trend towards increased bleeding risk [31]. The exact mechanism for benefits in REDUCE-IT remain unclear and debated, with another omega-3 fatty acid trial**,** which studied a combination of EPA and DHA, failed to show a benefit despite achieving similar EPA levels; questions have been raised regarding the agent used in the control groups between REDUCE-IT versus STRENGTH and whether the agent used in REDUC E-IT (mineral oil) was as neutral as the corn oil used in STRENGTH [32–34].

Peroxisome Proliferator-Activated Receptor-β/δ: Widely Expressed, But Still Incompletely Understood

Although PPAR-β/δ is widely expressed in most cell types, its role is less fully characterized. PPAR-β/δ participates in fatty acid oxidation and utilization, as seen on in vitro studies in skeletal muscle and adipocytes. In the cardiomyocyte, specifically, overexpression of PPAR-β/δ results in increased glucose metabolism and

diminished lipid accumulation in the presence of a high-fat diet. In turn, decreased expression of PPAR-β/δ has been suggested during hyperglycemic in diabetes mellitus, suggesting that PPAR-β/δ activation may help limit diabetic cardiomyopathy. PPAR-β/δ-deficient mice exposed to a high-fat diet were prone to obesity, while PPAR-β/δ activation helped protect against either nutritionally or genetically triggered obesity [5]. PPAR-β/δ has also been found to play an important part in wound healing and inflammatory responses in skin [35]. One report suggested that PPAR--β/δ activation might limit inflammation by sequestering the pro-inflammatory co-activator BCL-6 in macrophages [36]. Bezafibrate, a traditional PPAR-α agonist, may also activate PPAR-β/δ but with low potency and low affinity to it [5]. Of note, unlike PPAR-γ and PPAR-α, no PPAR-β/δ agonist has ever made it to clinical use, perhaps because of side effects or other issues, which may underlie the more the more limited research into PPAR-β/δ.

Peroxisome Proliferator-Activated Receptors in Vascular Biology and Atherosclerosis

The effects of PPAR-γ and PPAR-α agonists on vascular biology and atherosclerosis are an obvious issue given the patient populations that receive these drugs. Thiazolidinediones are used in patients with DM, and thus in patients with well-defined increased risk for cardiovascular events [10]. Fibrates are used to treat patients with increased triglycerides and often low HDL, a profile with increased cardiovascular risk and often seen among patients with insulin resistance and/or diabetes [24]. Theoretically, PPAR agonists could have vascular benefits based on their various metabolic effects—improving insulin sensitivity, lowering glucose and raising HDL. An alternative but not mutually exclusive hypothesis has been direct vascular and inflammatory effects, given the evidence that PPARs are expressed in those cell types [4, 37]. All PPAR isoforms are now known to be expressed in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and monocytes/macrophages and T-lymphocytes [38–40]. An increasing amount of data continues to identify various PPAR-regulated target genes known to be involved in atherosclerosis. Moreover, this data extends to in vivo studies in both rodents and humans.

Peroxisome Proliferator-Activated Receptor-γ in Inflammation, Vascular Biology and Atherosclerosis

Early reports established not only that PPAR-γ was expressed in monocytes, macrophages and human atherosclerosis, but also that PPAR-γ agonists could repress expression of inflammatory cytokines and matrix metalloproteinases (MMPs) implicated in atherosclerosis and/or its complications [40, 41]. To some extent, the clinical trial evidence with PPAR-γ agonists has pushed the field beyond

consideration of the preclinical effects of these agents. Nevertheless, review of such data is of value given insight provided into vascular biology and atherosclerosis, potential relevance as the generation of endogenous PPAR ligands continues to be considered, and potential insight into additional PPAR therapeutics.

The initial observations regarding PPAR-γ inflammation and atherosclerosis were countered by reports that PPAR-γ agonists could also increase the expression of CD36, a receptor mediating uptake of oxidized LDL, possibly promoting foam cell formation [42]. Subsequent studies identified coordinated induction by both PPAR-γ and PPAR-α of ABCA1, an important effector of cholesterol efflux, which would potentially offset concerns regarding any CD36 effects [43–45].

Interestingly, TZDs have opposite effects on CD36 in vivo, decreasing their expression levels [46]. Aside from macrophages, PPAR-γ activation in VSMC decreases the proliferation and migration of these cells and their production of MMPs and endothelin-1 [47–50]. The latter target suggests one possible mechanism accounting for the small but reproducible decrease in blood pressure seen with PPAR-γ agonists [51]. Consistent with the effects seen in macrophages, PPAR-γ agonists repress inflammatory cytokine production in T-lymphocytes [52]. In ECs, PPAR-γ may decrease adhesion molecule expression although the results are variable, pointing out a limitation of a field that has depended heavily on synthetic agonists as experimental tools, with all the attendant concerns of pharmacological studies: physiological relevance, receptor dependence, dose dependence, and toxicity effects to name a few [53, 54]. One example of the potential complexities involved is evident in the reported relationship between PPAR-γ ligands and plasminogen activator inhibitor 1 (PAI-1) levels. Several reports indicate that PPAR-γ ligands may increase the expression of PAI-1, a pro-coagulant, pro-atherosclerotic response. Other laboratories find a PPAR-γ-mediated repression of PAI-1 [55–57]. Others reported the inhibition of PAI-1 expression [58]. In humans, PPAR-γ ligands appear to decrease circulating PAI-1, although this may be a manifestation of improved glycemic control, less insulin resistance, or lower triglycerides [51]. In vivo, PPAR-γ ligands have been given to various different mouse models of atherosclerosis. In general, these studies have all shown decreases in the atherosclerotic lesions with PPAR-γ agonists [41]. Surrogate marker studies in humans suggest PPAR-γ agonists in clinical use may lower the levels of circulating MMP9, replicating the responses seen in vitro with VSMCs and macrophages and in alignment with the IRIS clinical trial results noted earlier [47, 59–61]. PPAR-γ agonists also decrease circulating levels of C-reactive protein (CRP) and levels of CD40 ligand (CD40L), both suggestive of anti-inflammatory effects [60]. Several PPAR-γ agonists have been found to decrease carotid intimal-medial thickness, a parameter linked with cardiovascular risk [62, 63]. Independent of these direct effects on atherosclerosis, it remains possible that PPAR-γ agonists could limit atherosclerosis and/or inflammation indirectly by delaying or even preventing diabetes, as has been suggested in some studies [64]. Any such benefits must be gauged against any potential toxicity or adverse outcomes seen with TZDs, like edema and weight gain. The recent significant positive CV outcome data with other diabetes medications

have tempered enthusiasm for the role of these agents in reducing CV risk although their potent effects on directly decreasing insulin resistance have not been supplanted with other therapeutic options.

Peroxisome Proliferator-Activated Receptors in Vascular Biology, Inflammation and Atherosclerosis

A similar but distinct picture as to the one described for PPAR-γ, emerged for PPAR-α and its potential role in the vasculature and inflammation. PPAR-α is also known to be expressed in most vascular and inflammatory cells [37]. PPAR-α activation can favorably alter the expression of a number of genes that are involved in well-established pathways strongly implicated in atherosclerosis and inflammation. As with PPAR-g, attention has shifted away from fibrates as clinical agents although interest remains, especially given continued work on unique agents like pemafibrate, the possibility of icosapent ethyl acting via PPAR-a, the mechanistic insight provided through PPAR-a studies and the prospect of PPAR mechanisms having relevance in other disease settings with unmet needs, like non-alcoholic steatohepatitis.

PPAR-α ligands clearly limit the inflammatory cytokine induction of adhesion molecules in endothelial cells [48, 53], an effect that is absent in microvascular cells lacking PPAR-α [38, 39]. The salutary benefits of omega-3 fatty acids may derive in part from PPAR-α activation with certain fatty acids limiting adhesion molecule expression and leukocyte adhesion in vivo in wild-type but not PPAR-α-deficient mice (Fig. 4.1) [65]. Interestingly, both omega-3 fatty acids and PPAR-α ligands can also limit the expression of tissue factor, a protein found in macrophages and thought to be a major contributor to plaque thrombogenicity [66, 67]. PPAR-α has also been found in VSMCs in which it represses the responses to inflammatory cytokines and, in limited data, decreased CRP levels [68]. Similar PPAR-α effects on CRP have been recently suggested in transgenic mice as well [69, 70]. Like PPAR-γ, PPAR-α ligands have been found to induce expression of the cholesterol efflux mediator ABCA1 [43]. In T-lymphocytes, PPAR-α ligands repress the expression of inflammatory cytokines like interferon-L, tumor necrosis factor-F and interleukin-2, suggesting the potential for proximal upstream anti-inflammatory modulation [52]. One way in which PPAR-α activation may exert these effects is by limiting NF-kB activation. The clinical trials using fibrates, initially reviewed earlier, offer some insight into the cardiovascular effects of PPAR-α agonists. In the Veterans Affairs HDL Intervention Trial, patients with a prior history of cardiovascular disease and a relatively average LDL, low HDL, and only modestly elevated triglycerides, experienced fewer recurrent cardiac events in response to the fibrate gemfibrozil as compared to placebo, as especially evident in those with diabetes, while clinical trials with fenofibrate or fenofibrate added to statins showing only secondary endpoints with CV benefit [28].

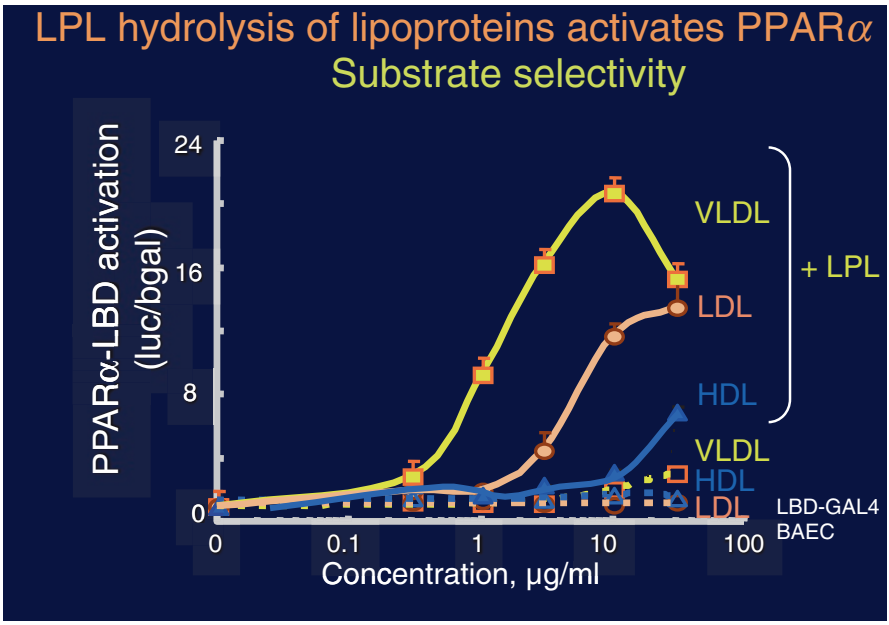
Endogenous Peroxisome Proliferator-Activated Receptor Activation: New Connections Between Fatty Acids, Lipid Metabolism and Peroxisome Proliferator-Activated Receptor Responses

The metabolic benefits seen with synthetic PPAR agonists along with their potential effects on inflammation and atherosclerosis combine with the importance of PPARs in regulating energy balance frames a fundamental question: what endogenous molecules does the body make to activate these receptors? Presumably, such natural ligands replicate or even surpass the effects of synthetic PPAR drugs, possibly protecting individuals from diabetes mellitus, dyslipidemia and/or atherosclerosis. Early studies into endogenous PPAR agonists focused mainly on specific candidate molecules. Oxidized linoleic acid in the form of 9 or 13 hydroxyoctadecanoic acid (HODE) appears to activate PPAR-γ [71], although it also has PPAR-α activity as well [38]. The prostaglandin metabolite 15-deoxy-D12, 14-prostaglandin J2 (15d-PGJ2) [23, 72] reportedly activates PPAR-γ agonist, although it can also act on IPB kinase and is of unclear physiologic significance [73, 74]. The greater biological effects seen with 15d-PGJ2 despite its lower PPAR-γ-binding affinity may result from its PPAR-independent effects on IPB kinase [73, 75]. Oxidized linoleic acid (HODE) is generated by 15 lipoxygenase [76] and activates PPAR-γ and PPAR-α [38, 42, 77, 78]. Leukotriene B4 may be an endogenous PPAR-α ligand that terminates inflammation [79]. The identity of endogenous PPAR-α ligands has also been investigated. Early landmark experiments reported that certain fatty acids could activate PPARs, a great advance in the field [80–82]. However, the physiological significance of those important observations was less clear, because the fatty acid effects seen required high concentrations of fatty acids (100–300 mM) and were not tested in vivo. Moreover, the link between endogenous lipid metabolism and subsequent PPAR activation remained obscure as did the mechanisms that might underlie selective PPAR isoform activation by natural ligands-like fatty acids.

Given that PPAR isoforms are differentially regulated, it seems unlikely that endogenous PPAR activation is indiscriminate as to PPAR isotype. Subsequent work advanced insight into endogenous PPAR activation. McIntyre and colleagues reported that lysophosphatidic acid could bind to and activate PPAR-γ [83]. Oleylethanolamide, a fatty acid analog, was found to regulate feeding by activating PPAR-α [84]. An alternative approach to understanding PPAR agonists is to investigate not specific candidate molecules but rather pathways that generated endogenous PPAR ligands. Through such studies, insight might be gained into PPAR function under more physiological conditions, connect pathways of lipid metabolism to PPAR activation and perhaps account for selective PPAR responses. It has been established that lipoprotein lipase (LPL), the primary enzyme in triglyceride metabolism, acts on triglyceride-rich lipoproteins like very low-density lipoprotein (VLDL) to generate PPAR ligands [39, 44]. These effects depended on intact LPL catalytic activity and were absent in response to LPL's known non-catalytic lipid uptake [39]. Moreover, these studies revealed striking specificity in regard to lipid

substrate (VLDL ≫ LDL > HDL) (Fig. 4.2). LPL hydrolysis may also explain selective PPAR activation, perhaps as a function of different cells and tissues. Although we observed LPL acted on VLDL to preferentially generate PPAR-α ligands, Evans and colleagues reported that LPL treatment of VLDL could also activate PPAR-β/δ in macrophages [44]. Of note, mouse macrophages may have relatively low levels of PPAR-α, which may contribute to the greater PPAR-β/δ response seen [85]. Lipolytic PPAR activation may also be specific in regard to different lipases and specific fatty acids. For example, we found that other lipases, like phospholipases D, C, A2, failed to activate PPAR-α despite releasing equivalent amounts of fatty acids as LPL [86]. This was presumed to be a result of the release of different fatty acids, as defined by both the lipase and the lipoprotein substrates. Interestingly, LPL action also replicated the effects of synthetic PPAR-α agonists on inflammation, decreasing VCAM-1 expression in a PPAR-α-dependent manner [38, 39]. This data suggests an anti-inflammatory role for LPL, a mechanism that could explain the protection against atherosclerosis enjoyed by individuals with intact, efficient lipolytic pathways (i.e., individuals with normal triglyceride and higher HDL levels). Such data may also connect to findings, for example, with individuals with a genetic loss of ApoC3, an endogenous LPL inhibitor. Interestingly, extensive data established that patients with DM typically have elevated free fatty acids [87]. Other lines of well-done and carefully executed studies indicate that LPL overexpression in muscle induces insulin resistance [88, 89]. Several possibilities might help reconcile these two sets of data. First, fatty acids are often referred to in a generic sense when, in fact, great differences exist between various fatty acids, for example, ranging from the responses to omega-3 fatty acids, with their likely cardio-protective effects, to saturated fatty acids and their reported pro-atherosclerotic effects [90, 91]. Thus, the elevated fatty acids in the circulation of patients with diabetes may differ from fatty acids produced by LPL, a significant percentage of which would be taken up by tissues as opposed to being present in the circulation. Moreover, these elevated fatty acids arise not out of the physiological function of LPL but rather abnormal metabolism. The DM seen in animal models overexpressing LPL in skeletal muscle is also associated with massive accumulation of triglycerides in these tissues [92]. Thus, the important observations from these experiments may not necessarily be a result of intact physiologic LPL action. Indeed, humans with LPL mutations that confer a gain of LPL function are associated with lower triglyceride levels, higher HDL and apparent protection against atherosclerosis [93]. The observations regarding the role of mitochondria as the main site of fatty acid oxidation in humans by Shulman and colleagues only add insight into the role of fatty acids in determining biological responses [94–96].

As mentioned, new formulations of PPAR agonists have been developed, along the lines of SPPARM agents, including dual PPAR-α/γ agonists, which may combine anti-diabetic, anti-inflammatory, anti-coagulant and/or anti-atherosclerotic action. Whether any of these novel PPAR agents will avoid prior or new reported adverse effects and offer benefits that allows them to be brought forward remains to be seen [17].



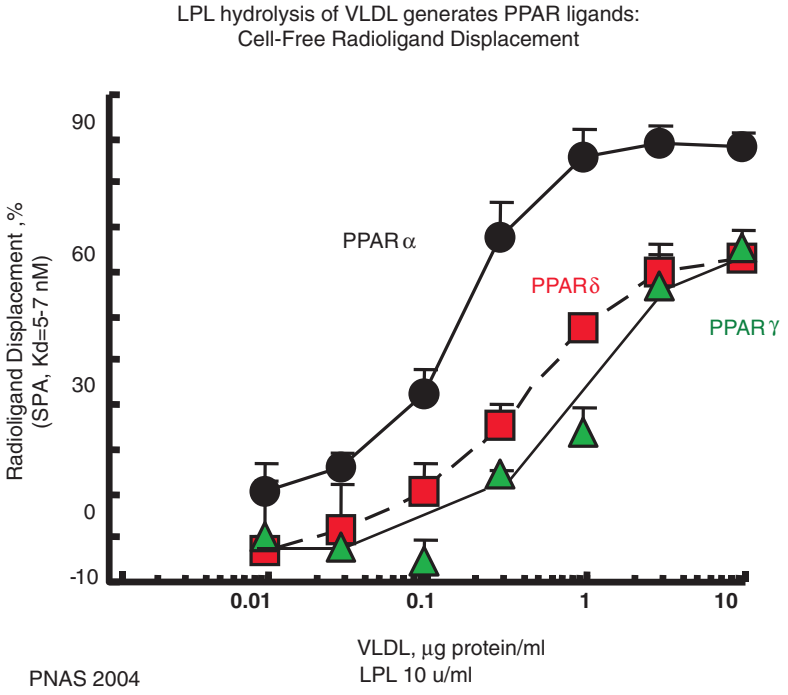


Fig. 4.2 LPL hydrolysis of VLDL generates PPAR ligands: cell-free radioligand displacement

Conclusion

From a biologic perspective, PPARs are clearly central mediators that control gene expression and transcriptional programs that are at the crossroads of metabolism, inflammation and atherosclerosis. As such, PPARs are of inherent relevance to common clinical scenarios where such issues arise, including diabetes, prediabetes and cardiovascular disease, while the prospect of the action of these nuclear receptors leading to therapeutic opportunities remains. Even with the controversies seen with clinical trial data using prior PPAR agonists, advancing science will continue to return to PPARs given the centrality of their involvement in metabolism, inflammation and atherosclerosis, as is already evident with data regarding omega-3 fatty acids, genetic variants associated with decreased CV risk, e.g., LPL function, and ongoing clinical trials with novel agents, like pemafibrate, and consideration of other clinical settings, like NASH.

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Chapter 5
Diabetes and Thrombosis

David J. Schneider

Diabetes and Vascular Disease

Complications of macrovascular disease are responsible for 50% of the deaths in patients with type 2 diabetes mellitus, 27% of the deaths in patients with type 1 diabetes for 35 years or less, and 67% of the deaths in patients with type 1 diabetes for 40 years or more [1, 2]. The rapid progression of macroangiopathy in patients with type 2 diabetes may reflect diverse phenomena; some intrinsic to the vessel wall; angiopathic factors such as elevated homocysteine [3], uncoupled nitric oxide synthase and superoxide generation [4], and hyperlipidemia; deleterious effects of insulin resistance [5] and dysinsulinemia, and excessive or persistent microthrombi secondary to a prothrombotic and antifibrinolytic state with consequent acceleration of vasculopathy secondary to clot-associated mitogens [6, 7]. As a result of these phenomena, cardiovascular mortality is as high as 15% in the 10 years after the diagnosis of diabetes mellitus becomes established [8]. Because more than 90% of patients with diabetes have type 2 diabetes and because macrovascular disease is the cause of death in most patients with type 2 as opposed to type 1 diabetes, type 2 diabetes will be the focus of this chapter. In addition to coronary artery disease, patients with type 2 diabetes have a high prevalence and rapid progression of peripheral arterial disease, cerebral vascular disease, and complications of percutaneous coronary intervention including restenosis [9].

Diabetes mellitus is associated with diverse derangements in platelet function, the coagulation, and the fibrinolytic system, all of which can contribute to

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Table 5.1 The potential impact of insulin resistance and diabetes on thrombosis

Factors predisposing to thrombosis
• Increased platelet mass
• Increased platelet activation
– Platelet aggregation
– Platelet degranulation
– Platelet cAMP and cGMP
– Thromboxane synthesis
• Increased procoagulant capacity of platelets
• Elevated concentrations and activity of procoagulants
– Fibrinogen
– von Willebrand factor and procoagulant activity
– Thrombin activity
– Factor VII coagulant activity
• Decreased concentration and activity of antithrombotic factors
– Antithrombin III activity
– Sulfation of endogenous heparin
– Protein C concentration

Modified from Schneider DJ and Sobel BE. *Coron. Artery Dis*. 3:26–32, 1992

Table 5.2 The potential impact of insulin resistance and diabetes on fibrinolysis

Factors attenuating fibrinolysis
• Decreased t-PA activity
• Increased PAI-1 synthesis and activity
– Directly increased by insulin
– Increased by hyperglycemia
– Increased by hypertriglyceridemia and increased FFA (free fatty acids)
– Synergistically increased by hyperinsulinemia combined with elevated triglycerides and FFA
• Decreased concentrations of α_2-antiplasmin

Modified from Schneider DJ and Sobel BE. *Coron. Artery Dis*. 3:26–32, 1992

prothrombotic state (Tables 5.1 and 5.2). Some are clearly related to metabolic derangements, particularly hyperglycemia. Others appear to be related to insulin resistance and associated hyperinsulinemia. In the material to follow, we will consider mechanisms exacerbating thrombosis as pivotal factors in the progression of atherosclerosis and their therapeutic implications.

Thrombosis and Atherosclerosis

Thrombosis appears to be a major determinant of the progression of atherosclerosis. In early atherosclerosis, microthrombi present on the luminal surface of vessels [10, 11] can potentiate progression of atherosclerosis by exposing the vessel wall to

clot-associated mitogens. In later stages of atherosclerosis, mural thrombosis is associated with growth of atherosclerotic plaques and progressive luminal occlusion, exacerbated in diabetes because of impaired compensatory vascular remodeling [12].

The previously conventional view that high-grade occlusive, stenotic coronary lesions represent the final step in a continuum that begins with fatty streaks and culminates in high-grade stenosis has given way to a different paradigm because of evidence that thrombotic occlusion is frequently the result of repetitive rupture of minimally stenotic plaques. Thus, as many as two thirds of lesions responsible for acute coronary syndromes are minimally obstructive (less than 50% stenotic) at a time immediately before plaque rupture [13, 14]. Multiple episodes of disruption of lipid-rich plaques and subsequent thrombosis appear to be responsible for intermittent plaque growth that underlies occlusive coronary syndromes [15, 16]. While plaque erosion has been recognized as an important cause of acute coronary syndromes [17, 18], characterization of plaque morphology in patients with diabetes plus acute coronary syndrome demonstrates a greater incidence of thin-capped atheroma with a large lipid core none to be prone to plaque rupture [18].

The extent of thrombosis in response to plaque rupture depends upon factors potentiating thrombosis (prothrombotic factors), factors limiting thrombosis (antithrombotic factors), and the local capacity of the fibrinolytic system reflecting a balance between activity of plasminogen activators and their primary physiologic inhibitor, and plasminogen activator inhibitor type-1 (PAI-1). Activity of plasminogen activators leads to the generation of plasmin, an active serine proteinase, from plasminogen, an enzymatically inert circulating zymogen present in high concentration (~2 μM) in blood. The activity of plasmin is limited by inhibitors such as α_2 macroglobulin.

When only limited thrombosis occurs because of active plasmin-dependent fibrinolysis at the time of rupture of a plaque, plaque growth may be clinically silent. When thrombosis is exuberant because of exaggerated thrombin generation, enhanced platelet activation typical of diabetes [19], and limited fibrinolysis, an occlusive thrombus can give rise to an acute coronary syndrome (acute myocardial infarction, unstable angina, or sudden cardiac death).

The principle components of thrombi are fibrin and platelets. Other plasma proteins and white blood cells are incorporated to a variable extent. The rupture of an atherosclerotic plaque initiates coagulation and adhesion of platelets because of exposure to blood of surfaces denuded of endothelium and to constituents of the vessel wall such as collagen. Coagulation is initiated in the tissue factor pathway, activated by hyperinsulinemia and hyperglycemia [20], by tissue factor, a cell membrane-bound glycoprotein [21–23]. Membrane-bound tissue factor binds circulating coagulation factor VII/VIIa to form the coagulation factor "tenase" complex that activates both circulating coagulation factors IX and X expressed on activated macrophages, monocytes, fibroblasts, and endothelium in response to cytokines in the region of the ruptured plaque. Subsequent assembly of the "prothrombinase" complex on platelet and other phospholipid membranes leads to generation of thrombin. Availability of platelet factor Va is a key constituent of the initial prothrombinase complex. Subsequently, thrombin activates coagulation

factor V in blood to form Va. Thrombin, in turn, cleaves fibrinogen to form fibrin. The generation of thrombin is sustained and amplified initially by its activation of circulating coagulation factors VIII and V. Thrombin generation is sustained by activation of other components in the intrinsic pathway including factor XI. Platelets are activated by thrombin, and activated platelets markedly amplify generation of thrombin.

A complex feedback system limits generation of thrombin. The tissue factor pathway becomes inhibited by tissue factor pathway inhibitor (TFPI) previously called lipoprotein-associated coagulation inhibitor (LACI). Furthermore, thrombin attenuates coagulation by binding to thrombomodulin on the surface of endothelial cells. The complex activates protein C (to yield protein Ca) that, in combination with protein S, cleaves (inactivates) coagulation factors Va and VIIIa.

Exposure of platelets to the subendothelium after plaque rupture leads to their adherence mediated by exposure to both collagen and multimers within the vessel wall of von Willebrand factor [24, 25]. The exposure of platelets to agonists including collagen, von Willebrand factor, ADP (released by damaged red blood cells and activated platelets), and thrombin leads to further platelet activation. Activation is a complex process that entails shape change (pseudopod extension that increases the surface area of the platelet); activation of the surface glycoprotein IIb/IIIa; release of products from dense granules such as calcium, ADP, and serotonin and from alpha granules such as fibrinogen, factor V, growth factors, and platelet factor 4 that inhibits heparin; and a change in the conformation of the platelet membrane that promotes binding to phospholipids and assembly of coagulation factors.

Activation of surface glycoprotein IIb/IIIa results in a conformational change that exposes a binding site for fibrinogen on the activated conformer [26]. Each molecule of fibrinogen can bind two platelets, thereby leading to aggregation. Surface expression of P-selectin leads to the formation of platelet–leukocyte aggregates and to the activation of monocytes and neutrophils [27].

After activation, the plasma membranes of platelets express negatively charged phospholipids on the outer surface that facilitate the assembly of protein constituents and subsequently activity of the tenase and prothrombinase complexes [23]. Thus, platelets are participated in thrombosis by (1) forming a hemostatic plug (shape change, adherence to the vascular wall and aggregation with other platelets and with leukocytes); (2) supplying coagulation factors and calcium (release of alpha and dense granule contents); (3) providing a surface for the assembly of coagulation factor complexes; and (4) simulating vasoconstriction by releasing thromboxane and other vasoactive substances.

As noted previously, thrombosis-complicating plaque rupture can occlude the lumen entirely or, when limited, contribute in a stepwise fashion over time to progressive stenosis. Mechanisms by which thrombi can contribute to plaque growth include incorporation of an organized thrombus into the vessel wall [28]. Exposure of vessel wall constituents to clot-associated mitogens and cytokines can accelerate neointimalizaiton and migration and proliferation of vascular smooth muscle cells in the media. Fibrin and fibrin degradation products promote the migration of vascular smooth muscle cells and are chemotactic for monocytes [29]. Thrombin itself and

growth factors released from platelet alpha granules such as platelet-derived growth factor and transforming growth factor beta activate smooth muscle cells potentiating their migration and proliferation [30–33]. The powerful role of platelets has been demonstrated by a reduction in the proliferation of smooth muscle cells after mechanical arterial injury in thrombocytopenic rabbits with atherosclerosis [34].

Both local and systemic factors can influence the extent of thrombosis likely to occur in association with plaque rupture. The morphology and biochemical composition of the plaque influence thrombogenic potential. Atheromatous plaques with substantial lipid content are particularly prone to initiate thrombosis in contrast to the antithrombotic characteristics of the luminal surface of the normal vessel wall [35].

Both the severity of vascular injury and the extent of plaque rupture influence the extent to which blood is exposed to subendothelium and consequently to thrombogenicity. The balances between the activity of prothrombotic factors and antithrombotic factors in blood and between thrombogenicity and fibrinolytic system capacity are important determinants of the nature and extent of a thrombotic response to plaque rupture. In subjects with type 2 diabetes, the balances between determinants are shifted toward potentiation and persistence of thrombosis and, hence, toward acceleration of atherosclerosis [36]. The same is true in patients with syndromes of insulin resistance (the metabolic syndrome) [37].

Platelet Function in Subjects with Diabetes Mellitus

The activation of platelets and their participation in a thrombotic response to rupture of an atherosclerotic plaque are critical determinants of the extent of thrombosis, incremental plaque growth, and the development of occlusive thrombi. Increased adherence of platelet to vessel walls manifesting early atherosclerotic changes and the release of growth factors from alpha granules can exacerbate the evolution of atherosclerosis. Evidence of increased activity of platelets in patients with diabetes is reflected by increased concentrations in blood of soluble CD40 (a platelet released mediator of thrombosis and inflammation) and P-selectin (reflecting platelet activation) [36]. Patients with diabetes, particularly those with macrovascular disease, have an increased circulating platelet mass secondary to increased ploidy of megakaryocytes [37]. Activation of platelets is increased with type 2 diabetes, mediated in part by increased VLDL and remnant lipoprotein particles [38, 39]. This is reflected by increased concentrations in urine of a metabolite of thromboxane A2, thromboxane B2, and by the spontaneous aggregation of platelets [40–42] in blood. The prevalence of spontaneous aggregation of platelets correlates with the extent of elevation of concentrations of HbA1c [41]. Stringent glycemic control decreases concentrations in urine of thromboxane B2 [40, 42]. In addition, platelets isolated from the blood of subjects with diabetes exhibit impaired vasodilatory capacity [43], apparently mediated by release of a short-acting platelet-derived substance(s) that interferes with the ADP-induced dilatory response seen in normal vessels with intact endothelium [44].

Evidence of Increased Platelet Reactivity

Platelets from subjects with both type 1 and 2 diabetes are hyperreactive [45–49] and in normal subjects subjected to combined hyperglycemia and hyperinsulinemia [50]. Platelet aggregometry performed with platelet-rich plasma and with suspensions of washed platelets in buffers from people with diabetes and control subjects has demonstrated increased aggregation of platelets in response to agonists such as ADP, epinephrine, collagen, arachidonic acid, and thrombin. In addition, spontaneous (in the absence of added agonists) aggregation of platelets from subjects with diabetes is increased compared with aggregation of those from nondiabetic subjects [48], mediated in part by increased expression of platelet FcgammaRIIa receptor [51–54] and by the collagen receptor, glycoprotein VI [55, 56].

Platelets from subjects with diabetes exhibit increased degranulation in response to diverse stimuli. The capacity to promote growth of smooth muscle cells in vitro is greater as shown by exposure of vascular smooth muscle cells to platelets from subjects with poorly controlled compared with well controlled diabetes [57, 58]. Because alpha granules contain growth factors, the enhanced growth promoting activity of platelets from subjects with poorly controlled diabetes appears likely to be secondary to increased alpha granule degranulation.

The threshold for induction of release of substances residing in dense granules in response to thrombin is lower in platelets from diabetic compared with nondiabetic subjects [59]. In addition, the procoagulant capacity of platelets from subjects with diabetes mellitus is increased [60, 61]. Thus, the generation of coagulation factor Xa and of thrombin is increased by three- to sevenfold in samples of blood containing platelets from diabetic compared with those from nondiabetic subjects [61].

In patients with diabetes, adhesion of platelets is increased because of increased surface expression of glycoprotein Ib-IX [62]. The binding of von Willebrand factor multimers expressed on endothelial cells to glycoprotein Ib-IX mediates adherence and promotes subsequent activation of platelets. Adherence is promoted also by increased concentrations of and activity of von Willebrand factor [62, 63]. Circulating von Willebrand factor stabilizes the coagulant activity of circulating coagulation factor VIIIa [64].

An altered cellular distribution of guanine nucleotide binding proteins (G-proteins) appears to contribute to the increased reactivity of platelets in people with diabetes mellitus [65]. Platelet reactivity would be expected to be increased by the decreased concentrations of inhibitory G-proteins that have been reported [66]. In addition, platelet reactivity would be increased by the greater turnover of phosphoinositide and consequent intraplatelet release of calcium that have been seen [67, 68].

As noted above, activation of platelets leads to the expression of specific conformers of specific glycoproteins. Determination of the percentage of platelets expressing activation-dependent markers with flow cytometry can be used to delineate the extent of platelet activation that has occurred in vivo. Increased surface expression of CD63 (a marker of lysosomal degranulation), thrombospondin (a

marker of alpha granule degranulation), and CD62 (also called P-selectin), another marker of alpha granule degranulation, has been observed with platelets isolated from patients with newly diagnosed diabetes and those with advanced diabetes regardless of whether or not overt macrovascular complications were present [69, 70]. The increased plasminogen activator type-1 inhibitor (PAI-1) in plasma (see below) in patients with diabetes is associated with a paradoxically decreased platelet content of PAI-1 [71], consistent with the possibility that release of PAI-1 from the platelets may contribute to the increased PAI-1 in blood.

Platelet survival is reduced in subjects with diabetes. The reduction is most pronounced in those with clinical evidence of vascular disease [72]. Thus, it appears to be more closely correlated with the severity of vascular disease [73] than with the presence of diabetes per se. Accordingly, the decreased survival of platelets may be both a marker of extensive vascular disease and a determinant of its severity.

Adherence of platelets to vessel walls early after injury resulting in de-endothelialization is similar in diabetic and nondiabetic animals [74]. By contrast, increased adherence of platelets to injured arterial segments 7 days after injury occurs in diabetic BB Wistar rats compared with that in control animals. A continued interaction of platelets with the vessel wall after injury is likely to be related to a decreased rate of healing and re-endothelialization in diabetic animals rather than to an increased propensity for adherence per se [75]. Regardless, continued interaction of platelets with the vessel wall and continued exposure of the vessel wall to growth factors released from alpha granules of platelets are likely to accelerate and exacerbate atherosclerosis.

Mechanisms Responsible for Hyperreactivity of Platelets in People with Diabetes

Increased expression of the surface glycoproteins Ib and IIb/IIIa has been observed in platelets from subjects with both type 1 and type 2 diabetes [62]. Glycoprotein Ib/IX binds to von Willebrand factor in the subendothelium and is responsible for adherence of platelets at sites of vascular injury. Interaction between glycoprotein Ib/IX and von Willebrand factor leads to activation of platelets. Activation of glycoprotein IIb/IIIa leads to the binding of fibrinogen and aggregation of platelets. Thus, increased expression of either or both of these two surface glycoproteins is likely to contribute to the increased reactivity that has been observed platelets from people with diabetes. Overexpression of the FcgammaRIIa [51–56] and increased GP VI signaling [55, 56] may contribute as well.

Winocour and his colleagues have shown an association between decreased membrane fluidity and hypersensitivity of platelets to thrombin [76]. Reduced membrane fluidity may be a reflection of increased glycation of membrane proteins. A reduction in membrane fluidity occurs following incubation of platelets in media containing concentrations of glucose similar to those seen in blood from subjects

with poorly controlled diabetes. Because membrane fluidity is likely to alter membrane receptor accessibility by ligands, reduced membrane fluidity may contribute to hypersensitivity of platelets. Accordingly, improved glycemic control would be expected to decrease glycation of membrane proteins, increase membrane fluidity, and decrease hypersensitivity.

Intracellular mobilization of calcium is critical in several steps involved in the activation of platelets. Platelets from subjects with type 2 diabetes exhibit increased basal concentrations of calcium [77]. Increased phosphoinositide turnover, increased inositide triphosphate production, and increased intracellular mobilization of calcium are evident in response to exposure to thrombin of platelets from subjects with type 2 diabetes [78]. The increased concentrations of several second messengers may contribute to the hypersensitivity seen in platelets from patients with diabetes. In addition, increased production of thromboxane A_2 may contribute to the increased platelet reactivity [43, 45].

We have found that the osmotic effect of increased glucose concentrations increases directly platelet reactivity [79]. Exposure of platelets in vitro to increased concentrations of glucose is associated with increased activation of platelets in the absence and presence of added agonist. Exposure of platelets to isotonic concentrations of glucose or mannitol increases platelet reactivity to a similar extent [79]. Thus, the osmotic effect of hyperglycemia on platelet reactivity may contribute to the greater risk of death and re-infarction that has been associated with hyperglycemia in patients with diabetes and myocardial infarction [80–82].

Insulin alters reactivity of platelets [83]. Exposure of platelets to insulin decreases platelet aggregation in part by increasing synthesis of nitric oxide that, in turn, increases intraplatelet concentrations of the cyclic nucleotides, cyclic guanosine monophosphate, and cyclic adenosine monophosphate. Both of these cyclic nucleotides are known to inhibit activation of platelets. Thus, it is not surprising that an insulin concentration-dependent increase in nitric oxide production exerts anti-aggregatory effects. Insulin deficiency typical of type 1 diabetes and seen in advanced stages of type 2 diabetes may contribute to increased platelet reactivity by decreasing the tonic inhibition of platelet reactivity otherwise induced by insulin. Furthermore, abnormal insulin signaling may contribute in subjects with type 2 diabetes. Insulin decreases platelet aggregation and adhesion in patients without diabetes, an effect not seen in those with type 2 diabetes [84]. Accordingly, the increased resistance to insulin typical of type 2 diabetes may contribute to increased platelet reactivity by decreasing tonic inhibition of platelets that would have been induced otherwise by the high prevailing concentration of insulin.

Constitutive synthesis of nitric oxide is reduced in platelets from subjects with both type 1 and type 2 diabetes [85]. The oxidative stress associated with diabetes and its uncoupling of nitric oxide synthase from arginine can exacerbate endothelial dysfunction and activation of platelets secondary to generation of superoxide [4, 86]. Thus, tonic inhibition of platelets as well as insulin-dependent suppression of reactivity may be reduced in subjects with diabetes.

Antiplatelet Therapy and Diabetes

While several studies have identified beneficial cardiovascular effects of aspirin for primary prevent in people with diabetes, a meta-analysis that included results from 33,679 demonstrated that the use of aspirin for primary prevention of cardiovascular disease in patients with diabetes mellitus increases the risk of total bleeding without reducing the risk of major adverse cardiovascular outcomes [87]. Considered together, data acquired in vitro and in vivo suggest that platelets from subjects with diabetes are hypersensitive to diverse agonists. Unfortunately, currently available antiplatelet therapy does not restore normal responsiveness to platelets from subjects with diabetes. In animal preparations simulating selected aspects of diabetes, platelets remain hypersensitive to thrombin despite administration of aspirin [88]. Furthermore, responses to aspirin are suboptimal in people with diabetes [89–91]. These observations suggest that the hypersensitivity is not a reflection of generation of thromboxane A_2, and that the treatment of subjects with diabetes with aspirin (as is being done often inferentially) is unlikely to decrease platelet reactivity to the level typical of that seen with platelets from nondiabetic subjects. Because hyperglycemia per se appears to increase platelet reactivity, improved glycemic control is a critical component of the antithrombotic regimen.

Platelet hyperreactivity has been observed before and during treatment with clopidogrel [48, 92, 93]. This increased platelet reactivity has been associated with a greater risk of subsequent cardiovascular events [92]. Higher doses of clopidogrel suppress platelet reactivity in patients with diabetes to a greater extent, but the effect remains heterogeneous and uniform suppression of platelet hyperreactivity was not seen [94]. Furthermore, withdrawal of cloplidogrel is associated with proinflammatory and prothrombotic effects [95]. These results suggest that patients with diabetes are likely to benefit from more powerful antiplatelet regimens. Ticagrelor, a more powerful $P2Y_{12}$ antagonist was shown to be more effective than clopidogrel (both agents were used in combination with aspirin) in preventing recurrent heart attack, stroke, and death in patients with acute coronary syndromes, and the beneficial effects of ticagrelor were similar in patients with and without diabetes [96]. Prasugrel plus aspirin was more effective than clopidogrel plus aspirin in patients with acute coronary syndromes, and the beneficial effects were maintained in patients with diabetes [97]. Accordingly, for patients with diabetes and acute coronary syndrome, dual-antiplatelet therapy with aspirin plus either ticagrelor or prasugrel is preferred.

The Coagulation System and Diabetes Mellitus

Activation of the coagulation system leads to the generation of thrombin and thrombin-mediated formation of fibrin from fibrinogen. The generation of thrombin depends on activation of procoagulant factors. It is limited by antithrombotic factors

and inhibitors. Fibrinopeptide A (FPA) is released when fibrinogen is cleaved by thrombin. It has a very short half-life in the circulation and is cleared promptly by the kidneys. Elevated concentrations in blood are indicative of thrombin activity in vivo [98]. Subjects with diabetes mellitus (both types 1 and 2) have increased concentrations of FPA in blood and in urine compared with corresponding concentrations in nondiabetic subjects [99–102]. The highest concentrations are observed in patients with clinically manifest vascular disease [100, 102].

The increased concentrations of FPA seen in association with diabetes reflect an altered balance between prothrombotic and antithrombotic determinants in subjects with diabetes mellitus favoring thrombosis. This interpretation is consistent with other observations suggesting that generation of thrombin is increased with diabetes resulting in increased concentrations in blood of thrombin-antithrombin complexes [103]. The steady-state concentration of thrombin-antithrombin complexes in blood is a reflection of the rate of formation of thrombin being generated over time.

The increased generation of thrombin in people with diabetes is likely to be dependent on increased activity of factor Xa. This has been observed in patients with type 1 diabetes [104]. Factor Xa, a major component of the prothrombinase complex, is formed from components including circulating coagulation factor X assembled on phospholipid membranes in association with the tissue factor VIIa complex. Thrombin is generated by the prothrombinase complex comprising factors Xa, Va, and II assembled on phospholipid membranes. The activity of this complex is reflected by prevailing concentrations in blood of prothrombin fragment 1 + 2, a cleavage product of factor II (prothrombin). Increased concentrations of prothrombin fragment 1 + 2 in blood from patients with type 1 diabetes have been observed, consistent with the presence of a prothrombotic state.

Mechanisms Responsible for a Prothrombotic State Associated with Diabetes

Patients with diabetes mellitus have increased concentrations in blood of the prothrombotic factors fibrinogen, von Willebrand factor, and factor VII coagulant activity [105–107]. Among the three coagulation factors, fibrinogen has been most strongly associated with the risk of development of cardiovascular disease [108]. Although the mechanisms responsible for increased concentrations of fibrinogen and von Willebrand factor have not yet been fully elucidated, elevated concentrations in blood of insulin and proinsulin may be determinants in people with type 2 diabetes. This possibility is suggested by the close correlation between concentrations of fibrinogen with those of insulin and proinsulin in healthy subjects [109]. Because prediabetic subjects and people with early stages of diabetes have marked insulin resistance that leads to a compensatory increase in the concentrations in blood of insulin and proinsulin [110–112], the hyper(pro)insulinemia of type 2 diabetes is likely to underlie, at least in part, the typically increased concentrations of fibrinogen. Improvement in metabolic control per se (euglycemia and amelioration

of hyperlipidemia) has not been associated with normalization of the increased concentrations in blood of fibrinogen, von Willebrand factor, or factor VII coagulant activity [107]. By the same token, the extent of elevation of concentrations in blood of prothrombin fragment 1 + 2 is not closely correlated with the concentration of hemoglobin A1c, a marker of glycation of proteins [113]. First-degree nondiabetic relatives of subjects with type 2 diabetes exhibit increased concentrations of fibrinogen and factor VII coagulant activity in blood compared with values in age-matched controls [114]. Thus, the increases in fibrinogen and factor VII coagulant activity are associated with other, presumably independent features of insulin resistance. Consistent with this observation, controlling hyperglycemia reduces tissue factor expression in patients with type 2 diabetes [115]. Infusion of insulin increased tissue factor procoagulant activity, and the combination of hyperinsulinemia plus hyperglycemia increased tissue factor procoagulant activity to the greatest extent [115]. Accordingly, increased concentrations of prothrombotic factors seen typically in subjects with type 2 diabetes mellitus are likely to reflect the combination of metabolic derangements typical of the diabetic state in combination with insulin resistance and hyperinsulinemia. In fact, hormonal abnormalities, particularly insulin resistance and hyper(pro)insulinemia, appear to underlie the prothrombotic state [109–114].

As mentioned in the preceding section on platelet function, procoagulant activity is increased in platelets from subjects with diabetes. Procoagulant activity of monocytes is increased as well [116]. The negatively charged phospholipid surface of platelets and monocytes catalyzes both formation and activity of the tenase and prothrombinase complexes. Thus, increased procoagulant activity of platelets and monocytes can potentiate thrombosis.

Decreased activity of antithrombotic factors in blood can potentiate thrombosis. Of note, concentrations in blood of protein C and activity of antithrombin are decreased in diabetic subjects [117–120], although not universally [103]. Unlike changes in concentrations of prothrombotic factors, altered concentrations and activity of antithrombotic factors appear to be reflections of the metabolic state typical of diabetes, either type 1 or type 2, especially hyperglycemia. Thus, decreased antithrombotic activity has been associated with nonenzymatic glycation of antithrombin.

To recapitulate, functional activities of the prothrombinase complex and of thrombin itself are increased consistently in blood of people with diabetes. The increased activity is likely to be a reflection of increased procoagulant activity of platelets and monocytes in association with increased concentrations of fibrinogen, von Willebrand factor, and factor VII. Diminished activity in blood of antithrombotic factors secondary to glycation of antithrombin and protein C may contribute to the prothrombotic state. To the extent that glycation of proteins contributes to a prothrombotic state, optimal glycemic control should attenuate it. Accordingly, the most effective mechanism available to attenuate a prothrombotic state is normalization of the hormonal and metabolic abnormalities in patients with diabetes. Results in the DCCT trial are consistent with this interpretation. Despite the fact that the trial focused on microvascular complications of diabetes, known to be influenced by

hyperglycemia, a trend toward reduction of macrovascular events was seen with stringent and glycemic control [121]. This trend is consistent with reduction of the intensity of the prothrombotic state and hence attenuation of atherogenesis, determinants of its sequela, or both.

A prespecified analysis of the Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) compared the effects of rivaroxaban (2.5 mg twice daily) plus aspirin (100 mg daily) versus placebo plus aspirin in patients with diabetes mellitus versus without diabetes mellitus in preventing major vascular events [122]. A consistent and similar relative risk reduction was seen for benefit of rivaroxaban plus aspirin ($n = 9152$) versus placebo plus aspirin ($n = 9126$) in patients both with ($n = 6922$) and without ($n = 11{,}356$) diabetes for the primary efficacy end point (hazard ratio, 0.74, $p = 0.002$; and hazard ratio, 0.77, $p = 0.005$, respectively, $p_{\text{interaction}} = 0.77$) and all-cause mortality (hazard ratio, 0.81, $p = 0.05$; and hazard ratio, 0.84, $p = 0.09$, respectively; $p_{\text{interaction}} = 0.82$). Despite the prothrombotic state associated with diabetes, the incidence of bleeding was similar in patients with and without diabetes [122]. In the treatment of atrial fibrillation, results show similar efficacy and safety of direct-acting anticoagulants (DOACs) compared with warfarin in patients with or without diabetes. Treatment with DOACS in patients with diabetes has been associated with a significant relative reduction in vascular death compared to warfarin [123].

Diabetes and Fibrinolysis

Decreased fibrinolytic system capacity is observed consistently in blood from patients with diabetes mellitus, particularly those with type 2 diabetes [124–127]. It has been known for many years that obesity is associated with impaired fibrinolysis [128]; that elevated blood triglycerides and other hallmarks of hyperinsulinemia are associated with increased activity of PAI-1 [129]; and that elevated PAI-1 is a marker of increased risk of acute myocardial infarction as judged from its presence in survivors compared with age-matched subjects who had not experienced any manifestations of overt coronary artery disease [130]. Recently, the 4G/5G PAI-1 polymorphism has been found to increase the risk of recurrence of myocardial infarction in nonhyperlipidemic subjects [131] and that increased PAI-1 is associated with increasing concentrations of glucose within the normal range in nondiabetic subjects [132]. We have found that impaired fibrinolysis in subjects with type 2 diabetes mellitus, not only under baseline conditions but also in response to physiologic challenge, was attributable to augmented concentrations in blood of circulating PAI-1. Furthermore, obese diabetic subjects exhibited threefold elevations of PAI-1 in blood compared with values in nondiabetic subjects despite tissue-type plasminogen activator (t-PA) values that were virtually the same. The observation of an impairment of fibrinolysis not only under basal conditions but also in response to physiologic stress implicates the pathophysiologic import of the abnormality [125]. Subsequently, we found that precursors of insulin including proinsulin and

des[30,31]- and des[63,64]proinsulin-induced time- and concentration-dependent elevation in expression of PAI-1 by human hepatoma cells in culture [133]. In addition, we found that concentrations of PAI-1 can be elevated in blood in normal subjects rendered hyperglycemic, hyperinsulinemic, and hyperlipidemic [134]. Furthermore, women with the polycystic ovarian syndrome, known to be associated with hyperinsulinemia, have increased concentrations of PAI-1 in blood that can be reduced by administration of troglitazone, an insulin sensitizer [135].

Thus, people with type 2 diabetes exhibit a decreased fibrinolytic system capacity secondary to increased PAI-1 in blood. Similar derangements are evident in association with other states of insulin resistance and compensatory hyperinsulinemia in conditions such as obesity [125, 128], hypertension [136], and the polycystic ovarian syndrome [135, 137, 138].

Because the endogenous fibrinolytic system influences the evolution of thrombosis and the rapidity and extent of lysis of thrombi when vascular damage is repaired, overexpression of PAI-1 is likely to exacerbate development and the persistence of thrombi. Results in transgenic mice deficient in PAI-1 compared with wild-type animals are consistent with this hypothesis. Thus, 24 h after arterial injury, persistence of thrombosis and the residual thrombus burden were greater than in wild-type mice that were not deficient in PAI-1 [139]. Thus, increased expression of PAI-1 typical of that seen with the metabolic changes present in type 2 diabetes [140] is likely to be a determinant of increased and persistent thrombosis. Consistent with this observation, higher concentrations of PAI-1 in blood have been independently associated with a greater risk of coronary heart disease [141].

Mechanisms Responsible for the Overexpression of PAI- in Diabetes

Increased expression of PAI-1 in diabetes is undoubtedly multifactorial. A direct effect of insulin on the expression of PAI-1 has been suggested by a positive correlation between the concentration of insulin and PAI-1 in vivo [124, 125, 129, 136–138, 142]. Triglycerides and their constituents (fatty acids) appear to contribute to the overexpression of PAI-1 in view of the fact that both insulin and triglycerides independently increase expression of PAI-1 by human hepatoma cells in vitro [140, 143–145]. Liver steatosis is another determinant of elevated concentrations of PAI-1, perhaps indicative of the response of both to derangements in the TNF-signaling pathway [146]. Insulin and triglycerides exert a synergistic increase in accumulation of PAI-1 in conditioned media when both are present in pathophysiologic concentrations [140]. Analogous results are obtained with insulin in combination with VLDL-triglyceride, emulsified triglycerides, or albumin-bound-free (nonesterified) fatty acids. Thus, the combination of hyperinsulinemia and hypertriglyceridemia increases expression of PAI-1 consistent with the possibility that the combination is a determinant of the increased PAI-1 in blood in vivo in people with diabetes. Furthermore, because elevated concentrations of glucose increase

expression of PAI-1 by endothelial cells and vascular smooth muscle cells in vitro [147, 148], the metabolic state typical of diabetes may elevate concentrations of PAI-1 in blood emanating from release of PAI-1 from vessel wall cells.

A combination of hyperinsulinemia, hypertriglyceridemia, and hyperglycemia increases the concentration of PAI-1 in blood in normal subjects [134]. Although neither the infusion of insulin with euglycemia maintained by euglycemic clamping nor the infusion of triglycerides without induction of hyperinsulinemia in normal subjects increases the concentration of PAI-1 in blood, the induction of hyperglycemia, hypertriglyceridemia, and hyperinsulinemia by infusion of glucose plus emulsified triglycerides plus heparin (to elevate blood free fatty acids) does increase concentrations of PAI-1 in blood. Of note, the infusion of insulin under euglycemic clamp conditions results in a marked decrease in the concentration of blood triglycerides and free fatty acids. Thus, results obtained in the infusion studies demonstrate that the combination of hyperinsulinemia, hyperglycemia, and hypertriglyceridemia is sufficient to increase expression of PAI-1 in healthy subjects. However, results in these studies do not answer the question of whether, as in the case in vitro, insulin increases expression of PAI-1 when concentrations of glucose, triglycerides, and free fatty acids are all maintained within normal ranges. What is clear is that a combination of hormonal (hyperinsulinemia) and metabolic (particularly hypertriglyceridemia) derangements typical of type 2 diabetes mellitus elevate the concentration of PAI-1 in blood. The elevations of PAI-1 may subject people with diabetes to double jeopardy because the ratio of PAI-1 activity to the concentration of PAI-1 protein increases when the latter is high. This appears to reflect a slower rate of loss of PAI-1 activity associated with higher concentrations of PAI-1 protein [149].

Adipose tissue is another potential source of the increased blood PAI-1 in subjects with type 2 diabetes mellitus. Studies performed on genetically obese mice demonstrated that PAI-1 mRNA expression was increased four- to fivefold in mature adipocytes [150]. The injection of insulin into lean mice increased expression of PAI-1 in adipocytes, an effect seen also with 3T3-L1 adipocytes in vitro. We have found that elaboration of PAI-1 from adipocytes is increased by TGF-β, known to be released from activated platelets [151] secondary to increased transcription and furthermore that caloric restriction per se lowers elevated PAI-1 in blood in obese, nondiabetic human subjects [152]. Thus, the elevated concentrations of PAI-1 in blood seen in subjects with type 2 diabetes appear to be secondary to effects of hyperinsulinemia, particularly in combination with hypertriglyceridemia, and to effects of other mediators implicated in the prothrombotic state seen with diabetes on expression of PAI-1 by hepatic, arterial, and adipose tissue.

In addition to elevated PAI-1 in blood, expression of PAI-1 in vessel walls with subsequent elaboration into blood is increased by insulin [153]. Pathophysiologic concentrations of insulin increase the expression of PAI-1 by human arteries in vitro [154], an effect seen in both arterial segments that appear to be grossly normal and those that exhibit atherosclerotic changes [155]. The increased expression of PAI-1 is seen in arterial segments from subjects with or without insulin-resistant states. Augmented expression of PAI-1 is seen in response to insulin with vascular smooth muscle cells in culture [156] and with co-cultured endothelial cells and smooth

muscle cells [153]. Insulin increases expression of PAI-1 by vascular tissue in vivo. Local elaboration of PAI-1 follows perfusion with insulin in forearm vascular beds of healthy human subjects [157].

With the use of a co-culture system, one mechanism by which insulin increases arterial wall expression of PAI-1 has been characterized [153]. In vivo, insulin present in the luminal blood is known to be transported from the luminal to the abluminal surface of endothelial cells. In vitro, smooth muscle cells exposed to insulin have been shown to release a soluble factor(s) that increases endothelial cell expression of PAI-1. Thus, it appears likely that insulin in vivo alters expression of PAI-1 in arterial walls through a direct effect on vascular smooth muscle cells that, in turn, increases endothelial cell expression of PAI-1 in a paracrine fashion.

Therapy designed to reduce insulin resistance, the resultant hyperinsulinemia, or both have been shown to reduce PAI-1 in blood as well. Thus, treatment of women with the polycystic ovarian syndrome with metformin or troglitazone decreased concentrations in blood of insulin and of PAI-1 [138]. Changes in the concentrations of PAI-1 in blood correlated significantly with those of insulin [135]. Treatment of patients with type 2 diabetes with an insulin secretagogue, repaglinide, was associated with greater concentrations in blood of PAI-1 compared with treatment with metformin [158]. Similarly, treatment of patients with type 2 diabetes with pioglitazone decreases the concentration of PAI-1 [159]. One mechanism by which thiazolidinediones may decrease expression of PAI-1 is through induction of adiponectin [160]. The effect of rosiglitazone on expression of PAI-1 correlates positively with changes in the concentration of adiponectin and the effect of rosiglitazone of concentrations of PAI-1 in blood is attenuated in mice genetically deficient in adiponectin [160]. The concordance supports the view that insulin contributes to the increased PAI-1 expression seen in vivo. Despite meta analyses that spanned intense controversy [161–163], there is no convincing evidence that rosiglitazone increases mortality [164–168], and there is evidence that another thiazolidinedione, pioglitazone, diminishes it [169].

Human subjects who participate in relatively large amounts of leisure time physical activity have low levels of PAI-1 activity in blood [170]. After adjustment for variables indicative of syndromes of insulin resistance such as high body mass index and waist:hip ratio in addition to advanced age and elevated concentrations of triglycerides, the association of PAI-1 activity with physical activity was no longer significant. This observation, particularly in combination with the results seen after therapy with troglitazone and metformin in women with the polycystic ovarian syndrome, demonstrates that interventions designed to attenuate insulin resistance will lower concentrations of PAI-1 in blood and increase fibrinolytic system capacity.

The exposure of human hepatoma cells to gemfibrozil decreases basal and insulin-stimulated secretion of PAI-1 [171]. This inhibitory effect has been observed in vitro but not in vivo [172, 173] despite reductions in vivo in the concentration of triglycerides in blood by 50–60%. No changes in insulin sensitivity or concentrations of insulin in the blood were seen after treatment of patients with gemfibrozil. Thus, unlike therapy with agents that reduce insulin resistance and lower concentrations of insulin, therapy with gemfibrozil that reduces triglycerides without

affecting concentrations of insulin does not lower PAI-1 in vivo. These observations support the likelihood that insulin is the critical determinant of altered expression of PAI-1 in subjects with insulin resistance such as those with type 2 diabetes mellitus. As judged from results in studies in which human hepatoma cells were exposed to insulin and triglycerides in vitro, modest elevations in the concentrations of triglycerides and free fatty acids in the setting of hyperinsulinemia may be sufficient to augment expression of PAI-1. Thus, although the concentration of triglycerides in patients treated with gemfibrozil was decreased by 50%, the prevailing concentration of triglycerides may have been sufficient to lead to persistent elevation of PAI-1 in blood in the setting of hyperinsulinemia. Recent results in studies with several statins including atorvastatin fail to show concordant changes in PAI-1 in blood, consistent with this possibility [174].

Fibrinolysis and Arterial Mural Proteolysis

In addition to their role in blood, plasminogen activators and PAI-1 appear to be involved in the evolution of macroangiography in the arterial wall itself [175]. Intramural plasminogen activators and PAI-1 influence proteolytic activity of matrix metalloproteinases (MMPs) that are activated from zymogens by plasmin. Cell surface plasmin-dependent proteolytic activation of MMPs promotes migration of smooth muscle cells and macrophages into the neointima and tunica media. Activation of MMPs appears to be a determinant of plaque rupture in complex atheroma and advanced atherosclerotic lesions, particularly in the vulnerable acellular shoulder regions of plaques [176].

Conversely, overexpression of PAI-1, by inhibiting intramural proteolysis and turnover of matrix, may contribute to accumulation of extracellular matrix particularly in early atheromatous lesions. Overexpression of PAI-1 and the resultant accumulation of extracellular matrix have been implicated as a substrate for activation and migration of smooth muscle cells, chemotaxis of macrophages, and hence, acceleration of early atherosclerosis. Analogously increased expression of PAI-1 has been observed in zones of early vessel wall injury after fatal pulmonary thromboembolism [177].

Taken together, these observations imply that an imbalance between the activity of plasminogen activators and the activity of PAI-1 can contribute to progression of atherosclerosis in diverse directions under diverse conditions. In early lesions, excess activity of PAI-1 may potentiate accumulation of matrix and its consequences. In complex lesions and late atherosclerosis, excess activity of plasminogen activators may exacerbate plaque rupture. Our observations regarding the relative amounts of plasminogen activators and of PAI-1 in association with the severity of atherosclerosis are consistent with both [178]. The tissue content of PAI-1 is increased in early atherosclerotic lesions exemplified by fatty streaks. By contrast, the tissue content of plasminogen activators is increased in more complex lesions at a time when smooth muscle cell proliferation is prominent.

The effects of PAI-1 in vessel wall repair have been clarified in animals genetically modified to be deficient in PAI-1 (PAI-1 knockout mice). Removal of noncellular debris and migration of smooth muscle cells are accelerated after mechanical or electrical injury of arteries in PAI-1-deficient mice [179]. However, clot burden and persistence are increased. Thus, it appears likely that excess of either plasminogen activator or PAI-1 activity in the vessel wall may potentiate atherosclerosis. Excess PAI-1 may potentiate mural thickening secondary to accumulation of extracellular matrix and noncellular debris with diminished migration into the neointima of vascular smooth muscle cells during evolution of plaques destined to be vulnerable to rupture. Excess plasminogen activator activity may potentiate degradation of matrix and plaque rupture [175] in mature, vulnerable plaques. Consistent with this view, we have found increased immunoassayable PAI-1 and decreased urokinase plasminogen activator (u-PA) in atherectomy specimens from occlusive coronary lesions in patients with diabetes with or without restenosis compared with values in corresponding specimens from nondiabetic subjects [154]. Conversely, immunoassayable urokinase in the atheroma was markedly diminished in association with diabetes.

It has been demonstrated that people with type 2 diabetes are remarkably prone not only to primary coronary lesions but also to restenosis after angioplasty [9, 180, 181]. Our observations with extracted atheroma suggest that restenosis, especially that following iatrogenic injury to vessel walls associated with percutaneous coronary intervention, may develop, in part, because of increased expression of PAI-1. Although increased PAI-1 attenuates cell migration, it augments proliferation and inhibits apoptosis [182]. Thus, restenosis may be exacerbated by increased PAI-1 resulting in increased proliferation and decreased apoptosis of smooth muscle cells within the arterial wall [183].

Therapeutic Implications

Consideration of the derangements in platelet function, the coagulation system and the fibrinolytic system, and their contributions to exacerbation of macrovascular disease in type 2 diabetes gives rise to several therapeutic approaches. Because many of the derangements contributing to a prothrombotic state in diabetes are caused by hyperglycemia, rigorous glycemic control is essential. Accordingly, the use of diet, exercise, oral hypoglycemic agents, insulin sensitizers, and if necessary insulin itself are appropriate to lower hemoglobin A1c to <7%. Because other derangements contributing to a prothrombotic state such as attenuation of fibrinolysis appear to be related to insulin resistance and hyper(pro)insulinemia, the use of insulin sensitizers as adjuncts to therapy with insulin or with other oral hypoglycemic agents is likely to be helpful.

Agents that enhance sensitivity to insulin and thereby promote glycemic control but limit hyperinsulinemia merit particular emphasis. Thiazolinediones lower elevated PAI-1 in patients with hyperinsulinemia by attenuating insulin resistance,

increasing peripheral glucose disposal, and modifying transcription of genes with protein products that are involved in carbohydrate and lipid metabolism as well as in fibrinolytic system activity. This class of agents exerts favorable effects on intimal medial thickness of carotid arteries in people with type 2 diabetes [184, 185] and appears to reduce the progression of macrovascular disease [169].

Use of metformin may attenuate abnormalities in the fibrinolytic system as well as improving glycemic control, although the primary mechanism of action of the drug differs from that of troglitazone. Metformin and its congeners decrease hepatic glucose output thereby normalizing carbohydrate and lipid metabolism and reducing requirements for insulin and lowering circulating endogenous insulin levels. Side effects are usually minor gastrointestinal disturbances, but lactic acidosis can be encountered particularly in patients with renal dysfunction, congestive heart failure, liver disease, or any condition predisposing to metabolic acidosis including diabetic ketoacidosis or excessive consumption of alcohol. Metformin should be discontinued temporarily when contrast agents are used (e.g., coronary angiography) to avoid lactic acidosis. In contrast to thiazolidinediones, metformin can produce hypoglycemia, particularly when it is used with sulfonylureas.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors favorably affect cardiovascular outcomes. SGLT2 inhibitors function through a novel mechanism of reducing renal tubular glucose reabsorption, producing a reduction in blood glucose without stimulating insulin release. Other benefits may include favorable effects on blood pressure and weight. A meta-analysis [186] that assessed cardiovascular outcomes of all four available SGLT2 inhibitors in patients with type 2 diabetes demonstrated that SGLT2 inhibitors were associated with a reduced risk of major adverse cardiovascular events (hazard ratio 0.90; 95% confidence interval 0.85–0.95) as well as hospitalization for heart failure and cardiovascular death (hazard ratio 0.78; 95% confidence interval 0.73–0.84). Based on this convincing class effect, SGLT2 inhibitors should be considered for all patients with type 2 diabetes, particularly those with cardiovascular disease.

Glucagon-like peptide 1 (GLP-1) receptor agonists stimulate glucose-dependent insulin release from the pancreatic islets and slow gastric emptying, inhibit inappropriate post-meal glucagon release, and reduce food intake. A meta-analysis [187] demonstrated that GLP-1 receptor agonist treatment reduced the incidence of major adverse cardiovascular events by 12% (hazard ratio 0.88, 95% confidence interval 0.82–0.94; $p < 0.0001$). Consistent reductions were apparent in death from cardiovascular cause (hazard ratio 0.88; 95% confidence interval 0.81–0.96; $p = 0.003$), fatal or non-fatal stroke (hazard ratio 0.84; confidence interval 0.76–0.93; $p < 0.0001$), and fatal or non-fatal myocardial infarction (hazard ratio 0.91; confidence interval 0.84–1.00; $p = 0.043$).

While aspirin is no longer recommended for primary prevention [87], it should be used in patients with established coronary artery disease (secondary prevention). Patients with diabetes and acute coronary syndrome should be treated with dual-antiplatelet therapy that combines aspirin plus either ticagrelor or prasugrel [96, 97]. The combination of aspirin plus low-dose rivaroxaban reduced cardiovascular events in the COMPASS trial and should be considered for long-term treatment [122].

Several complications and concomitants of diabetes can exacerbate a prothrombotic state and accelerate vascular disease. Thus, hypertriglyceridemia, hypertension, and hyperglycemia must be ameliorated. A target-driven, long-term, intensified intervention aimed at multiple risk factors in patients with type 2 diabetes and microalbuminuria reduces the risk of cardiovascular and microvascular events by about 50% [188].

Hypertension should be treated vigorously, generally with ACE inhibitors because of the demonstrated reduction of progression of renal disease accompanying their use. An alternative may be angiotensin receptor-blocking agents. Despite the ominous portent of macrovascular disease in type 2 diabetes, nephropathy continues to be a dominant life-threatening complication with an extraordinarily high incidence. Its occurrence is clearly related to hyperglycemia and may contribute to a prothrombotic state and acceleration of macrovascular disease through diverse mechanisms. Accordingly, rigorous glycemic control is essential.

Life-style modifications including implementation of a regular exercise program, reduction of obesity through dietary measures, and avoidance or cessation of cigarette smoking should be implemented to reduce the intensity of a prothrombotic state and the progression of macrovascular disease.

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Chapter 6
Genetics of Coronary Artery Disease in Diabetes Mellitus

Mario Luca Morieri and Alessandro Doria

Leveraging Genetics to Decrease the Cardiovascular Burden of Diabetes

Despite the improvements in cardiovascular preventive strategies and the resulting overall decrease in cardiovascular mortality and morbidity that have occurred during the past few decades, patients with diabetes remain at higher risk of cardiovascular disease (CVD) than non-diabetic subjects [1, 2]. This, combined with the ongoing worldwide increase in diabetes prevalence, represents a global health threat with important social and financial implications. Patients with type 2 diabetes (T2D), who are 90–95% of diabetic subjects, often have other cardiovascular risk factors such as hypertension, dyslipidemia, and obesity; however, the increased CVD risk associated with T2D is independent of these other predisposing clinical characteristics, meaning that patients with T2D are at higher CVD risk than patients without diabetes even after accounting for these classic cardiovascular risk factors—an observation that is also true for hyperglycemia [2]. Understanding the mechanisms underlying this increased cardiovascular risk is of pivotal importance in order to achieve a meaningful reduction of CVD in T2D.

One approach to expand knowledge in this field is to search the human genome for variants that are associated with an increased risk of CVD in diabetes, and use the information about the location and function of these variants to infer about the mechanisms involved in the diabetes-induced acceleration of atherogenesis. This information

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can also be used to improve the identification of T2D individuals who are at especially high risk of CVD and to personalize the preventive interventions that can be targeted at them. Although 99.5% of the 3 billion base pairs of the human genome are identical among different individuals, the 0.5% that is variable translates into millions of genetic variants potentially affecting susceptibility to common disorders. Since the complete sequencing of the human genome in the early 2000, our capability to investigate this genetic variability, and its relationship with CVD in the general population as well as in patients with diabetes, has exponentially increased. Over the past 20 years, we have moved from family-based studies, including a few hundred subjects, to large population-based studies including thousands or even millions of individuals. At the same time, we have gone from studying few variants at pre-specified loci to genotyping millions of variants covering the entire genome at progressively lower costs. Such extraordinary increase in capabilities has led to the discovery of hundreds of new genes involved in the pathophysiology of CVD, and especially of coronary artery disease (CAD). These discoveries have revealed a remarkably complex polygenic background of CAD, and although we are far from completely dissecting such complexity, we have started to leverage these findings to develop new approaches to improve prevention and treatment of CAD in patients with type 2 diabetes as discussed above.

Genetic Determinants of CAD in Diabetes

A large body of evidence points to CAD as a complex, multifactorial disease resulting from the combined effects of genetic and environmental factors. Indication of a genetic component of CAD has come from studies showing that a positive family history increases CAD risk, independently from other traditional risk factors. This has been shown to be equally the case in the general population and among patients with diabetes [3–7], with estimates of the proportion of CAD variability explained by genetic variation ranging from 40% to 60% [6, 7]. The genome-wide association studies (GWAS) that have been carried out during the past decade have shown that a large proportion of CAD heritability (40–70%) is explained by common variants (i.e., Single Nucleotide Polymorphisms—SNPs), having population frequencies greater than 5% [7–9]. However, infrequent or rare variants have also been implicated, although their effect has been more difficult to demonstrate [10–12]. The interplay between rare and common variants in shaping CAD risk is well illustrated by studies on Familial Hypercholesterolemia (FH), showing that the penetrance of the CAD phenotype of this monogenic disorder due to rare mutations is largely influenced by the polygenic component of CAD determined by common variants. For instance, in a recent study of the general population of the UK Biobank, the probability of having a CAD event by age 75 among subjects carrying rare FH pathogenic variants in the *LDLR*, *APOB*, or *PCSK9* genes varied from 17% to 78% depending on their overall genetic predisposition to CAD as estimated by a polygenic risk score combining millions of genome-wide common variants [13, 14]. Thus, from a clinical perspective, genetic susceptibility to CAD should be viewed as the combined effect of common and rare variants (Fig. 6.1). Two individuals might have a similar genetic risk of CAD, but this may derive from different combinations of rare mutations and common polymorphisms.

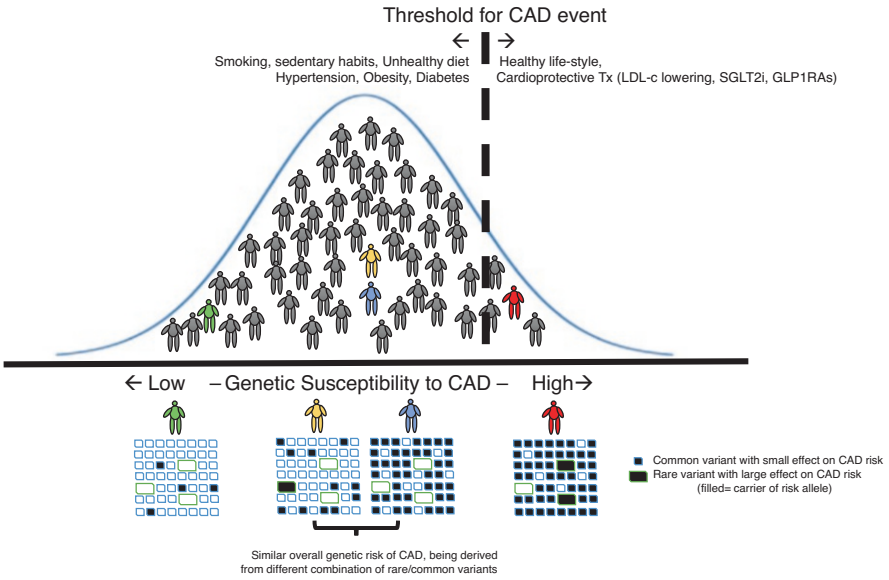

Fig. 6.1 Genetic susceptibility to CAD according to different combinations of common and rare variants at multiple genetic loci

Such genetic susceptibility to CAD acts together with the susceptibility induced by other modifiable and non-modifiable risk factors, such as male gender, smoking habits, un-healthy diet, sedentary lifestyle, presence of obesity, hypertension, and diabetes. The combined effect of these factors can move the theoretical threshold of CAD events towards the left (i.e., increasing the risk of CAD events and/or decreasing the age at which they occur), while preventive strategies (medication or health lifestyle) can move the threshold to the right (i.e., delaying or avoiding the CAD events).

As schematically illustrated in (Fig. 6.2), and discussed in more detail below, the genetic architecture of the susceptibility to coronary artery disease in patients with diabetes can be viewed as being composed by the following groups of genes: (1) Genetic variants increasing CAD risk in the general population that also increase CAD risk in subjects with diabetes. (2) Genetic variants predisposing to both T2D and CAD (in agreement with the so-called "common soil" hypothesis), and (3) Genetic variants increasing the risk of CAD specifically in the presence of T2D or hyperglycemia (i.e., through gene-by-environment interactions).

Genetic Variants Increasing CAD Risk in the General Population and in T2D

To date, common genetic variants at more than 160 loci have been found to be independently and consistently associated with CAD in GWAS that were mainly conducted in the general population and in subjects of European or Asian ancestries [15]. Whether these loci are associated with increased CAD risk also in patients

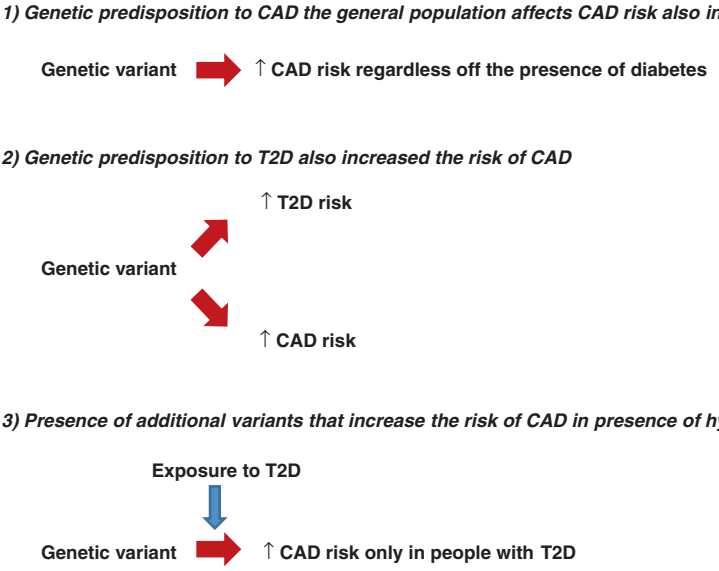

Fig. 6.2 Genetic architecture of increased susceptibility to coronary artery disease in diabetes

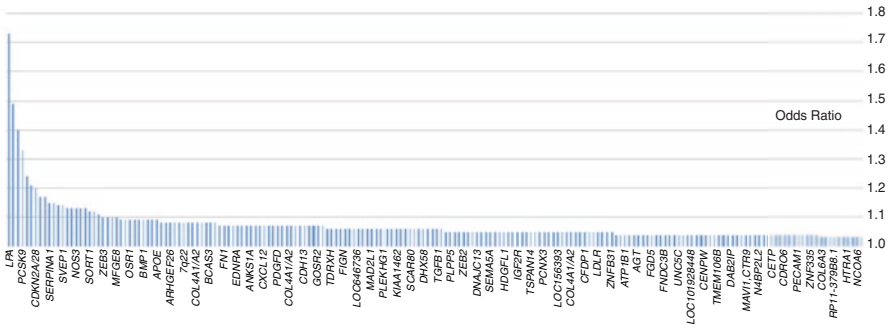

Fig. 6.3 Graphical representation of 160 CAD risk loci discovered in the general population. Loci are sorted by the strengths of their association with CAD (expressed as odds ratio per risk-allele copy). For graphical purposes, only some representative genes/loci are indicated with their names

with type 2 diabetes has been the topic of several studies [16–19]. Given the relatively small effect of each of these variants (most of them increase the risk of CAD by 5–10% per each risk allele variants, and very few increase the risk above 20%, as shown in Fig. 6.3) their validation in subjects with diabetes requires large sample sizes and well-defined phenotypes (e.g., diabetes diagnosis predating the CAD event). For this reason, the analysis of single variants has been often replaced by the analysis of genetic risk scores (GRS) capturing the overall polygenic burden of each individual. GRS can be estimated as "crude GRS," i.e., a score equal to the sum of the number of risk allele for each polymorphism, or as "weighted GRS," where each risk allele is weighted by its strength of association with CAD.

The first of these studies, conducted a decade ago, was a case-control study combining three different populations of subjects with T2D and CAD from the Joslin Heart Study, the Nurses' Health Study, and the Health Professional Follow-up Study [16]. In a combined analysis of these three cohorts, 5 of the 12 CAD loci that had been identified in the general population at that time (2011) were found to be nominally associated with CAD also among patients with T2D. A GRS derived from these SNPs showed a significant association with CAD, with a 19% increase in risk (95% CI 13–26%) for each additional risk allele in the GRS. Similar results were reported in 2015 in a post hoc analysis of the Look-AHEAD study (Action for Health in Diabetes) [17], including 4016 overweight or obese subjects with T2D who were followed for a median of 9.6 years. In this cohort, each standard deviation of a GRS derived from 153 SNPs associated with CAD in the general population was associated with a 19% increase in risk of incident CVD (95% CI 10–28%, $p = 1 \times 10^{-5}$). This GRS was also associated with classic CVD risk factors such as higher LDL-cholesterol, blood pressure, and HbA1c, but the association with CVD remained significant after adjustment for these factors.

More recently, our group has tested the association of major coronary events (combining fatal CAD events, non-fatal myocardial infarction, and unstable angina) with a GRS composed of 204 SNPs representative of the 160 CAD loci known as of 2018 to be associated with CAD in the general population at genome-wide significance level ($p < 5 \times 10^{-8}$) [19]. This analysis was conducted in white participants from the "Action to Control Cardiovascular Risk in Diabetes" (ACCORD, $n = 5360$) and "Outcome Reduction with Initial Glargine Intervention" (ORIGIN, $n = 1931$) studies. In the ACCORD study, 32 SNPs were found to be nominally associated with CAD, although they did not reach study-wide significance levels because of the limited power to analyze individual loci. In addition, 151 out of the 204 SNPs that were tested (i.e., 74% of them, corresponding to a p value 2×10^{-12} for deviation from the null hypothesis of 50%) showed the same trend of association with prevalent CAD as that reported in the general population. A weighted GRS derived from the 204 SNPs was associated with 27% ($p = 4 \times 10^{-10}$) and 35% ($p = 2 \times 10^{-4}$) higher risks of incident major CAD events per standard deviation in the ACCORD and ORIGIN studies, respectively. This GRS was also associated with family history of CVD, prevalent CVD, and HDL-cholesterol at baseline, but its association with incident CAD persisted, although slightly attenuated, after adjustment for these and other classic CVD risk factors (including the ACC/AHA Pooled Cohort Equations 10-year CVD risk model). Moreover, the association was similar in subjects in primary or secondary CVD prevention and with or without a family history of CVD.

Altogether these studies indicate that the genetic factors predisposing to CAD in the general population do so also in people with T2D, suggesting that the presence of a powerful CVD risk factor such as diabetes does not override these genetic effects. In fact, exposure to the diabetic milieu may enhance the effect of some of these genes, as exemplified by the finding of synergism between poor glycemic control and the CAD locus on 9p21 [20]. As discussed in the following section, diabetes may also provide an additional genetic burden since some of the genetic variants that predispose to T2D, and are, therefore, over-represented among diabetic subjects, may also predispose to CAD.

Genetic Variants Predisposing to Both T2D and CAD

Several studies have suggested the existence of a common genetic background ("common soil") shared by T2D and CAD [21]. Two such studies evaluated the results from separate GWAS on CAD and T2D and, after accounting for sample overlaps between studies and the linkage disequilibrium between variants, found a significant positive correlation between allelic effects on T2D and CAD risk across the genome (r_g = 0.39–0.40) [22, 23]. Of note, in one of these studies, the correlation between CAD and T2D (r_g = 0.39) was higher than that between CAD and other traits such as LDL-c, HDL-c, triglycerides, and BMI [23]. Another approach to assess the shared genetic background of T2D and CAD has been to test whether variants identified as being strongly associated with T2D are enriched with variants that are also associated with CAD. Following this strategy, Jansen et al. analyzed 22,233 CAD cases and 64,762 controls from the CARDIOGRAM genome-wide dataset (derived from the combination of several worldwide studies and consortia) and found that 10 of 44 variants (23%) known in 2015 to be associated with T2D at genome-wide significant level were nominally associated with an increased risk of CAD. This proportion was much higher than that expected by chance under the null hypothesis of no association (23% vs. 5%, $p = 5 \times 10^{-5}$) [24]. In the same study, the number of SNPs with an effect that was consistent between T2D and CAD risk (odds ratio per risk allele >1 for both conditions) was significantly higher than that expected by chance (i.e., 64% [29/44] vs. 50%, $p = 0.02$). Of note, the enrichment of T2D SNPs with variants associated with CAD was unaffected by the exclusion of those SNPs with profound effects on other known CAD risk factors. Similar conclusions were drawn by another paper conducted on an overlapping, but larger, population and with slightly higher number of SNPs [25]. A more recent study (including 106 variants associated with T2D as of 2017) confirmed the enrichment of T2D variants (31/106, 29%) with SNPs significantly and concordantly associated also with CAD risk (binomial test for chance observation $p < 5 \times 10^{-15}$) [26]. Other studies have confirmed the association between genetic predisposition to T2D and increased CAD risk in East Asian populations [27, 28].

The "Common Soil" Hypothesis and Insulin Resistance

In the studies above, the enrichment of T2D SNPs with CAD variants was similar for subset of genes affecting different T2D pathways (e.g., reduced beta cell function, reduced insulin sensitivity, or altered insulin secretion). However, more recent studies (based on the increasing number of genetic variants found to be associated with T2D) have suggested that CAD risk may not be equally increased by the different genetic mechanisms determining increased T2D risk [26, 29]. Specifically, genetic variants that increase T2D through pathways related to insulin resistance appear to be among those that are most strongly associated with CAD [30]. From the moment when the "common soil" hypothesis was put forward, insulin

resistance has been proposed as the most important link between T2D and CAD [21]. Compelling evidence for this concept has been provided by GWAS studies, showing that the genetic locus of *IRS1* (encoding insulin receptor substrate-1) harbors multiple variants increasing the risk of both CAD and T2D [22, 24, 26, 30–34]. Despite been placed more than 500,000 base pairs from the *IRS1* coding region, these variants are located in an enhancer site with long-range effects on *IRS1* expression [35] and have been associated with *IRS1* expression in human adipose tissue [33, 34] as well as with several traits related to insulin resistance, such as fasting insulin, HDL-cholesterol, triglycerides, and adiponectin levels, as well as body fat distribution [34, 36, 37]. Altogether, these data provide strong evidence of a causal role of insulin resistance not only in T2D but also in CAD. This concept is also supported by the recent discovery of novel CAD loci (e.g., rs11057401 p.Ser70Cys in *CCDC92*) associated with insulin resistance-related phenotypes such as body fat percentage, HDL, triglycerides, and adiponectin levels [33, 38].

Genetic Variants Increasing the Risk of CAD Specifically in the Presence of T2D

Beyond the genetic factors cited in the section on "Genetic Variants Increasing CAD Risk in the General Population and in T2D", some additional factors has been found to increase CAD risk specifically in the presence of diabetes or diabetes-related metabolic traits (e.g., hyperglycemia or insulin resistance). These variants therefore show a significant "gene by environment" interaction, by which the association with CAD is stronger, or exclusively present, among patients with diabetes as compared to the general population.

The 1q25 Locus

As of today, the strongest and most replicated signal showing a significant "gene by diabetes" interaction is represented by a genetic variant at the 1q25 locus associated with the expression of *GLUL* (coding for glutamate-ammonia ligase, a.k.a. glutamine synthase, converting glutamic acid to glutamine) [39–41]. This locus was initially identified by our group in a genome-wide association study specifically aimed at identifying genetic variants associated with increased CHD risk (defined as fatal or non-fatal myocardial infarction, revascularization procedures, or angiography evidence of significant coronary stenosis) in patients with type 2 diabetes [39]. The study combined a total of 1517 CHD cases and 2671 CHD-negative controls with type 2 diabetes derived from five independent population (the Nurses' Health Study—NHS, the Health Professionals Follow-up Study—HPFS, the Joslin Heart Study—JHS, the Gargano Heart Study, and the Catanzaro Study). After testing 2.5 million common variants, a genome-wide significant association was identified

between SNP rs10911021 (on chromosome 1q25) and CHD risk. The association, observed consistently across the five datasets, was such that each copy of the risk allele "C" was associated with a 36% higher risk of CHD (OR 1.36, 95% CI 1.22–1.51, $p = 2 \times 10^{-8}$) in patient with diabetes, but not among patients without diabetes (OR 0.99, 95% CI 0.87–1.13), yielding a significant "SNP by diabetes" interaction ($p = 2 \times 10^{-4}$). A similar association, although not reaching statistical significance, was found in a study by the *University College, London School of Hygiene and Tropical Medicine, Edinburgh and Bristol* (UCLEB) Consortium (including 12 prospective studies in patients mainly of European ancestries), in which the C-allele showed a non-significant trend for increasing CHD risk in patients with diabetes and without previous history of CAD (HR 1.25; 95% CI 0.94–1.66) [42]. As reported in Fig. 6.4, the meta-analyses of these results with those from our studies yielded an increase in the significance of the association with CHD (HR 1.35; 95% CI 1.24–1.47, $p = 8 \times 10^{-10}$). Also in this study, rs10911021 was not associated with CHD in patients without diabetes [42]. The association with increased CHD was confirmed also among 3295 patients with diabetes on primary CVD prevention enrolled in the prospective Look-AHEAD study [40]. In that study, conducted over 9.7 years of follow-up, the "C" risk allele was significantly associated with a 17% increased risk of CVD (HR 1.17, 95% CI 1.01–1.36, with CVD defined as a composite outcome of death from cardiovascular causes, non-fatal

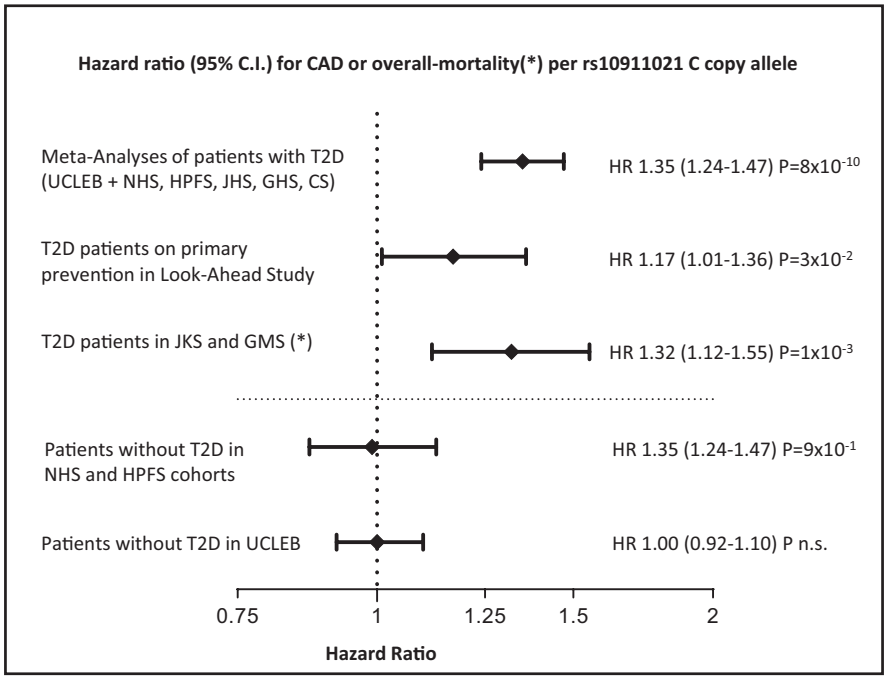

Fig. 6.4 Association between variant rs10911021 at the *GLUL* locus and clinical outcomes in longitudinal studies of patients with and without diabetes

myocardial infarction, non-fatal stroke, or hospitalization for angina). Another study, combining 1242 white subjects with type 2 diabetes from the Joslin Kidney Study (JKS) and the Gargano Mortality Study (GMS), confirmed the association of the C-allele with a higher risk of all-cause mortality (HR 1.32; 1.12–1.55, $p = 0.0011$) that appeared to be driven by cardiovascular mortality [41].

It is worth mentioning that two studies failed instead to detect a significant interaction between rs10911021 and diabetes on CAD risk [43, 44]. The design of these studies, however, was strongly biased towards the null hypothesis (i.e., finding no differences in the effect of gene on CAD between T2D and non-T2D, when instead a true difference is present) since prevalent cases of CAD were defined as having occurred among patients with diabetes regardless of whether the CAD events had occurred before or after the diagnosis of diabetes. Instead, a proper test of "gene × environment" interaction requires that the environmental factor (in this case, diabetes) is present before the onset of the outcome (in this case, CAD), and that this exposure is long enough to influence the genetic effect (meaning that duration of diabetes should be taken into account). Therefore, by including only prevalent data, and considering CAD events prior to diabetes equivalent to those after diabetes, these studies introduced a crucial misclassification that biased results towards the null hypothesis.

More recently, in support of the generally consistent and well-replicated findings of the *GLUL* locus, functional studies have increased our understanding of the mechanism of this genetic effect, suggesting a dysfunction of the γ-glutamyl cycle, leading to intracellular alteration of glutathione levels that increases susceptibility to oxidative stress. These findings point to a new potential pharmacological target to reduce CAD disease in diabetes, as described in more detail in the section on "Development of New Preventive Interventions" [45].

The *HP* Locus

Another genetic variant with a potential effect on CAD only in the presence of diabetic milieu has been identified in the *HP* gene (coding for Haptoglobin—a plasma protein binding-free hemoglobin, which, in physiological conditions, reduces hemoglobin-induced oxidative damage). This variant is a common biallelic Copy Number Variant (CNV—rs72294371), determining Haptoglobin proteins with distinct forms and lengths, leading, therefore, to three different genotypes (HP 1/1, HP 1/2, and HP 2/2) [46–48]. The HP 2/2 genotype was found to increase the risk of incident CAD risk only in patients with HbA1c above 6.5% [49]. A similar trend was reported in other prospective studies [50], and HP 2\2 was found to increase CAD risk also in patients with type 1 diabetes [51]. However, in these studies, the interaction with Hba1c levels was not formally tested, or if it was, it was not significant [50]. The evidence has been inconclusive also for the interaction between HP 2/2 genotype and intensive glycemic control on incident of CAD events among patients with type 2 and type 1 diabetes [52–54]. Therefore, further validation and replication studies are required. Nonetheless, given the role of Hp in regulation of

hemoglobin-induced oxidative damage, these findings may be consistent with those on *GLUL*, as they also seem to suggest that anti-oxidant homeostasis plays a pivotal role in modulating the onset of coronary artery disease in patients with diabetes [50].

Translating Genetic Findings to Clinical Practice

The discovery of genetic variants associated with CAD provides several opportunities to translate these findings into actionable items to improve the care of patients with diabetes. A straightforward application is the use of these genetic markers to improve prediction of CVD risk. From this standpoint, genetic markers can be considered and tested for validity, as it is done with other novel biomarkers, with the major advantages of (1) being stable over time (one test in a lifetime is sufficient) and (2) not being susceptible to reverse causation as no disease, treatment, or other modifiable factor can influence the presence of one or the other allele since these are inherited at conception. Another application relates to the discovery of new genes or pathways involved in CAD risk, which may point to new targets for cardiovascular prevention. Despite the challenges of moving from genetic associations to the identification of causal variants, causal genes, and the design of new drugs, this process has already being successful in some cases. Finally, pharmacogenetics is one of the most exciting and challenging field of investigation, holding the promise to use genetics variants to identify those subjects who might benefit the most from a specific treatment and distinguish them from those patients who would not derive benefit or may be even harmed by it. The following Sections provide some examples in each of these fields of translational research.

Improving CV Risk Assessment

Using genetic findings to improve risk prediction is an obvious application of genetic research on CVD/CAD. While individual SNPs cannot be used for this purpose due to their small effects, the GRS combining multiple SNPs that were described above may provide this opportunity. Indeed, this has been showed to be the case in a recent study of the ACCORD cohort [19], in which the GRS combining 160 CAD loci significantly improved the prediction of future CAD events when added to conventional risk factors. Although the C-statistics only minimally increased (+1%), there was a significant improvement in the correct classification of subjects in those who did and those who did not develop events during the follow-up, as shown by the substantial increase in relative Integrated Discrimination Index (rIDI + 8%, $p = 7 \times 10^{-4}$) and in the Net Reclassification Index (NRI = 0.16, $p < 1 \times 10^{-4}$). From a clinical perspective, the AHA and ACC committee used an rIDI threshold of at least 6% to evaluate whether it was useful or not to add a new biomarker to the Pooled Cohort Equations CVD risk model [55]. Therefore, this

GRS based on 204 variants (160 loci) would have passed that threshold and would have been considered for inclusion in the CVD risk equation.

An important determinant of the predictive performance of GRS's is the number of loci that are included in the scores. This is illustrated well by a retrospective analysis of the performance of the GRSs that could have been built at different times during the past decade based on the CAD-associated SNPs that were known at each point in time. As can be seen in Fig. 6.5, the increase in the number of known CAD loci that could be included in the GRS [56] has been paralleled by an increase in the prediction and discrimination provided by this tool [19]. However, since the new CAD loci that are discovered have increasingly smaller effect on CAD risk, an increasingly larger number of SNPs is required to further increase the GRS performance. This has led, over the past year, to the idea of building genome-wide polygenic risk score (GPRS) for CAD based on up to six million common variants, i.e., a GRS including all available common variants regardless of the *p* value for their association with CAD [57]. In the general population, these GPRS show better performance in discriminating subjects at very high CAD risk as compared to "classic" GRS using only genome-wide significant variants. For instance, a GPRS based on six million variants could identify a significant (8%) proportion of the population having a cardiovascular risk equivalent to that of subjects with a rare monogenic form of CAD such as familial hypercholesterolemia [57, 58]. Several studies have then confirmed the usefulness of these GPRS on top of classic risk factors for the prediction of incident cardiovascular events [59–62]. However, some other studies have yielded negative or mixed results [63, 64], highlighting the need for further research with larger sample size and multiancestry representation [12]. Also, few studies have evaluated the role of these large GPRS in patients with type 2 diabetes. In a subset of 21,102 subjects from the UK Biobank, each S.D. of a GPRS based on more than six million common variants was associated with a 50% higher risk of prevalent CAD risk (OR per SD 1.50, 95% CI 1.43–1.57), and among 352 subjects with type 2 diabetes from the McGill Cardiac Complications in Diabetes cohort (MCCD) who underwent coronary angiography, it was associated with a 65%

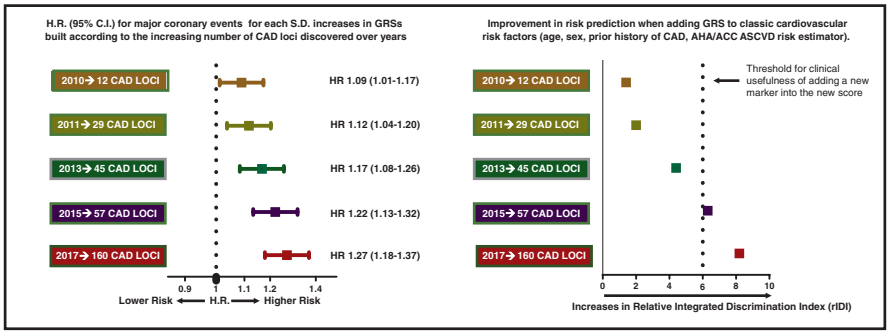

Fig. 6.5 Progressive improvement of genetic risk scores (GRS) for prediction of incident CAD events in patients with type 2 diabetes from ACCORD. Note: AHA/ACC ASCVD: 10-year American College of Cardiology (ACC)/American Heart Association (AHA) atherosclerotic cardiovascular disease (ASCVD) risk estimator. (Adapted from Morieri et al. Diabetes Care 2018 [19])

higher risk of multivessel stenosis (95% CI: 1.25–2.20) and larger number of major stenotic lesions (OR = 1.35; 95% CI 1.08–1.69) [65]. However, the performance of this GPRS in terms of improving risk prediction and discrimination over that provided by traditional risk factor has not been determined.

Overall, while more research is needed and further improvements can be expected, we can conclude that the genetic prediction tools that are available at this time, such as the GRS based on 204 SNPs [19], have reached the threshold for being adopted in clinical practice and efforts should be made to facilitate this process by incorporating them in the current clinical management guidelines.

Development of New Preventive Interventions

Several of these CAD-associated variants affect genes with well-known links to classic factors (e.g., LDL-cholesterol or blood pressure) that are known to increase CAD risk both in patients with and without diabetes [66]. However, the majority of these genetic variants are located in proximity of genes with as of yet undefined function [12]. This provides many opportunities for novel discoveries on the mechanisms regulating atherogenesis and the development of CAD in T2D, which in turn may potentially uncover novel targets for preventive strategies. At the same time, converting genetic associations into causal genes and atherogenetic pathways, and finding old or new drugs to target these, present several challenges that may delay the translation of genetic findings into clinical practice. These challenges are related to different factors, which are discussed below by describing three different genetic findings that are at different stages of translation into clinical practice: one still very far from this goal (locus 9p21), one half-way through achieving this goal (locus 1q25, *GLUL*), and one for which this goal has been achieved (PCSK9 inhibitors).

9p21 Locus

One of the clearest examples of how hard it can be to translate genetic associations into new preventive interventions is the 9p21.3 locus. This genomic region hosts the first genetic variants that were discovered to be associated with CAD by a genome-wide study back in 2007 [67, 68]. It is one of the most replicated genetic associations with CAD, including in subjects with diabetes [20, 22]. A recent meta-analysis of multiple genome-wide dataset of CAD, reported a summary Odds Ratio of 1.21 (OR = 1.21, 95% CI 1.19–1.22, $p = 5 \times 10^{-204}$) for the leading variants in this locus (rs4977574), meaning that the odds of CAD are increased by ~21% for each risk allele carried by an individual [38]. Such increased risk is unaffected by adjustment for other cardiovascular risk factors, implying that this effect is independent from known risk pathways [69]. Yet, despite these consistent and replicated findings, and despite multiple functional studies [70–75], the mechanisms linking these variants to CAD are still unclear, making the translation of this finding into an actionable target far from being achieved [12]. One of the reasons for these disappointing

results relate to the large size of the locus harboring these CAD-associated variants—a 60 kb linkage disequilibrium block with no protein-coding genes. Current evidence supports the involvement of the long non-coding RNA *CDKN2B-AS1* (a.k.a. ANRIL, for antisense non-coding RNA in the INK4 locus), located in this region and expressed in many cell types relevant to the atherosclerotic process [70, 71]. The CAD-associated variants have been found to influence ANRIL expression and splicing, increasing the supposedly pro-atherogenic, short linear ANRIL isoform and decreasing the long-circular anti-atherogenic isoform [72]. The closest protein-coding genes, which are placed outside the 60 kb locus where the CAD-associated variants are located, but could be influenced by them, code for cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A* and *CDKN2B*). The products of these genes control cell proliferation, cell aging, and apoptosis, are expressed at high levels in endothelial and inflammatory cells and may be therefore also involved in the genetic association [73–75]. *CDKN2A* and *CDKN2B* are also expressed in pancreatic islets where they play a role in regulating islet cells regenerative capacity [75], consistent with in vitro experiments showing that reduced expression of *CDKN2A* significantly modifies insulin secretion [76]. Indeed, the same locus also harbors at least two distinct genetic signals of association with a higher risk of diabetes, one of which is correlated with the CAD-associated variants [70, 77, 78]. Another element of complexity is that the 9p21 locus has been reported to have a larger effect on CAD risk among individuals with type 2 diabetes than in the general population, and among those with diabetes, to have a larger effect on among those with the worst glycemic control (as shown in Fig. 6.6) [20]. Such synergism between diabetes and the 9p21.3 locus on CVD suggests at least a partial overlap between

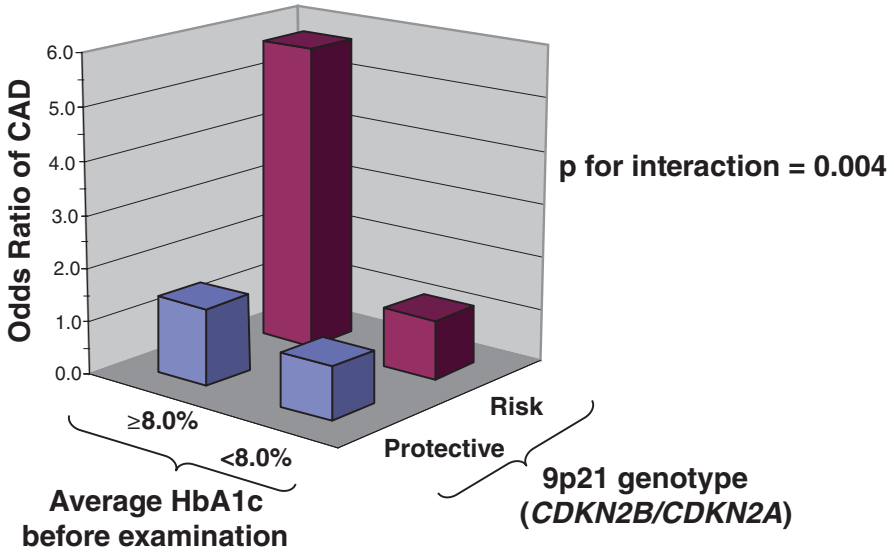

Fig. 6.6 Interaction between 9p21 CAD locus and poor glycemic control on risk of CAD in the Joslin Heart Study

the pathways through which this locus and diabetes increase CAD risk. This interaction, however, has not been replicated in other studies, some of these reporting inconclusive results [70, 79–81], and other reporting significant interactions, but in the opposite direction for glycemic control [82] and the presence of diabetes [22]. Altogether, the lack of identification of the causal variant(s), causal gene(s), and tissue(s) that are involved in the association between the 9p21.3 locus and CAD clearly illustrate the challenges of translating genetic findings into clinically actionable items. Nonetheless, the overlap between variants influencing CAD and T2D, the possible gene by diabetes and gene by glycemic control interactions, and the fact that this is the strongest CAD loci identified to data in general population, clearly warrant further studies of this locus.

1q25 Locus

In contrast with the 9p21 locus, the 1q25 locus associated with CAD in patients with diabetes provides an example of a genetic finding with a more promising path towards its possible translation into novel treatments for cardiovascular prevention. Already in the first report of the association between this locus and CAD risk in patients with diabetes, the rs10911021 C-risk allele was shown to be associated with lower endothelial expression of the nearby gene *GLUL* [39] coding for glutamine synthase—the catalytic enzyme-converting glutamate to the amino acid glutamine [83]. Although the SNP was not associated with neither glutamate nor glutamine levels, it was found to be associated with lower pyroglutamic/glutamic ratio. These two metabolites are intermediates in the γ-glutamyl cycle, which is responsible for the production and homeostasis of the anti-oxidant glutathione (GSH). On this basis, the genetic variants affecting CHD risk in patients with diabetes have been postulated to acts through this pathway [39]. This hypothesis has been further investigated and confirmed in a recent study of a large collection of human umbilical vein endothelial cells (HUVECs) naturally carrying different genotypes of rs10911021 and exposed to different glucose concentrations [45]. This study confirmed the association between the SNP and *GLUL* expression as well as its effect on a variety of metabolites related to glutamic acid metabolism and the γ-glutamyl cycle. In particular, the C-risk allele was associated with reduced GSH/glutamate ratio and was found to be inversely related to S-lactoyl-glutathione, which originates from the GSH-mediated detoxification of methylglyoxal—a glycolysis byproduct and precursor of AGEs implicated in the pathogenesis of CVD in diabetes [84]. This raised the hypothesis that the detoxification of methylglyoxal is impaired among carriers of the rs10911021 C-allele risk and is responsible for the increase in CVD risk observed among these subjects. In support of this hypothesis, the study found (1) an increase of methylglyoxal levels per each C-allele copy in HUVEC cells and (2) a significant increase in methylglyoxal levels following *GLUL* down-regulation through shRNA interference. As summarized in Fig. 6.7, these findings support the following chain of events: C-risk allele → lower *GLUL* expression → impaired γ-glutamyl cycle and glyoxalase system → and higher,

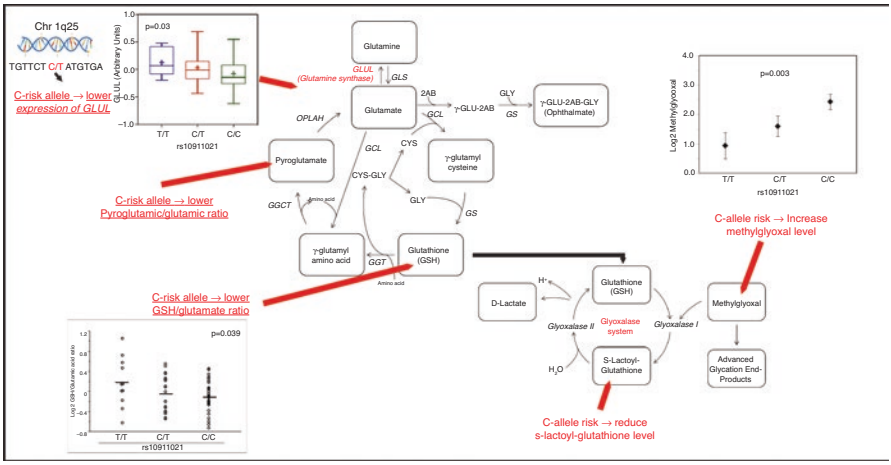

Fig. 6.7 Graphical representation of the effect of 1q25 locus variant on γ-glutamyl cycle and glyoxalase system. Amino acid levels and ratios influenced by the rs10911021 C-risk allele are indicated in red. (Adapted from Pipino et al. Diabetes 2020 [45])

pro-atherogenic, methylglyoxal levels [39]. Most importantly, from a translational point of view, the study showed that the increase in methylglyoxal levels induced by GLUL deficiency was completely prevented by exposing cells to high concentration of glutamine (the product of the GLUL regulated enzymatic reaction). Notably, oral supplementation with L-glutamine (a precursor of NAD) is an already FDA-approved treatment for sickle cell disease, in which this treatment was found to raise the NAD redox ratio in red blood cells and reduce oxidative stress [85–87]. Therefore, while further functional studies are required, these results may support the use of glutamine supplementation for cardiovascular prevention in patients with type 2 diabetes carrying the 1q25 risk allele—an approach whose usefulness can be easily investigated through a clinical trial.

PCSK9

The *PCSK9* locus is an example of a successful and completed translation of genetic finding into clinical activity, which, in less than 15 years from its discovery, has led to the development and clinical use of a highly effective new cardiovascular prevention treatment, i.e., proprotein convertase subtilisin–kexin type 9 inhibitors. The role of the *PCSK9* gene in autosomal-dominant hypercholesterolemia was initially discovered in 2003 [88] and subsequent studies quickly led to the identification of the protein coded by this gene as a regulator of LDL-receptor (LDL-r) degradation. By binding to the LDL-r, PCSK9 promotes degradation of this molecule in hepatocytes, decreasing the clearance of LDL from circulation and therefore increase circulating LDL-cholesterol levels. In only a few years, the identification of rare loss-of-function PCSK9 mutations associated with reduced

LDL-cholesterol levels and CAD risk [89, 90], in particular in African Americans, led to the development of two monoclonal antibodies targeting the PCSK9 protein and reducing LDL-cholesterol levels [91–93]. Approved for treatment of hypercholesterolemia in 2015, these inhibitors are very effective in reducing the incidence of cardiovascular events and mortality in subjects at high cardiovascular risk [94–96]. The quick translation of the genetic finding with PCSK9 into new treatments has been due to the initial link of this gene to a well-known CV risk factor (i.e., the LDL-cholesterol). It is reasonable to expect that developments will be much slower for those genetic findings for which the causal genes and pathways are not so clear. Nonetheless, the PCSK9 inhibitors story is encouraging and also provides additional hints concerning the usefulness of genetic studies. For instance, the findings of subjects carrying two loss-of-function PCSK9 alleles (i.e., with no or much reduced PCSK9 function) having no adverse health consequences supported the safety of PCSK9 pharmacological inhibition before the development of specific inhibitors and before clinical trials [97, 98]. One of the factors currently limiting the use of this cardiovascular preventive treatment is its high cost. One way to overcome this problem is to improve selection of subjects who will benefit the most from this treatment in order to prioritize treatment. In this regards, two distinct post hoc studies of randomized clinical trials have recently shown that subjects with a higher genetic risk of CAD, as identified with the use of a polygenic risk score for CAD similar to those described in the previous section, experience a higher relative and absolute risk reduction when treated with PCSK9 inhibitors [99, 100]. These early data provide the rationale for a successful pharmacogenomics approach as described in detail for other interventions in the next section.

Personalization of Therapy

Over the past few years, several studies have tried to identify new approaches to personalize cardiovascular prevention treatment in patients with type 2 diabetes. One of these approaches has been based on the hypothesis that the response to cardiovascular preventive treatment is partially determined by the genetic background of each patient. For this reason, there has been an increased interest, as part of the wider field of precision medicine, in pharmacogenetic studies aimed at identifying genetic variants associated with better or worse response to treatments. One may search for these variants through a genome-wide unbiased approach (i.e., without a priori hypotheses or set of genes being specified) or following a candidate-gene strategy (i.e., studying a set of pre-specified gene(s) or variant(s)). These approaches are complementary, since each of them has advantages and limitations. Below, we discuss two examples, one for each approach, showing promising results for implementation in clinical practice.

Genetics Determinants of Cardiovascular Response to Intensive Glycemic Control

Epidemiological studies have clearly shown the relationship between worst glycemic control and increased incidence of macro- and micro-cardiovascular disease in patients with diabetes [101]. These observational findings were confirmed in randomized controlled trials, in which interventions aimed at achieving intensive glycemic control clearly showed a benefit in reducing the incidence of micro-vascular complications [101, 102]. Results were less clear for macro-vascular disease. While meta-analyses showed that intensive glycemic control was associated with a 15% reduction in the risk of myocardial infarction and 9% reduction in major cardiovascular events [103], the effects on total and cardiovascular mortality were found to be neutral and in some cases even detrimental [101, 104]. For instance, the ACCORD clinical trial, which enrolled over 10,000 subjects, showed that participants randomized to intensive (Hba1c < 6.0%) rather than standard glycemic control (Hba1c between 7% and 8%, in line with recommendation at the time the study was conducted) experienced a significant reduction in myocardial infarction risk (−18%). However, this benefit was completely offset by a significant and paradoxical increase in total (+22%) as well as cardiovascular (+35%) mortality associated with intensive glycemic control, which led to an early termination of the trial [104]. Following these results, the achievement of intensive glycemic control with Hba1c < 6.0% has not been recommended by guidelines. In addition, the more recently discovery and approval of innovative cardioprotective glucose-lowering treatments, such as SGLT2 inhibitors and GLP-1Receptor Agonists, have not changed this recommendation. Indeed, although these drugs have shown a consistent cardiovascular benefit (including on mortality) as compared to placebo [66, 105], such an effect appears to be only in minor part related to HbA1c reduction [106–109], and these drugs are currently recommended for cardiovascular prevention according to the cardiovascular risk of patients and not to achieve lower HbA1c targets [66, 105]. Therefore, the question as to which patients might experience benefit or harm from intensive reduction of HbA1c, e.g., below 6.0%, is still unanswered.

Through genetic studies, we were able to provide an initial and promising answer to this question. Specifically, through a genome-wide analysis of over seven million common variants in self-reported white participants randomized to intensive glycemic control in the ACCORD clinical trial, we have identified two distinct genetic signals that were associated with higher risk of cardiovascular mortality at genome-wide significance levels [110]. The first of these loci is located in an intron of the *MGMT* (O-6-methylguanine-DNA methyltransferase) gene on chromosome 10 while the second is located upstream and proximal to three long intergenic non-coding (LINC) RNAs (LINC1335, LINC1333, and LINC1331) on chromosome 5. The leading SNPs at these loci were associated in the intensive glycemic control arm with 3.6- and 2.7-fold increases in risk of cardiovascular death per each copy of its allele (rs9299870, HR: 3.58; 95% CI 2.32–5.55 and rs57922, HR: 2.65 with 95%

CI 1.88–3.72, respectively). Combining the two variants together in a GRS ranging from 0 to 4 risk alleles, we found that those subjects carrying at least two risk alleles (around 30% of the population) had the double disadvantage of not deriving any benefit with respect to non-fatal myocardial infarction and experiencing a threefold increase in mortality when exposed to intensive glycemic control (red line on Fig. 6.8). By contrast, subjects with 0 or 1 risk allele experienced a reduction in non-fatal myocardial infarctions without any increase in mortality or even with a possible reduction in this outcome (green and yellow lines on Fig. 6.8). Similar effects were found in an observational cohort (the Joslin Kidney Study), in which only subjects carrying 0 or 1 risk alleles had a reduction of cardiovascular mortality when exposed to better glycemic control (defined as an HbA1c below the median level of 7.5%) [110]. These results were mechanistically enriched by the findings of an association between these variants and circulating fasting GLP-1 levels (glucagon-like peptide 1, active) [111]. Specifically, in the intensive glycemic control arm, subjects with 0 risk alleles for rs57922 (C/C homozygotes), i.e., those who derived the maximum cardiovascular benefits from intensive treatment, had a 22% increase in GLP-1 levels during follow-up, whereas subjects carrying two risk alleles (T/T homozygotes) had a 28% reduction in GLP-1 levels. These differences were not observed in the standard glycemic control arm, leading to a significant gene-by-intervention interaction. Altogether, these results suggest that a simple genetic test may allow the identification of those patients who might experience a cardiovascular benefit from more intense glycemic control than it is currently recommended (i.e., <6.0% vs. <6.5%). These data also suggest that those patients with detrimental cardiovascular response to intensive glycemic control might be identified by measuring GLP-1 levels after treatment intensification, and that these patients may especially benefit from the use of GLP1R agonists to lower their blood

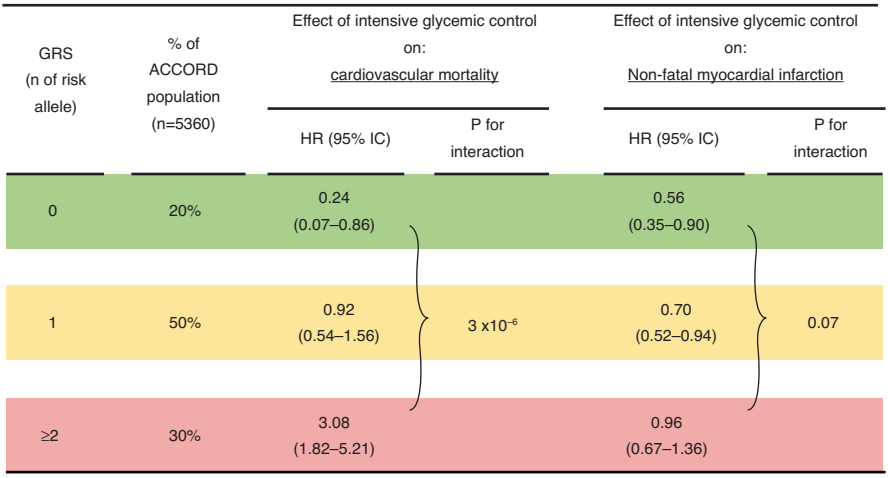

Fig. 6.8 Effect of intensive glycemic control on cardiovascular mortality and non-fatal myocardial infarction among participants in the ACCORD clinical trial stratified by a Genetic Risk Score derived from the combination of risk alleles at the 10q26 and 5q13 loci

glucose. These data require additional validation before getting to the clinic, especially with regard to non-white populations, since these were not included in the genetic studies of ACORD because of their small sample size. Nonetheless, these findings illustrate the potential for using genetic markers in a clinical setting.

Pharmacogenetic Studies on the Cardiovascular Effectiveness of Fenofibrate

Over the past few decades, several studies have investigated the cardiovascular effect of fibrates, including fenofibrate, in the general population and in particular in patients with type 2 diabetes [112–116]. Through the activation of the transcriptional factor peroxisome proliferator-activated receptor alpha (PPAR-alpha), fibrates improve lipid profile, and in particular, they contrast the so-called atherogenic dyslipidemia (defined by high triglycerides and low HDL-cholesterol levels)—a condition that is often present in patients with diabetes [117]. Fibrates, including fenofibrate, have also anti-inflammatory and anti-platelet actions that are independent from their lipid-lowering effect [118, 119]. However, despite these promising features, results from clinical trial on cardiovascular outcomes have been disappointing, in particular in those studies testing the efficacy of fibrates as adds-on to statin treatment [113, 114, 120], which have shown a heterogeneous response, with beneficial treatment only in the group of patients with atherogenic dyslipidemia [121–124]. Therefore, fibrates are not generally recommended by guidelines for cardiovascular prevention and might be considered only in those subjects with atherogenic dyslipidemia [66, 125]. Since several studies have shown that the lipid and anti-inflammatory response to fenofibrate might be partially genetically determined [126, 127], we tested whether genetic variants could also be used to identify subjects with diabetes having a better cardiovascular response to fenofibrate. To maximize our chances of success, we leveraged the many studies on fenofibrate and PPAR-alpha activation and followed a candidate-gene approach using the data from ACCORD-Lipid clinical trial [128, 129], in which more than 4000 patients with type 2 diabetes were randomized to fenofibrate or placebo on top of statin therapy, and for whom genetic data were available. First, we found that the cardiovascular effectiveness of fenofibrate was influenced by a common gain-of-function genetic variant (p.S447*) in the *LPL* gene, encoding for lipoprotein lipase (whose activity is enhanced by fenofibrate-induced PPAR-alpha activation [130, 131]), and already known to lower CAD risk [129, 132]. Specifically, we found that those subjects already carrying the allele increasing LPL activity did not derive any cardiovascular benefit from randomization to fenofibrate (RR 1.56; 95% CI 0.98–2.47), whereas all other subjects experienced a 19% risk reduction in the risk of major cardiovascular events (MACE) (RR 0.81%; 95% CI 0.66–1.00, p for interaction 0.01). This finding, given the only nominally significance of the negative interaction and the lack of additional evidence to date, should be considered as merely hypothesis generating. However, this observation was important as it suggested that genetic variants in the PPAR-alpha pathway could be used to identify subjects with better response to fenofibrate. This was confirmed in another study, in which we tested more than 400 common variants at the

PPARA locus, finding a variant (rs6008845) showing a study-wide significant interaction with fenofibrate on MACE ($p = 4 \times 10^{-4}$) [128]. This interaction was discovered in Whites patients, validated in African-Americans patients, and confirmed in observational cohorts. When all the observations from these different settings were combined together, they yielded a p value for interaction of 1×10^{-6}. The interaction was such that, as shown in Fig. 6.9 Panel (a), those subjects carrying the T/T genotype (about one third of the population included in ACCORD trial) had a 51% MACE risk reduction over a median follow-up of 4.7 years of treatments (HR 0.49, 95% CI 0.34–0.72), while subjects carrying other genotypes had no reduction in risk of MACE in response to this treatment. More importantly, the benefit of treatment with fenofibrate among those with T/T genotype was confirmed also in the subgroup of patients without atherogenic dyslipidemia, i.e., those for whom there was not current indication for treatment with fenofibrate. Based on these results, we estimated that the clinical benefit of fenofibrate, as assessed by the number of patients needed to be treated to avoid 1 MACE over the following 5 years (NNT), was similar among subjects without dyslipidemia but with the rs6008845 T/T genotype to that among subjects with atherogenic dyslipidemia. These results, while requiring further validation, clearly point to a pharmacogenetic approach towards optimal prescription of fenofibrate in patients with type 2 diabetes, which as shown in Fig. 6.9 Panel (b), would double the proportion of subjects who would benefit from this treatment.

Beyond the identification of a marker allowing the identification of subjects with better response to fenofibrate, these data have also provided important new insights

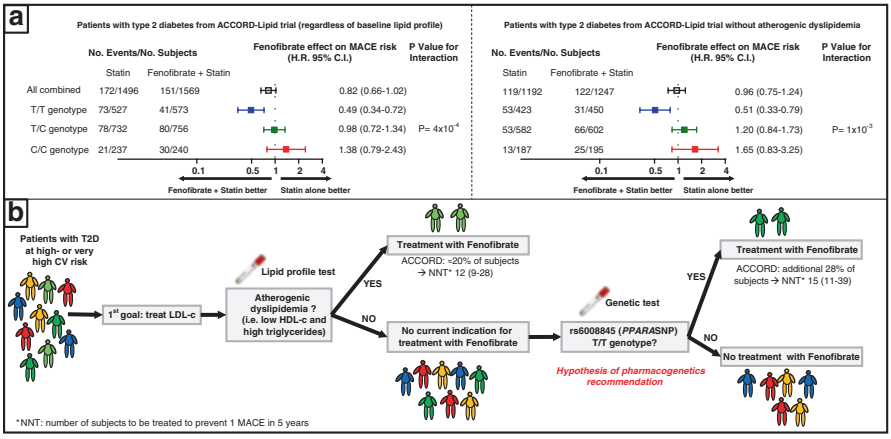

Fig. 6.9 Genetic variant (rs6008845) at the *PPARA* locus influencing the cardiovascular response to fenofibrate in ACCORD-Lipid. Panel (**a**): effectiveness of fenofibrate in reducing major cardiovascular events (MACE) risk in self-reported white patients from the ACCORD-Lipid trial. The results in the entire population are on the left and the results among participants without atherogenic dyslipidemia (i.e., those among whom fenofibrate is not currently recommended for cardiovascular prevention) are on the right. Panel (**b**): hypothesis of pharmacogenetic approaches allowing the identification of a larger proportion of patients deriving benefit from fenofibrate as compared to patients identified solely through lipid profile. (Adapted from Morieri et al. Diabetes 2020 [128])

into the mechanism of action of fenofibrate. First, the SNP modulating the response to fenofibrate, which is located ~25 kb from the *PPARA* starting site, was associated with PPAR-alpha expression in multiple tissues. Although the relevant tissue(s) involved in the genetic effect have not yet been identified, this supports the hypothesis that genetically determined PPAR-alpha expression (and activity) can influence the cardiovascular effectiveness of fenofibrate [128]. Second, we found that the differences in cardiovascular risk reduction across genotypes were not paralleled by differences in fenofibrate-induced changes in lipid profile as captured by HDL-cholesterol, triglycerides, or LDL-cholesterol levels. Although counterintuitive, these findings support those of a previous study showing that the lipid-lowering actions of fibrates explain only a small fraction of their cardiovascular effects (as small as 25% in the VA-HIT trial) [133] and that other metabolic effects of fibrates are probably involved. Indeed, in a small subset of ACCORD patients, we found that participants with the T/T genotype, i.e., those with a better cardiovascular response, had significantly lower levels of a pro-atherogenic chemokine (CCL11 or Eotaxin) after treatment with fenofibrate [128]. Whether these findings can be confirmed in larger populations, whether they explain the observed genetic effect, and what is the nature of the tissue(s) and cell type(s) involved in this genetic modulation is being currently investigated.

Conclusions and Future Directions

Over the past 15 years, there has been an exponential increase in our understanding of the genetic background of coronary artery disease in diabetes. Hundreds of CAD-associated loci have been discovered in the general population, and although most of them have yet unknown function, they have been found to increase CAD risk also in patients with diabetes. Genetic studies have provided clear validation of the long-standing hypothesis of a "common soil" between CAD and T2D, by identifying genetic variants, in particular those linked to insulin resistance, increasing the risk for both conditions. Genetic variants that increase CAD risk specifically in the presence of diabetes have also been identified and are currently under further investigation to develop new CVD-preventing treatments in diabetes. The combination of genetic variants into GRS's has been shown to improve prediction of future cardiovascular events that might be useful for research and clinical purposes. Most importantly, the discovery of genetic variants associated with CAD has allowed the design of new drugs (e.g., PCSK9 inhibitors), and is paving the way to genetic-guided approaches to prescribe cardiovascular prevention treatments more precisely and effectively.

Yet, this is most likely just the beginning of the use of genetic findings to improve treatment and prevention of CAD in patients with diabetes and in the general population. Despite these exciting results, the genetic analyses performed to date still have many limitations, which offer additional opportunities and point to new directions for future research. For instance, we are still far from being able to explain all the heritability and genetic susceptibility to CAD. The main reasons for this are the relatively small effects of individual variants and the need to apply stringent significance

threshold ($p < 5 \times 10^{-8}$) to avoid false-positive findings. The combination of these factors has translated into limited statistical power, despite the relatively large sample size of the studies conducted thus far. Further increasing sample size, as it is being done by initiatives such as the UK Biobank, will provide a better definition of the association of the CAD variants identified to date and will likely lead to the identification of many other ones, including those with a low frequency, which have been thus far overlooked. Larger studies will also allow evaluation of possible gene-by-gene interactions (epistasis) affecting the risk of CAD. Indeed, we are currently estimating the genetically determined risk conferred by variants considered individually, whereas the effects of some of them might depends on the presence of other variants. Testing this hypothesis on genome-wide scale, including millions of variants, will require very large sample sizes (i.e., millions of subjects) to achieve adequate power to reject the null hypothesis of no epistasis [134]. At the same time, sample sizes will need to be increased without sacrificing the quality on the phenotypic information, making sure, for instance, that diabetes is properly defined and precedes the onset of CAD in order to avoid the problems discussed in the section on "Development of New Preventive Interventions". Finally, current studies are still mainly focused on European populations with inadequate representation of individuals of other ancestries. In fact, the majority of CAD loci discovered in the general population has been derived from studies of non-Hispanic whites, and GRS based on these variants show a poor performance in other racial groups. This problem has been reported not only in the general population [12] but also among subjects with diabetes. For instance, the two GRS for CAD developed in the Look-Ahead study and the ACCORD trial were not associated with increased risk in African-American subjects with type 2 diabetes [17, 19]. There is therefore the need to develop GRS including ancestry-specific loci and variants [12]. Moreover, as it has been the case for studies of the genetics of type 2 diabetes [22], these multi-ancestry approaches may foster the discovery of additional genetic variants associated with CAD. A case in point is the identification of the loss-of-function mutation of PCKS9 in African-American individuals, which prompted the development of PCSK9 inhibitors. Overcoming these challenges will be essential to continue the path towards a fast and highly effective translation of genetic findings into better strategies to prevent CAD and decrease the burden of this health problem among patients with diabetes.

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Chapter 7
Nitric Oxide, Its Role in Diabetes Mellitus and Methods to Improve Endothelial Function

Mariia Nikolaeva and Michael Johnstone

Introduction

Diabetes mellitus (DM) is a major source of morbidity in the United States (US), affecting over 34 million people, which is more than 10% of US population [1]. The cause of much of this morbidity and mortality is vascular disease. Vascular disease in diabetics affects both large vessels and microvasculature, manifested as atherosclerosis and microangiopathy, respectively [2–7]. As discussed elsewhere in this text, atherosclerosis occurs earlier in diabetics than in non-diabetics, its severity is often greater, and its distribution is more diffuse [8, 9].

Because diabetes is a vascular disease, much attention has been given to the vascular endothelium. It has a pivotal role in maintaining homeostasis of the blood vessels. The endothelium's functions include modulating blood cell–vessel wall interactions and regulating blood fluidity, angiogenesis, lipoprotein metabolism, and vasomotion. One mediator that serves a significant function in maintaining vascular homeostasis is nitric oxide (NO), also known as endothelium-derived relaxing factor (EDRF). Alterations in its elaboration, activity, or degradation play an important role in the initiation and progression of vascular diseases.

In 1980, Furchgott discovered that the endothelium is responsible for the vasodilator action of acetylcholine [10]. This finding has fostered a great number of investigations on the role of the endothelium on the initiation and development of vascular

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disease and its subsequent clinical sequelae. Further research indicated that acetylcholine released a soluble factor from the endothelium termed EDRF and that this substance was released by other agents, including bradykinin, substance P, serotonin, and adenosine triphosphate (ATP), and shear stress [11]. Ignarro used spectral analysis of hemoglobin to prove that EGRF was identical to NO [12]. Shortly thereafter, Palmer and colleagues concluded that NO was derived from the terminal guanidino nitrogen of the amino acid L-arginine. The production of NO is catalyzed by the family of enzymes known as NO synthase (NOS) [13]. Three isoforms of NOS have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and cytokine-inducible NOS (iNOS) [14].

The first two NOS isoforms are constituently expressed, whereas the latter is inducible by inflammatory cytokines. The synthesis and release of NO is positively regulated by multiple agonists, such as acetylcholine, histamine, serotonin, bradykinin, thrombin, ADP, substance P, as well as by shear stress, which is considered the main endothelial physiologic stimulus for NO production. NO synthesis requires its main substrate L-arginine and NOS cofactors including tetrahydrobiopterin (BH4), nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) [15].

Vascular smooth muscle responds to NO via stimulation of soluble guanylate cyclase and the formation of cyclic guanosine monophosphate (cGMP) [12]. cGMP modulates the effect of cGMP-dependent protein kinases, including protein kinase G-1 (PKG-I) two isoforms, PKG-Iα in vascular smooth myocytes and cardiomyocytes and PKG-Iβ found in platelets [16]. Activation of PKG-Iα causes vascular smooth muscle relaxation by decreasing intracellular calcium and activating myosin light chain phosphatase, which leads to decreased interaction between myosin light chains and actin. In vascular myocytes, decreased intracellular calcium concentration is achieved by extrusion of calcium from the cell through specific calcium channels and its uptake by sarcoplasmic reticulum via sarcoplasmic/endoplasmic reticulum calcium-ATPase (SERCA). In cardiomyocytes, calcium concentration decreases as a result of inhibition of L-type calcium channels on surface membrane and phosphorylation of phospholamban in sarcoplasmic reticulum [16].

NO can affect systems that are cGMP independent; they include S-nitrosylation of proteins, involved in cell signaling [17], direct activation of adenylate cyclase, which has been described in cardiomyocytes [18], activation of cytosolic adenosine 5′-diphosphate (ADP)-ribosyl transferase in the platelets, which catalyzed the transfer of ADP ribose to glyceraldehyde 3-phosphate dehydrogenase [19]. Furthermore, NO can interact with reactive oxygen species, resulting in peroxynitrite ($ONOO^-$) formation, which induces inflammation and oxidative stress and will be explained in detail later in this chapter. The summary of the NO effects is shown in Fig. 7.1.

Although NO synthesis occurs in a wide variety of cell types and tissues other than vascular endothelium including platelets, macrophages, cardiac myocytes, and neuronal cells, the focus of this discussion is NO and the endothelium.

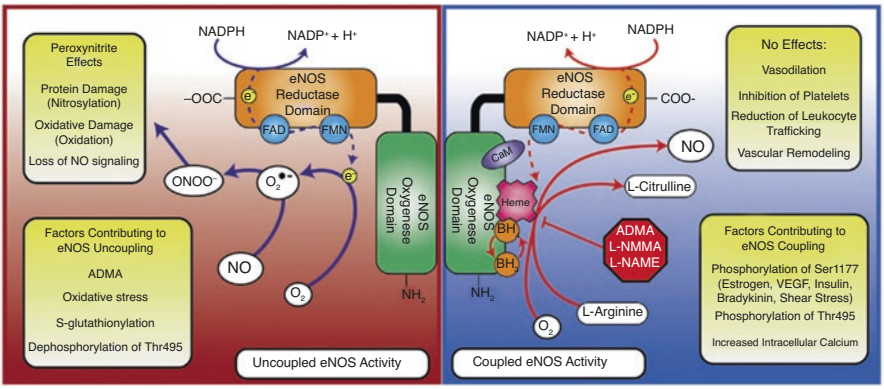

Fig. 7.1 Summary of NO effects during eNOS coupling and uncoupling (reproduced from Cyr, A. R., Huckaby, L. V., Shiva, S. S., & Zuckerbraun, B. S. (2020). Nitric Oxide and Endothelial Dysfunction. Critical Care Clinics, 36(2), 307–321). NO formed by a coupled eNOS activity (blue side) is produced from substrates L-arginine and O_2. The reaction requires the presence of heme and tetrahydrobiopterin. L-citrulline and NO are created as a result of this reaction. In the uncoupled state (red side), superoxide radicals (O_2^-) are generated, which combine with NO and result in peroxynitrite (ONOO) formation. Effects of NO and ONOO as well as factors contributing to eNOS coupling and uncoupling are listed. These are explained in greater detail in the body of the text. NO—nitric oxide, NADPH—nicotinamide-adenine-dinucleotide phosphate, BH4—tetrahydrobiopterin, eNOS—endothelial nitric oxide synthase, ADMA—asymmetric dimethylarginine, L-NMMA—NG-Monomethyl-L-arginine, L-NAME—N(G)-nitro L-arginine methyl ester, FMN—Flavin mononucleotide, FAD—flavin adenine dinucleotide, VEGF—vascular endothelial growth factor

Physiologic Effects of Nitric Oxide on the Vascular System

NO is released continuously by vascular endothelial cells through the action of eNOS, and this basal release regulates vascular tone. NO is important in the maintenance of resting vascular tone [20], in particular the regulation of coronary resistance vessels as well as pulmonary, renal, and cerebral vascular resistance [21, 22]. NO production is highest in the resistance vessels and may be important in the regulation of vascular tone of various vascular beds [23], as well as blood pressure (BP) control.

NO also regulates production and release of other vasodilator substances responsible for endothelial-dependent vasodilation by smooth vascular myocyte hyperpolarization (endothelium-dependent hyperpolarization, EDH). Vasodilation mediated by EDH is particularly important when NO synthesis is impaired, as it happens in endothelial dysfunction. In this situation, EDH takes over the vasodilatory function and partially compensates the loss of NO [24]. NO also modulates vascular tone by regulating the expression of various endothelial vasoconstrictors and growth factors, including platelet-derived growth factor-B and endothelin-1 (ET-1) [25].

NO is also involved in the regulation of myocardial contractility by a cGMP-dependent mechanism. This regulation occurs in the microvascular endothelium

which is in close proximity to cardiac myocytes. NO synthesized by eNOS and nNOS affects cardiac contractility by regulating excitation–contraction coupling, response to β1-adrenergic stimulation, mitochondrial respiration, and intracellular calcium concentration and sensitivity of cardiac sarcomeres to calcium. nNOS-mediated NO release leads to acetylcholine release in vagal neurons and inhibits norepinephrine release by sympathetic nerves in sinus node, which causes negative chronotropic effect [16] (Fig. 7.2).

NO also serves to maintain the integrity of the vascular endothelium through the interaction of both platelets and leukocytes with the vessel wall. Substances released during platelet activation (ADP, serotonin), or the coagulation cascade (thrombin) stimulate NO production [26]. NO is then released from the endothelium into the vessel lumen, in which it interacts with platelets and disaggregates

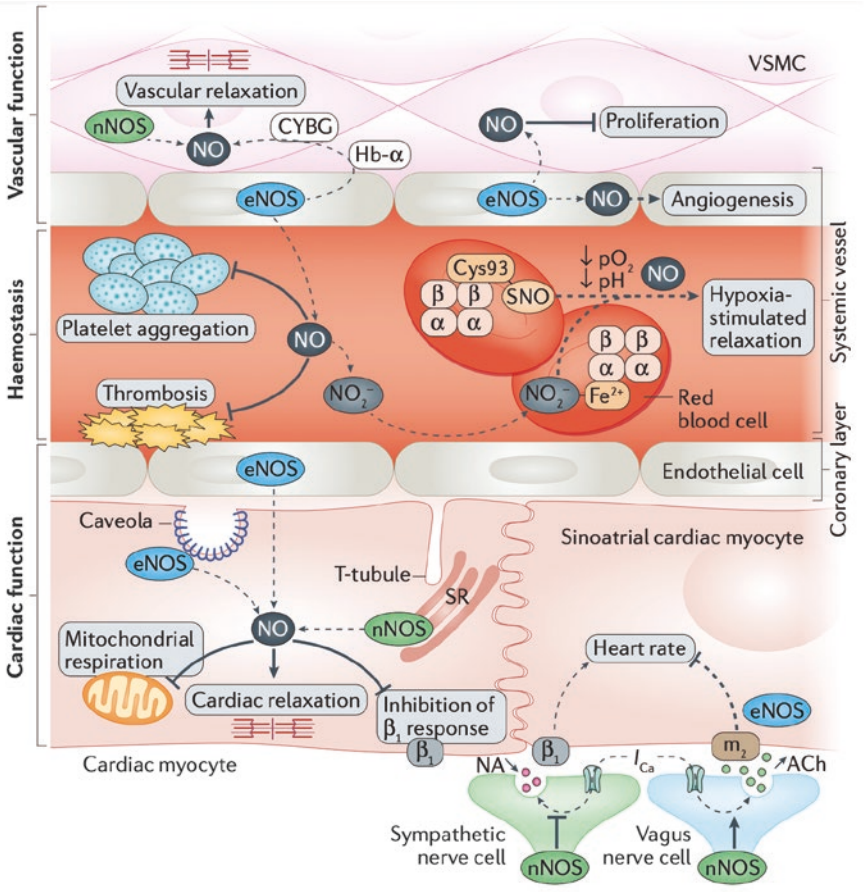

Fig. 7.2 Mechanisms of cardiovascular effects of NO (reproduced from ref. [16]). β1—adrenergic receptor β1; Ach—acetylcholine; m2—muscarinic acetylcholine receptor m2; NA—noradrenaline; SNO—S-nitrosothiol; SR—sarcoplasmic reticulum; T-tubule—transverse-tubule

them via a cGMP-dependent mechanism [27]. Thus, if platelet aggregation occurs in a coronary artery, serotonin and ADP released by platelets and thrombin production stimulates eNOS, resulting in NO formation and vasodilation. This process is different in mechanical injury of the vessel, when thromboxane A2 and serotonin released by thrombocytes cause vasoconstriction to provide hemostasis [24].

NO also serves to attenuate leukocyte–vascular wall interactions. Inhibition of NO promotes leukocyte adhesion to the endothelium and causes a rapid increase in microvascular permeability and vascular leakage that is characteristic of an acute inflammatory response [28].

In vitro [29] and in vivo [30] studies have demonstrated that NO can attenuate vascular smooth muscle proliferation. Animal studies have shown that L-arginine, the substrate for NOS, impairs neointimal proliferation after vascular injury [31].

Nitric Oxide and the Development of Atherosclerosis

All the major cardiovascular risk factors (including hypertension, high levels of low-density lipoprotein [LDL] cholesterol, tobacco use) are associated with decreased endothelium-dependent vasodilation prior to the development of clinically apparent vascular disease. This would suggest that the endothelial damage is implicated in the development of atherosclerosis [32]. After endothelial injury, platelets aggregate in those areas of cell damage, releasing growth factors and cytokines. As a result, the endothelium is more permeable to lipoproteins and other macromolecules, resulting in subendothelial accumulation of LDL-cholesterol, either directly or incorporated into macrophages. The LDL becomes oxidized, further promoting the development of atherosclerosis. This leads to leukocyte adhesion, vascular smooth muscle migration from the media to the intima, and consequent intimal proliferation and extracellular matrix production.

NO plays an essential role in vasodilation and vascular protection but also contributes to the atherosclerotic plaque formation in certain circumstances. Results from the experimental studies on animals indicate that the role of NO produced by endothelial and neuronal NOS isoforms are protective, whereas inducible NOS has proatherogenic properties. eNOS is a constitutively expressed enzyme in the vascular endothelium. In physiologic levels, it has an ability to prevent atherosclerosis and vascular spasm on many levels. The main functions of NO produced by eNOS include the relaxation of smooth myocytes and prevention of platelet aggregation. It also inhibits leukocyte adhesion and migration through the vascular wall, vascular smooth muscle proliferation, and LDL oxidation [33]. eNOS deficiency in apoE/eNOS-double knockout mice fed with a high-fat diet demonstrated significant increase in atherosclerotic burden compared to a control group of apoE knockout mice. Double knockout animals had coronary atherosclerosis, perivascular and myocardial fibrosis, thickened left ventricular wall, aortic aneurysms and

dissection, all of which was not observed in the control group [34]. Interestingly, eNOS overexpression was shown to be as dangerous as eNOS deficiency because of an uncoupling phenomenon. This occurs because of a decreased substrate (L-arginine) or cofactors (such as BH4) resulting in the reaction shifting toward superoxide generation instead of NO production. This, in turn, results in peroxynitrite formation, which is a very reactive molecule that causes lipid peroxidation and cellular damage. In the experimental mouse model with the overexpression of eNOS, supplementation of tetrahydrobiopterin inhibited the progression of atherosclerosis by restoration of NO synthesis [35].

iNOS is inactive in most tissues in normal circumstances. Its transcription is induced by inflammatory cytokines, leading to significant increase in NO levels. NO is produced in larger amounts by iNOS compared to eNOS, and because of that it interacts with superoxide radicals, resulting in above-mentioned peroxynitrite formation and free radical-mediated cellular damage. iNOS also competes for BH4 with eNOS, diminishing utilization of BH4 by eNOS for NO formation, and increasing eNOS uncoupling [36]. The generated reactive oxygen species (ROS) and reactive nitrogen species (RNS) induce the oxidative modification of LDL in endothelium and macrophages, resulting in endothelial dysfunction and initiating atherosclerotic plaque formation [37]. Miyoshi with colleagues demonstrated that atherosclerotic models of apoE knockout mice without iNOS gene had reduced LDL oxidation rate and had significantly lower atherosclerotic lesions after 12 weeks of high-fat diet compared with a control group [38].

nNOS can be primarily found in nervous system and is involved in neuronal signaling. It can be found in the vascular wall and atherosclerotic plaques. This enzyme generates NO and causes eNOS-independent vasodilation. This was demonstrated in studies on human forearm [39] and coronary arteries [40]. Animal studies on apoE knockout mice show that nNOS protects from spontaneous atherosclerosis development [34] and the absence of nNOS in apoE knockout animals leads to the increase in atherosclerotic plaque burden compared to mice with normal nNOS [41].

Endothelial Dysfunction and Diabetes Mellitus

The relative risk of cardiovascular mortality is doubled in diabetic individuals compared to non-diabetics [42]. The specific mediators in diabetes which cause vascular disease are likely multifactorial. These include chronic inflammation, insulin resistance, oxidative stress, and associated with diabetes hypertension and dyslipidemia. In diabetic individuals, endothelial dysfunction and insulin resistance usually coexist and reinforce each other, creating a vicious cycle leading to accelerating atherosclerosis and arterial stiffness.

Endothelium-Dependent Vasodilation in Animal Models

Endothelium-dependent vasodilation occurs as a response to vasodilator substances released by endothelial cells, which causes relaxation of underlying smooth myocytes. NO is the main player in this process, although recent studies revealed another important mechanism of endothelium-dependent relaxation, called endothelium-dependent hyperpolarization (EDH). It works independently of action of NO and results in hyperpolarization of the cell membrane of vascular smooth muscle, causing smooth muscle relaxation [24].

Studies using different animal models of diabetes in several vascular beds [43–46] suggest that there is a decrease in endothelium-dependent vasodilation in the diabetic state. In two such animal models of type 1 diabetes, rats are made diabetic with streptozocin or rabbits made diabetic with alloxan, pancreatic β-cells are destroyed, with a corresponding decrease in insulin secretion. Studies evaluating endothelium-dependent vasodilation in these animal models have demonstrated a decreased response to endothelial stimulators such as ADP, acetylcholine, or its analog methacholine [44].

Similarly, in an animal model of type 2 diabetes, the Zucker rat, which is characterized by hyperglycemia because of insulin resistance, abnormal endothelium-dependent vasodilation is also observed [43]. The early vascular dysfunction that occurs in type 1 diabetic animal models can be prevented with insulin therapy [47, 48]. The abnormal endothelial cell function that develops appears to be as a result of hyperglycemia rather than any other metabolic disturbance. This has been demonstrated by in vitro incubation experiments in which isolated arteries exposed to elevated glucose concentrations have similar decrease in endothelium-dependent vasodilation [49, 50]. This effect does not seem to be as a result of the hyperosmolarity because similar concentrations of mannitol have no effect on endothelium-dependent relaxation [49]. The decreased endothelium-dependent vasodilation that occurs may be as a result of decreased synthesis or release of NO, decreased responsiveness if the smooth muscle to NO, the inactivation of NO by superoxide radicals, generation of endothelial vasoconstrictive factors, or impaired endothelium-dependent hyperpolarization by altered potassium channel expression on smooth myocytes. This will be discussed in greater detail later in this chapter.

Early in the course of experimental diabetes, there is a selective decrease in the response to those endothelium-dependent vasodilators that are mediated by endothelial call receptors. The responsiveness of the endothelium to the direct endothelial vasodilator A23187, or the smooth muscle to nitrovasodilators, is preserved. Using a diabetic rabbit model, abnormal endothelium-dependent relaxation was also found [51] within 6 weeks of initiating the diabetic state. This may be explained by a decrease in the number of receptors, or in their function. These changes are specific to the diabetic state because these abnormal responses do not occur within

2 weeks after initiating the diabetic state and are not found in rabbits not made diabetic after alloxan treatment [52]. Yet, after a longer duration of diabetes, several groups have demonstrated a decrease in smooth muscle cGMP, suggesting a decrease in NO release or action over time [43, 53].

Endothelial cell dysfunction in diabetes may be explained in part not only to perturbations on NO activity or levels but the effect of vasoconstrictor prostanoids. There is increased expression of cyclooxygenase-2 mRNA and proteins levels with hyperglycemia in cultured human aortic endothelial cells but not cyclooxygenase-1. Cohen's group noted that endothelium-dependent relaxation in arteries of diabetic animals could be restored by the administration of cyclooxygenase inhibitors or thromboxane A2 receptor antagonists, suggesting the presence of vasoconstrictor prostanoids [45, 50]. The responsiveness of smooth muscle to direct smooth muscle vasodilators is similar in both diabetic and normal animal models, suggesting that decreased responsiveness to NO is not affected [44, 45].

There is an increase in oxygen-derived free radicals [54], either because if an increase in free radical production or because of a decrease in the free radical scavenger system. Furthermore, free radical scavengers have been shown to improve the abnormal endothelium-dependent vasodilation [55, 56], implying that such free radicals may contribute to the abnormal endothelium-dependent relaxations.

As was previously mentioned, endothelium-dependent hyperpolarization is another important mechanism of endothelium-dependent vasodilation, which also becomes disrupted in the diabetic state. This pathway is regulated by endothelium-dependent hyperpolarization factor, which causes opening of calcium-activated K^+ channels in smooth myocytes and plays an important role in basal and reactive changes in vascular blood flow. A study conducted by Misurski with colleagues showed that the activation of calcium-activated potassium channels may compensate for diminished NO-mediated vasodilation in diabetic rats. In a group of streptozotocin-treated diabetic Sprague–Dawley rats, the vasodilatory response of the mesenteric artery to acetylcholine was attenuated compared to control group, with preserved vasodilation in both groups in response to nitroprusside, suggesting an impairment in endothelium-dependent vasodilation. NOS inhibition showed less attenuated vasodilation to acetylcholine in the group with longer duration of diabetes (14 weeks vs. 2 weeks) and compared to non-diabetic controls, which demonstrated a significant role of non-NO-mediated endothelium-dependent mechanism of vasodilation in diabetes. NO-mediated acetylcholine-induced vasodilation, which was measured in the presence of potassium channel inhibitor tetrabutylammonium, in both 2-week and 14-week diabetic groups, demonstrated a similar degree of impairment in vasodilation. However, in diabetic rats, the degree of vasodilation in both NOS inhibition as well as calcium-dependent potassium channel inhibition was attenuated compared to controls, which showed that both NO-mediated and EDH-mediated components of endothelium-mediated vasodilation are impaired in diabetic state [57]. Similarly, in the experiment conducted by Mayhan et al., EDH-mediated vasodilation was shown to be attenuated in the diabetic state. In their in vivo study on rats, activation of inward-rectifier and calcium-activated potassium channels demonstrated impaired vasodilatory response in cerebral blood vessels in

diabetic compared to non-diabetic rats [58]. Earlier studies on cerebral vasculature of rats also demonstrated impairment in EDH-mediated vasodilation in diabetic state. In the diabetic group, the rats' pial arterioles showed less significant vasodilation in response to activators of ATP-sensitive potassium channels aprikalim and levcromakalim compared to a control group [59, 60]. Nitroglycerin did not demonstrate a statistically significant response on arterial diameter in both groups, suggestive of an alteration in ATP-sensitive potassium channels in the diabetic state [60].

Human Studies of Endothelium-Dependent Vasodilation in Insulin-Dependent Diabetes Mellitus

Human studies evaluating the effects of DM on endothelium-dependent vasodilation have yielded some conflicting results, although they generally corroborate those found in animal studies. Saenz de Tejada et al. [61] studied penile tissue excised from med with erective dysfunction and found that endothelium-dependent relaxation is reduced in the corpus cavernosa of men with erectile dysfunction with diabetes relative to those who are not diabetic.

However, in vitro studies involving human subjects with insulin-dependent diabetes have demonstrated both blunted and normal vasodilatory responses to acetylcholine, methacholine, or carbachol (the latter who being acetylcholine analogs) in forearm resistance vessels in patients with DM [62–64]. To evaluate in vivo endothelial function on these vessels, we and others have employed the venous occlusive plethysmography technique. Type 1 diabetic [62] individuals were shown to have impaired endothelium-dependent responses to methacholine in the forearm resistance vessels. The vasodilator response to both nitroprusside and verapamil, both endothelium-independent, were preserved. In this study, all the patients were taking aspirin, making it unlikely that vasodilator prostanoids were responsible for the altered endothelium-dependent relaxation. The degree of attenuation of forearm blood flow (FBF) response to methacholine was inversely correlated with the serum insulin level, but it did not significantly correlate with serum glucose concentration, glycosylated hemoglobin, or duration of diabetes.

Calver et al. [64] reported a decrease in responsiveness of *N*-monomethyl-L-arginine (L-MNNA), an inhibitor of NOS, suggesting a decrease in the basal NO release from the endothelium. Conversely, Smits et al. [63] and Halkin et al. [65] did not detect any impairment in endothelium-dependent vasodilation with type 1 diabetes. Both flow-mediated relaxation and endothelium-independent responses have also been found to the impaired in non-atherosclerotic peripheral conduit arteries and in angiographically normal coronary vessels in diabetic subjects [66, 67].

The reason for these contradictory results is unclear and probably multifactorial. Closer examination of these reports reveals that the subject population was not uniform between the various groups. Variations included the presence of absence of macrovascular or microvascular complications and autonomic dysfunction, the

gender studied (single sex vs. mixed), the degree of long-term glycemic control, the serum glucose concentration, the presence or absence of microalbuminuria, and the serum insulin concentration. Microalbuminuria, an early marker of diabetic nephropathy and a predictor of coronary artery disease (CAD), may correlate with the severity of endothelial dysfunction. Endothelial function in insulin-dependent diabetic subjects was normal in those studies that excluded individuals with microalbuminuria [63, 65] and abnormal in the study that included subjects with microalbuminuria [64].

The degree of glucose control may, in part, explain the variation in the data [62, 64, 68], because it has been established that glucose alone can alter endothelial function [69]. The serum insulin concentration was not routinely measured in most of these studies, although we found an inverse relationship between the serum insulin concentration and endothelial function [62]. Studies involving mixed genders might add further variation relative to studies with men alone because women appear to be protected against the adverse effects of risk factors of endothelium-dependent vasodilation compared with men [70]. Lastly, the presence of autonomic dysfunction in the study subjects may alter the response to the various agents administered.

Human Studies of Endothelium-Dependent Vasodilation in Non-insulin-Dependent Diabetes Mellitus

Reduced endothelium-dependent vasodilation was demonstrated in studies on different vascular beds of patients with type 2 diabetes [71–74]. Several studies also demonstrated that the impairment in endothelium-independent vasodilation was in type 2 DM [75–77]. These results would suggest that the mechanism of the impairment of vasodilation in type 2 diabetes might be different from that of type 1. It is important to note that patients with type 2 diabetes are usually older and have other cardiovascular risk factors, including dyslipidemia and hypertension [78] which, by themselves, can contribute to an impairment of endothelial function.

Possible Mechanisms of Impaired Endothelium-Dependent Vasodilation

The proposed mechanisms by which diabetes affects endothelial function may result from the changes in glucose metabolism, alterations in insulin signaling pathways and endothelial dysfunction, impaired NO synthesis due to oxidative stress, decreased availability of substrates and cofactors of NO synthesis, eNOS modifications causing its uncoupling, presence of endogenous eNOS inhibitors, and increased NO breakdown (Table 7.1).

Table 7.1 Mechanisms of endothelium-dependent vasodilation in diabetes

Alteration in insulin signaling pathways
• Attenuation of PI3K signaling (vasodilating) pathway
• Activation of MAPK-mediated (vasoconstricting) pathway
Impaired NO synthesis and/or sensitivity
• Oxidative stress and increase in NADPH oxidase activity
• Increase in oxidized LDL
• Deficiency in NO substrates and cofactors (e.g., L-arginine, BH4, NADPH)
• eNOS S-glutathionylation
Increased concentration of endogenous inhibitors of eNOS
• eNOS inhibition by ADMA
• eNOS inhibition by Caveolin-1
Increased NO inactivation (decreased bioavailability) and/or breakdown of NO
• NO inactivation by AGEs
• NO inactivation by peroxynitrite
eNOS inactivation by free fatty acids

PI3K—phosphatidylinositol 3-kinase; MAPK—mitogen-activated kinase; NO—nitric oxide; NADPH—reduced nicotinamide-adenine-dinucleotide phosphate; LDL—low-density lipoproteins; BH4—tetrahydrobiopterin; eNOS—endothelial nitric oxide synthase; ADMA—asymmetric dimethylarginine; AGEs—advanced glycosylation end-products

Alterations in the Insulin Signaling Pathways and Endothelial Dysfunction

Vascular endothelium plays an important role in maintaining vascular homeostasis. It has a control over vascular lumen size by mechanical transduction of frictional force from blood flow to the vessel wall, which regulates the vascular tone to meet tissue demand [79]. Insulin plays an important role in vascular homeostasis by increasing vascular compliance of conduit arteries, dilating resistant arterioles, and increasing vascular permeability to nutrients [80]. Insulin effect on vascular diameter depends on a signaling pathway that gets activated (Fig. 7.3) [82]. While this has been reviewed in detail earlier in this text (See Chap. 3), we will review it here again. Classically, insulin's effect on the endothelium results in vasodilation via increased NO production and bioavailability. When insulin resistance develops, the net effect of insulin stimulation results in vasoconstriction [81]. Insulin promotes eNOS activation via enhancement of eNOS expression in endothelial cells [83]. Activated insulin receptors stimulate intrinsic kinase activity, leading to phosphorylation of insulin receptor substrate (IRS) proteins. In turn, they activate phosphatidylinositol 3-kinase (PI3K)-mediated mechanism, which results in activation of different serine/threonine kinases, specifically protein kinase B, also known as Akt, which activates eNOS by phosphorylation of serine residue 1177 [84]. In addition to phosphorylation, eNOS undergoes other posttranslational modifications, including palmitoylation, nitrosylation, and addition of *N*-acetyl-glucosamine residue. These mechanisms provide basal and insulin-stimulated production of NO [85].

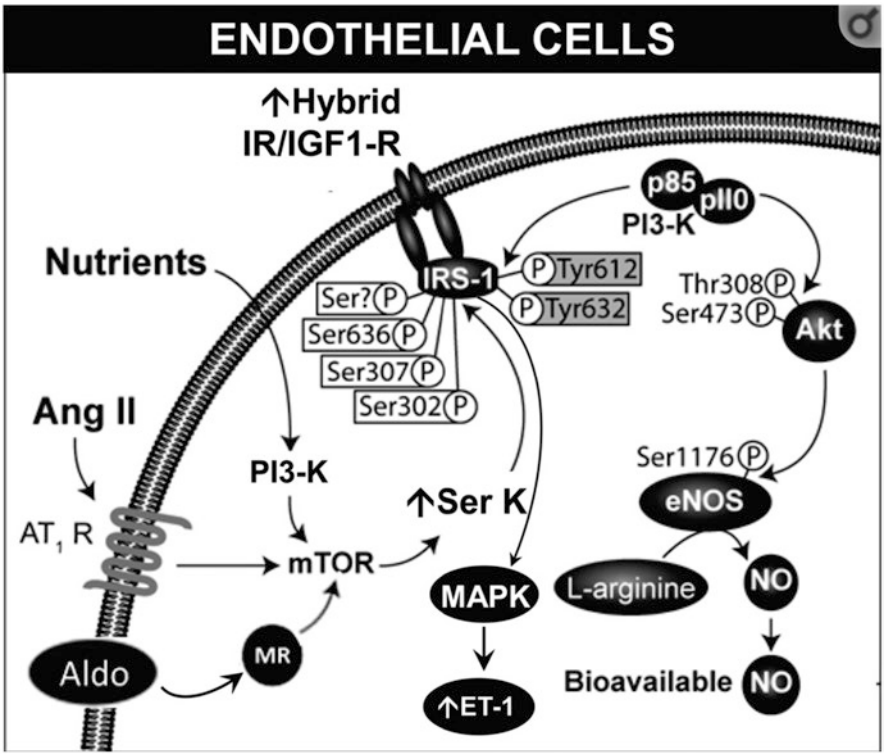

Fig. 7.3 Insulin receptor (IR) signaling pathways (reproduced from ref. [81]). Under normal conditions, stimulation of IR results in activation of the PI3K–Akt pathway, resulting in phosphorylation of eNOS, NO formation, and vasodilation. In case of insulin resistance (caused by increased activity of renin–angiotensin–aldosterone system and nutrient excess) there is increased phosphorylation of serine in insulin receptor substrate, which increases activation of MAPK signaling pathway. Aldo—aldosterone; Ang II—angiotensin II; AT1R—angiotensin II type 1 receptor; eNOS—endothelial NO synthase; ET-1—endothelin-1; IGF1-R—insulin-like growth factor-1 receptor; IR—insulin receptor; mTOR—mammalian target of rapamycin; MR—mineralocorticoid receptor; MAPK—mitogen-activated protein kinase; NO—nitric oxide; PI3K—phosphatidylinositol 3-kinase; p—phosphorylation; Akt—protein kinase B; Ser—serine; Ser K—serine kinase; Thr—threonine; Tyr—tyrosine

Interestingly, endothelial-dependent relaxation occurring through PI3K activation, also mediates glucose uptake in skeletal muscle, as well as glycogen and protein synthesis, which demonstrates the primary role of this pathway in metabolic actions of insulin. When endothelial dysfunction develops, it aggravates insulin resistance, which can be partially explained by attenuation of PI3K pathway [81].

The vasoconstricting effect of insulin is mediated by mitogen-activated (MAP) kinase-dependent signaling, which also promotes the mitogenic and growth effects of insulin. This signal transduction pathway results in an increase of endothelin-1 production and activates renin–angiotensin–aldosterone system (RAAS) [24]. Endothelin-1 is a potent vasoconstricting factor with profibrotic and inflammatory

properties, playing an important role in endothelial dysfunction in people with diabetes and obesity [80]. Endothelin-1 level was found to be increased in diabetes as demonstrated in both in vitro diabetic human umbilical vessel endothelial cells [86] as well as in clinical studies of diabetic patients [87]. Furthermore, studies involving diabetic Zucker rats demonstrate an upregulation of endothelial receptor ET-A, thereby augmenting endothelin's effect [88].

There is evidence that insulin resistance leads to preferential activation of MAPK-mediated vasoconstricting pathway, whereas vasodilatory mechanisms become disrupted. An imbalance of vasodilators and vasoconstrictors caused by insulin resistance is demonstrated in rats. In normotensive and insulin sensitive rats, insulin caused a vasodilatory and dose-dependent effect on mesenteric arteries, which were contracted by norepinephrine before the experiment. This vasodilatory insulin effect was diminished by addition of inhibitors of tyrosine kinase (genistein), PI3-kinase (wortmannin), and NO synthase (Nω-nitro-L-arginine methyl ester). Insulin-resistant hypertensive group of rats in the experiment showed impaired vasodilation by 20% compared to controls. Interestingly, ET-1 receptor blockade restored insulin-mediated vasodilation in this group, suggesting for ET-1 involvement in vasoconstriction in insulin resistance [89]. Selective attenuation of insulin signaling was shown in ex vivo and in vivo experiment conducted by Jiang and colleagues on obese Zucker rats. Obese diabetic rats had decreased PI3-kinase activity stimulated by insulin in the aorta compared to the controls. A downstream pathway was also inhibited, which decreased insulin-stimulated serine phosphorylation of Akt in isolated microvessels in the experimental group. At the same time, MAP-kinase pathway was equally increased in microvasculature in both groups of rats, but in obese rats its baseline activity was higher [84].

Impaired Nitric Oxide Synthesis and/or Sensitivity

Despite confirmed impairment in NO-mediated vasodilation in diabetes, studies have shown that hyperglycemia enhanced NO production and decreased its bioavailability, causing ROS synthesis [90, 91]. Studies on humans measuring serum NO concentration showed contradicting results. Because NO has very short half-life, nitrite and nitrate levels are usually measured as a surrogate of total NO concentration. A large systematic review and meta-analysis conducted by Assmann and colleagues in 2016, which reviewed 30 studies measuring NO levels in diabetic individuals, showed that NO levels were significantly increased in plasma of both T1DM and T2DM patients compared with non-diabetic controls [92]. The authors hypothesized that this finding is possibly explained by increased NO production by iNOS in the diabetic state and/or xanthine oxidoreductase upregulation, with corresponding increase in ROS formation [93]. Several studies showed correlation between higher HbA1c level and plasma NO elevation [93, 94], as well as lower NO levels in patients with better controlled type 1 diabetes compared with poorly controlled diabetes group [95]. Although majority of published studies describe

positive correlation between presence of diabetes and serum NO level, some studies found no correlation [96] or negative correlation [97, 98] between DM and NO level. The reasons of this inconsistency are unknown and require more studies on larger groups of patients.

As was previously mentioned, NO might have both protective and harmful effects on vasculature and endothelial function. On the one hand, it causes vasodilation, prevents smooth muscle proliferation and platelet aggregation, and decreases inflammatory response. On the other hand, it can cause detrimental effects on the endothelium by generating ROS. The hypothesis that the elevation in total plasma NO in diabetes occurs because of predominant iNOS-mediated NO rather that eNOS-mediated NO synthesis is controversial. Studies on diabetic mice demonstrated increased iNOS and decreased eNOS expression in coronary arterioles compared to non-diabetic controls [99]. On the contrary, in the experiment conducted by Blum with colleagues, higher eNOS expression was demonstrated in vitro when human umbilical vessel cell cultures were incubated with sera of patients with proliferative diabetic retinopathy (PDR), compared to sera of non-diabetic individuals or diabetic patients without PDR. This correlated with increased serum NO level in patients with PDR compared with two other groups. No difference was observed in iNOS expression [96]. These experiments demonstrate and confirm the complexity of NO metabolism in diabetic patients.

NO is continuously synthesized in endothelial cells by eNOS with L-arginine and oxygen, resulting in the formation of NO and L-citrulline. Any alternation in this pathway will result in decreased NO availability and impairment in NO synthesis. Increase in oxidized LDL, deficiency in BH4 or L-arginine, eNOS S-glutathionylation, and increase in NADPH oxidase activity result in eNOS uncoupling with subsequent chain of reactions resulting in decreased NO production and increased ROS generation [36].

Oxidative Stress and Role of NADPH Oxidases

Oxidative stress occurs in conditions of imbalance between ROS and antioxidant system, including enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase [100]. Hyperglycemia promotes ROS generation through activation of protein kinase C (PKC)-mediated NADPH oxidases, formation of advanced glycosylation end-products (AGEs), increased polyol synthesis, as well as eNOS uncoupling. It also causes a decrease in the free radical scavenger system and decrease in endogenous antioxidants SOD, catalase, and glutathione peroxidase, which was demonstrated on animal models [101, 102].

NADPH oxidases (NOX) are critical players in diabetes-related ROS formation. Type 1 and 2 NOX have been shown to cause endothelial dysfunction, whereas NOX type 4 appears to be an important component of normal glucose homeostasis by enhancing insulin signaling and preventing insulin resistance. NOX oxidizes BH4 causing its deficiency, which promotes eNOS uncoupling and turns it into a

superoxide-generating enzyme, with resulting decrease in the NO production. This leads to peroxynitrite formation and generation of oxidative and nitrosation–oxidative stress [80], which with impaired eNOS bioactivity and increased leukocyte adhesion cause endothelial dysfunction [36]. Furthermore, both hyperglycemia- and hyperinsulinemia-activated endothelial NOX2 alter insulin signaling pathways by the enhancement of Raf/MAPK-mediated vasoconstriction and simultaneous attenuation of PI3K/Akt-mediated vasodilation [80]. Besides causing endothelial dysfunction, ROS decrease vascular smooth muscle responsiveness to NO because it changes the β subunit Fe^{2+} to Fe^{3+} in soluble guanylate cyclase, leading to loss of responsiveness to NO [103].

Oxidized LDL (ox-LDL)

Type 2 diabetes is characterized by altered lipid metabolism and is associated with increased ox-LDL formation. Ox-LDL interacts with its receptor (lectin-like oxidized low-density lipoprotein receptor 1 or LOX-1), which attenuates akt-mediated phosphorylation of eNOS serine 1177 (serine 1176 in mice) and downregulates eNOS synthesis [36] in a diabetic mouse model. Restoration of eNOS phosphorylation improved endothelium-dependent vascular relaxation and decreased stroke size after middle cerebral artery occlusion [104] in this animal model. Activation of LOX-1 also leads to upregulation of intracellular eNOS inhibitor caveolin-1 and plasma eNOS inhibitor asymmetric dimethylarginine (ADMA), activation of arginase II, resulting in the attenuation of activity of eNOS and a decrease of the NO bioavailability [36].

Decreased L-arginine Availability

Another explanation for a decrease in NO synthesis in diabetes is the decreased availability of L-arginine, an important substrate for NO synthesis. L-arginine can be metabolized into NO and L-citrulline by NOS or can be converted into L-ornithine and urea by arginase enzyme [105]. Relative depletion of L-arginine due to its augmented breakdown with concomitant increase in L-ornithine and L-citrulline can be assessed by a global arginine bioavailability ratio (GABR). GABR is defined as [L-arginine]/([L-ornithine] + [L-citrulline]). In studies decreased GABR has been independently associated with more significant coronary artery disease, increase in major adverse cardiovascular events (MACE) [105], endothelial dysfunction, and increased risk of cardiovascular mortality [106]. Several studies demonstrated that in diabetic individuals GABR is decreased compared to non-diabetics [106, 107].

Besides L-arginine in the serum, its intracellular concentration has also been shown to be important in eNOS regulation. Studies show that endothelial cells are not dependent on L-arginine from the extracellular space and are able to obtain it

from L-citrulline and from other sources, like protein breakdown [33]. The proposed mechanism of intracellular L-arginine depletion and resulting decrease in NO synthesis is L-arginine breakdown by arginase enzyme, which metabolizes it into urea and L-ornithine [33]. L-arginine is a substrate for arginase I in liver, arginase II in peripheral tissues, and eNOS; therefore, arginases can reduce the available L-arginine to eNOS in cells and decrease NO generation. In the experiment on human corpora cavernosa, diabetic men with erectile dysfunction had increased arginase II gene and protein expression compared to the cavernosal tissue obtained from healthy non-diabetic men. Inhibition of arginase in the experiment significantly enhanced eNOS activity [108]. A significant elevation of the serum arginase activity was demonstrated in streptozocin-induced diabetic rats compared to controls and was strongly correlated with blood glucose level. Arginase inhibition with citrulline, norvaline, and ornithine significantly reduced AGEs concentration and showed decrease in diastolic blood pressure in diabetic rats. An impaired endothelium-dependent relaxation in response to acetylcholine in aortic rings observed in diabetic group of rats was prevented by arginase inhibition, suggesting an important role of arginase in decreased NO synthesis is diabetes [109].

Other mechanisms of L-arginine-dependent alterations in NO synthesis include its potential radical-scavenging properties, the cooperativity between L-arginine and BH_4-binding sites of NOS, and competition of L-arginine with endogenous eNOS inhibitor ADMA for eNOS L-arginine binding site [33].

Decreased Availability of Cofactors of NO Synthesis

Another requirement for the synthesis of NO is the availability of cofactors, including oxygen, calcium, calmodulin, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) [110]. Decreased availability of any of these cofactors would result in impaired synthesis of NO. One particular culprit that may be depleted in DM is NADPH, which undergoes increased consumption in hyperglycemic states and is restored via the pentose phosphate pathway. Hyperglycemia results in inhibition of this pathway, resulting in depletion of NADPH [111]. NADPH is a cofactor of aldose reductase enzyme, which converts glucose to sorbitol. Increased glucose conversion in the polyol pathway consumes NADPH, causing its depletion [112]. The second step is the oxidation of sorbitol to fructose, mediated by sorbitol dehydrogenase, which is coupled with the reduction of NAD^+ to NADH [113]. The increased cytosolic NADH/NAD^+ results in an altered redox state, which may alter the availability of tetrahydrobiopterin, an essential cofactor for NOS. If tetrahydrobiopterin is depleted, NO production is decreased [114, 115]. Wu et al. demonstrated decreased NADPH concentration in the lung tissue of diabetic rats. In this experiment, activity of glucose-6-phosphade dehydrogenase and levels of reduced glutathione were found to be suppressed, suggesting that NADPH depletion was caused by its consumption by the polyol pathway and functional impairment of G6PD [116]. A similar finding has been described in pancreatic tissue of diabetic rats [117].

S-glutathionylation of eNOS

One of the recently discovered mechanisms of eNOS uncoupling is eNOS S-glutathionylation. S-glutathionylation is a posttranslational modification of the protein in which the protein binds a glutathione tripeptide via a disulfide bond [118]. This process is involved in intracellular signaling and adaptation to oxidative stress. Oxidative stress enhances S-glutathionylation of proteins, including eNOS. When it happens, the ability of eNOS to produce NO decreases, shifting its enzymatic activity toward free radical generation and decreasing endothelium-dependent vasodilation [119]. This mechanism has been proposed to play a role in pathogenesis of endothelial dysfunction in diabetes [120] and hypertension [119] and was demonstrated in the experiment on spontaneously hypertensive rats [33]. Thus, eNOS S-glutathionylation represents another mechanism implicated in eNOS uncoupling and requires further research as a potential target for treatment of endothelial dysfunction in diabetes [33].

Increased Concentration of Endogenous Inhibitors of eNOS, ADMA, and Caveolin-1

Asymmetric dimethylarginine (ADMA) is a product of the degradation of methylated proteins containing L-arginine. L-arginine methylation in proteins is one of the posttranslational modifications, which determines signal transduction, histone function, and interaction of the resultant protein with other cellular proteins [121]. This process is mediated by the group of proteins called protein arginine methyltransferases (PRMT), which use *S*-adenosylmethionine as a methyl substrate. This results in the production of NG-Monomethyl-L-arginine (L-NMMA), ADMA, and symmetric dimethylarginine (SDMA). ADMA and L-NMMA are competitive inhibitors of all types of NOS. As a L-arginine analog, ADMA competes with it for eNOS L-arginine-binding site, thereby decreasing NO production in a dose-dependent fashion. In the vascular wall it also promotes free radical generation, which leads to eNOS uncoupling and decreased eNOS-derived NO bioavailability [122, 123].

There is evidence that ADMA levels are elevated in patients with type 2 diabetes, as well as in prediabetic individuals. Studies on ADMA level concentration in type 1 diabetes are conflicting, with some of them reporting elevated levels [124] and other showing decreased ADMA levels in type 1 diabetic individuals compared to non-diabetic controls [125]. One study showed an elevated plasma concentration of ADMA, inflammation, and adhesion molecules in prediabetic subjects [126]. In patients with type 1 diabetes and diabetic nephropathy, elevated plasma ADMA levels were correlated with all-cause mortality, incidence of cardiovascular events, and progression of kidney disease even after adjustment for CVD risk factors and baseline GFR [127]. In one study on Sprague–Dawley rats with induced type 2 diabetes, not only were ADMA levels elevated, but there was also reduced activity of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme responsible for

ADMA degradation. After exposure to hyperglycemic conditions, vascular smooth muscle cells (VSMC) and human endothelial cells demonstrated reduced DDAH activity. This was accompanied by ADMA accumulation cGMP depletion, which was suggestive for an impaired NO synthesis in these cells [128]. One study involving type 2 diabetic patients without known diabetic complications had significantly higher ADMA levels than a control group of healthy volunteers, despite similarities of two groups in age, gender distribution, BMI, and lipid levels [129]. It was also shown that in a population of type 2 diabetic individuals there is a positive correlation between higher ADMA levels and cardiovascular atherosclerotic disease independently on other cardiovascular risk factors, including homocysteine [130].

High ADMA levels and low arginine/ADMA ratios were associated with increased all-cause mortality in a large Framingham Offspring Study Cohort consisting of more than 3000 participants. Interestingly, in pre-specified analyses, this correlation was not observed in diabetic participants compared to those without diabetes. The Authors hypothesized that this phenomenon was caused by diabetes-related renal hyperfiltration with better renal clearance of ADMA at early stages of diabetic nephropathy (only participants with serum creatinine <2 mg/dL were included in the study), and potential more complex ADMA physiology in the presence of diabetes [131].

Another important inhibitor of eNOS activity described in literature is caveolin-1, a coat protein of caveolae, which are invaginations of plasma membranes. Caveolae are present in multiple cell types, including endothelial cells, VSMC, and adipocytes. Physiologically, they participate in maintaining plasma membrane integrity, signal transduction, and intercellular transport. Caveolin-1 upregulation decreases NO synthesis, whereas its downregulation causes the opposite effect. The disruption of caveolin-1 gene and absence of caveolae in mice increased the basal release of NO by 31% and caused threefold increase of its downstream mediator cGMP compared to mice with normal caveolin-1 function. Endothelium-dependent relaxation in aortic rings in the experimental group with disrupted caveolin-1 gene was markedly increased [132]. Caveolin-1 also plays an important role in insulin signaling pathway. An in vitro study on adipocytes cultured in a hyperglycemic environment, demonstrated increased caveolin-1 expression, as well as insulin receptor and PI3K dephosphorylation and therefore development of insulin resistance [133]. In both Type 1 and Type 2 diabetes, there is increase in caveolin-1 gene expression and as a result, eNOS inhibition and decreased NO synthesis [134].

Increased Nitric Oxide Inactivation (Decreased Bioavailability) and/or Breakdown of Nitric Oxide

Interposed between the endothelium and the smooth muscle cells of the media is a layer of subendothelial collagen. The auto-oxidation of glucose results in a non-enzymatic glycosylation reaction between glucose and the amino groups of

protein, termed advanced glycosylation end-products (AGEs). AGE-modified proteins interact with specific binding proteins, and trigger oxidation-enhancing reactions. Studies demonstrated an important role for AGEs in pathogenesis of diabetic vasculopathy. At concentrations similar to those found in the plasma of diabetic subjects, AGEs have been shown both in vitro and in vivo to inhibit eNOS activity [135].

Bucala and coworkers demonstrated NO inactivation by AGEs via a rapid chemical reaction both in vitro and in vivo [136]. Diabetic rats were shown to have decreased endothelium-derived vasodilation over time, and insulin did not reverse this effect. However, aminoguanidine, an inhibitor of advanced glycosylation both in vivo and in vitro, slowed the development of the vasodilatory impairment.

Another mechanism of direct NO inactivation is ROS-mediated peroxynitrite formation, which was described earlier. NO avidly reacts with superoxide and other ROS, resulting in peroxynitrite production. Peroxynitrite is highly cytotoxic and causes protein, lipid, and DNA damage. In the presence of oxidative stress caused by imbalance between ROS generation and antioxidant system defense, increase in ROS concentration causes more NO inactivation and peroxynitrite formation [137].

Free Fatty Acids and Nitric Oxide

Circulating Free Fatty Acids (FFAs) may play a role in the impairment of endothelial function in patients with DM. These FFAs are elevated in patients with DM because of excess liberation from adipose tissue and decreased uptake by skeletal muscle [138–140]. Patients with type 2 DM have increased abdominal adipose tissue that is often more insulin resistant and tends to release more FFAs than adipose tissue from other locations. Infusion of FFAs have been shown to reduce endothelial-dependent vasodilation in both animal and human subjects [141].

The FFAs act to decrease endothelial function probably by several pathways, including increased production of oxygen-derived free radicals, activation of PKC, and a decrease in insulin receptor substrate-1-associated PI3K activity [142–144]. Overproduction of superoxide induced by FFA leads to activation of pro-inflammatory signals and inactivation of prostacyclin synthase and eNOS, leading to decreased synthesis of prostanoids and NO [145]. In both insulin-resistant obese Zucker rats and high-fat diet-induced insulin-resistant mice, inhibition of FFA release from adipocytes prevented eNOS and prostacyclin synthase inactivation. This effect was also achieved by inhibition of the FA oxidation by blocking a rate-limiting enzyme of this pathway and inhibition of ROS synthesis [146].

Increased levels of FFAs cause increased VLDL production and cholesteryl ester synthesis. The resulting increased triglycerides found in diabetic subjects, coupled with the lower high-density lipoprotein (HDL), have also been associated with endothelial dysfunction [147, 148] and are discussed in greater detail below.

Other Risk Factors in Diabetic Endothelial Dysfunction

Dyslipidemia

Dyslipidemia is a common problem affecting patient with DM. Much evidence shows that elevated total and LDL-cholesterol levels are associated with impaired endothelial function, independent of the presence of other cardiac risk factors [149–153]. Furthermore, it remains unclear whether the mechanism of the endothelial dysfunction associated with dyslipidemia is the same as or different from that of DM. Possible mechanisms include decreased NO availability [154, 155], L-arginine deficiency [152, 156], increased NO inactivation via superoxide production [157], and the pro-inflammatory state [158]. It is therefore difficult to determine accurately the relative contribution that dyslipidemia has on diabetic endothelial dysfunction.

The dyslipidemia frequently affecting type 2 diabetics is characterized by elevated levels of small dense LDLs and triglycerides with low levels of HDL. The degree of impairment of endothelium-dependent relaxation in type 2 diabetics is significantly correlated with the serum triglycerides with low levels of HDL. The degree of impairment of endothelium-dependent relaxation in type 2 diabetics is significantly correlated with the serum triglyceride level [71] and inversely correlated with LDL size [11, 159, 160]. Skyrme-Jones and colleagues [161] have reported a similar deleterious effect of the small, dense LDLs and the reduced LDL vitamin E content on endothelium-dependent vasodilation in patients with type 1 diabetes. The diabetic state can result in the glycation of HDL, which may impair the protective effect of HDL on the endothelium [162].

Hypertension

Numerous animal and clinical studies have demonstrated that hypertension reduces endothelium-dependent relaxation [101, 163–166]. It was shown that basal production or release of NO is decreased in hypertensive patients [64, 167]. The possible mechanisms underlying the endothelial vasodilator dysfunction associated with hypertension include L-arginine deficiency [168], decreased muscarinic receptor function [169, 170], abnormalities in signal transduction [171], or NO inactivation by oxygen-derived free radicals [172–175].

As with dyslipidemia, hypertension is frequently associated with DM, making the relative contribution of either risk factor to the endothelial dysfunction found in the hypertensive diabetic person difficult to determine, Epidemiological studies have shown an association among obesity, insulin resistance, and hypertension [176, 177]. Further research has found that even lean individuals with essential hypertension are frequently insulin resistant. This finding led investigators to propose that insulin resistance and hyperinsulinemia may contribute to the pathogenesis of hypertension.

Lipotoxicity

Lipotoxicity refers to lipid accumulation in non-adipose tissue. This phenomenon is commonly observed in type 2 diabetes and metabolic syndrome. Lipotoxicity and glucotoxicity augment each other and stimulate the production of diacylglycerol (DAG) and ceramides [81]. DAG activates intracellular protein kinase C (PKC) isoforms β1 and β2 in endothelial cells, which inhibit intracellular effects of insulin, causing insulin resistance. In an experiment on insulin-resistant Zucker obese rats, treatment with the PKCβ inhibitor ruboxistaurin partially restored impaired Akt phosphorylation and cGMP depletion in diabetic animals. In this experiment, it was also shown that only PKCβ isoforms (and not PKCα, -δ, or -ζ) decreased insulin-stimulated Akt phosphorylation and eNOS expression [178]. Elevated PKCβ expression was also demonstrated in diabetic humans. Diabetic individuals also had altered insulin-mediated eNOS phosphorylation and decreased flow-mediated vasodilation compared to non-diabetic controls. Inhibition of PKCβ in diabetic patients improved eNOS phosphorylation at serine 1177 and reduced NFκB expression, which suggested that PKCβ impairs insulin-mediated eNOS activation and promotes an inflammatory response [179]. Lipotoxicity also impairs insulin-mediated NO production by stimulation of Toll-like receptor 2 (TLR2) and subsequent activation of the inflammatory cascade [81].

Potential Preventive and Therapeutic Options

Protein Kinase C Inhibitors

Hyperglycemia can activate PKC, which in turn increases oxidative stress by increasing iNOS expression. Inhibitors of different subtypes of PKC have been shown to decreased iNOS expression and iNOS-mediated NO release [180, 181] and increase eNOS expression.

An inhibitor of PKCβ LY333531 has been developed; in an experimental model, it normalizes retinal blood flow and glomerular filtration rate in parallel with the inhibition of PKC activity [182]. Moreover, experiments on other animal models showed that LY333531 reduced the incidence of diabetic nephropathy, retinopathy, neuropathy, and increased eNOS expression in cultured aortic endothelial cells and endothelium-derived relaxation and attenuated glucose-induced oxidative stress. These findings suggest that PKC-β inhibition can improve endothelial function and prevent micro- and macrovascular complications in diabetes [183].

Inhibitors of AGE Production

The production of AGE, as a result of prolonged exposure of proteins to chronic hyperglycemia, can result in direct quenching of NO and increasing the oxidative

stress. AGEs are formed in a reaction between ketones and aldehydes and protein amino groups. Activation of specific AGE receptors enhance NOX-1 activity, leading to ROS generation, which promotes inflammation, oxidative stress, and atherogenesis. Inhibition of AGE accumulation has shown to decrease the progression of diabetes-mediated atherosclerosis [184] and improve endothelial function [185] in animal models. Telmisartan was shown to decrease AGE-induced C-reactive protein (CRP) generation by decreased expression of AGE receptors [186]. Glucagon-like peptide-1 demonstrated a decrease in the production of pro-inflammatory cytokines by inhibition of AGE receptors [187]. AGE inhibition has also been demonstrated with ACE inhibitors, statins, bisphosphonates, ascorbic acid, alpha-lipoic acid, carnosine, glucagon-like peptide-1 receptor agonist exendin, and NF-κB inhibitor-pyrrolidine dithiocarbamate [188].

Vitamins C and E

As discussed earlier, one possible mechanism of endothelial dysfunction in both type 1 and type 2 DM is the inactivation of NO by oxygen-derived free radicals. Several clinical studies have reported a decrease in endogenous vitamin C [189, 190] and E [189, 191] levels in both type 2 and type 1 DM. Any means of decreasing the oxidative stress has the potential to improve endothelium-dependent vasodilation. Timimi et al. [192] and Ting and coworkers [193] found that intra-arterial infusion of vitamin C improved endothelium-dependent (but not endothelium-independent) relaxation in patients with type 1 and type 2 diabetes, respectively. Furthermore, the intra-arterial infusion of ascorbic acid restored the impaired endothelial vasodilation in healthy subjects exposed to hyperglycemic clamp [194]. In another experiment, oral vitamin C and E supplementation was shown to improve endothelial function in type 1 diabetic patients but not those with type 2 diabetes [195]. Vitamin C supplementation in patients with type 2 diabetes has been shown to improve glycemic control, blood pressure [196], lipid profile, and insulin sensitivity [197].

Tetrahydrobiopterin

Prolonged hyperglycemia and hypercholesterolemia both cause a depletion of tetrahydrobiopterin (BH4), an essential cofactor for NOS, resulting in an uncoupling of eNOS and lowered production of NO [198]. BH4 supplementation has been extensively studied to examine its effects on endothelial function by restoration of eNOS activity. In diabetic patients, BH4 supplementation showed improvement in endothelium-dependent dilation [199]. In a study on healthy subjects, BH4 supplementation showed to restore impaired endothelium-dependent vasodilation induced by an oral glucose challenge [200]. Short-term BH4 supplementation has been

shown to improve endothelial function on many human studies [201], but data on long-term effects of BH4 supplementation is lacking. Unfortunately, BH4 systemic effects are limited by its oxidation to dihydrobiopterin (BH2), which does not serve as an eNOS cofactor [201]. Since oxidative stress enhances BH4 to BH2 conversion, antioxidant supplementation (specifically, vitamin C) has been suggested to prevent this reaction. Vitamin C supplementation has been shown to increase BH4 levels and eNOS activity in animal studies [202, 203].

L-Arginine

As was mentioned earlier, a semi-essential amino acid L-arginine serves as a NO precursor, and deficiency of L-arginine or L-citrulline can cause a decrease in NO synthesis. Studies examining effects of L-arginine supplementation in diabetic animals showed improved NO availability, reduced tissue sorbitol accumulation, decreased oxidative stress [204, 205], reversal of endothelial dysfunction [206], and decrease in blood pressure [207].

Human trials studying the effects of arginine supplementation on endothelium-dependent vasodilation show conflicting results, with some showing a clear benefit of arginine supplementation [208, 209], while others show no effect [210], or in one study, even harm [211]. This latter study showed worse functional capacity in people with peripheral artery disease taking arginine compared to placebo.

Studies on diabetic patients with oral arginine supplementation demonstrated reduced lipid peroxidation [212], decreased blood pressure [213], and improved insulin sensitivity [214]. A meta-analysis conducted by Odrigues-Krause et al. of 13 randomized controlled trials comparing arginine supplementation with placebo in subjects with cardiovascular disease (coronary artery disease, peripheral artery disease, chronic heart failure, myocardial infarction, angina, etc.), obesity, and/or type 2 diabetes revealed no improvement in endothelium-dependent vasodilation, and no changes in NO or ADMA concentration with arginine supplementation. Interestingly, in a subgroup analysis, it was shown that patients with obesity or T2DM had increased NO concentration with arginine supplementation [215]. A meta-analysis of trials studying clinical effects of L-arginine supplementation in patients with myocardial infarction did not demonstrate increase in survival, success of cardiopulmonary resuscitation, or decrease in myocardial reinfarction, recurrent myocardial ischemia, heart failure hospitalizations, or shock in arginine group [216]. Another meta-analysis examining the effect of arginine supplementation on blood pressure revealed statistically significant decrease in systolic blood pressure on average by 5.39 mmHg and diastolic blood pressure by 2.66 mmHg [217]. Because of conflicting results, there is still not enough evidence to support L-arginine supplementation in patients with diabetes or cardiovascular disease. It is also not clear what target populations will benefit from this intervention the most, and what dose should be used to achieve the desired effect [218].

Estrogen

Epidemiological studies show that premenopausal females have a decreased incidence of CAD, stroke, and hypertension compared to age-matches males [219]. Estrogen has been proposed as a protective factor for cardiovascular health in premenopausal females. Diabetic women have the same cardiovascular risk as non-diabetic men, suggesting that they are denied the cardiovascular protection of estrogen enjoyed by other premenopausal women [220]. Estrogen's possible beneficial effects and antiproliferative effects include improvement of lipid metabolism with decrease in LDL and increase in HDL [221], inhibition of platelet aggregation [222], reduction in oxidative stress with increased NO bioavailability, improvement in vascular smooth myocyte sensitivity to vasodilators [24], increased NO production by increased eNOS activity and gene enhancement [223], and decreased in ADMA production [224]. Several investigators have demonstrated that estrogen improves endothelium-dependent vasodilation in ovariectomized animals [225, 226] and postmenopausal women [227–229]. Early observational studies, including both The Nurses' Health and Danish Studies, demonstrated a beneficial cardiovascular effect of estrogen therapy in postmenopausal women [230, 231]. Conversely, the randomized placebo-controlled trial Women's Health Initiative, which was completed in 2002, demonstrated that combined estrogen and progestin hormonal therapy in postmenopausal females did not protect them from adverse cardiovascular outcomes and may have resulted in a slightly increased risk of coronary events [232]. Because observational studies with hormonal therapy started early after the onset of menopause showed a mostly protective effect, while randomized trials with later initiation of hormonal therapy resulted in an increase in adverse cardiovascular events, it has been hypothesized that the beneficial cardiovascular effect of estrogen occurs only in early menopause, which is commonly called the "timing hypothesis" [233]. Subsequent randomized trials of hormonal therapy started in early menopause showed reduction in mortality, heart failure, myocardial infarction [233], with a series of meta-analyses supporting these findings [234, 235]. Despite that, the fear of potential complications of hormonal therapy, including breast cancer and thrombotic complications, limit healthcare providers from the routine prescription of estrogens to postmenopausal females, The initiation of hormonal therapy solely for cardiovascular protection is not recommended by the current guidelines [236].

Angiotensin-converting Enzyme (ACE) Inhibitors and Angiotensin Receptor Blockers

Angiotensin-converting enzyme (ACE) plays an important role in the renin–angiotensin–aldosterone system and the kinin–kallikrein system. The renin–angiotensin system is discussed in detail elsewhere in this volume. The kidney releases renin

into the systemic circulation in response to renal hypoperfusion, produced by hypotension or volume depletion, and increased sympathetic activity [237]. Renin converts angiotensinogen, made in the liver (and other organs including the kidney), to angiotensin I, which is inactive. Angiotensin I is then converted to angiotensin II. The reaction is catalyzed by an ACE, which presents in the pulmonary circulation, and also in the endothelial cells. Angiotensin II is a potent vasoconstrictor and promotes renal sodium and water reabsorption [238]. It also increases the production of aldosterone from the adrenal cortex, which also enhances sodium transport in the kidney. Furthermore, angiotensin II is a growth factor and potentiates thrombosis.

In the kinin–kallikrein system, bradykinin, a vasodilator, is produced in the kidney from an inactive precursor, kininogen. In the circulation, bradykinin is metabolized by kininases, one of which is ACE [239]. ACE inhibitor, therefore increases bradykinin levels and results in vasodilation, mediated in part by release of nitric oxide at vascular endothelial cells and in part of the stimulation of endothelial production of prostacyclin [240].

Angiotensin II type 1 receptor blockers (ARBs) have the theoretical potential to be efficient with respect to vascular endothelial function. ARBs can provide a sustained inhibition of the binding of angiotensin II to the Angiotensin-1 (AT_1) receptor, while during chronic ACE inhibitor therapy, angiotensin II levels may return to normal over time. Furthermore, ARBs do not affect the Angiotensin-2 (AT_2) receptor function that includes vasodilatory and antiproliferative ability. However, the absence of augmentation of bradykinin through inhibition of the kininase pathway may lead to differences between the effects of ARBs and ACE inhibitors [241].

ACE inhibitors and ARBs improve vascular function and cardiovascular outcomes in Type 2 diabetes. Both agents unequivocally improve endothelial function in patients with Type 2 diabetes [242–245]. Studies in our unit with an ARB, valsartan, showed an improvement in resting forearm skin blood flow and resting brachial artery diameter after 12 weeks in patients with type 2 diabetes [246]. In contrast, studies in subjects with Type 1 diabetes failed to be conclusive as they reported contradicting results [247, 248]. Regardless of their effect on endothelial function, it should be emphasized that ACE inhibitors and ARBs improve cardiovascular and all-cause mortality outcomes in patients with diabetes to a greater degree than in non-diabetics as noted in the subgroup analysis of the HOPE and LIFE studies (approximately 38% and 19%, respectively, of subjects had diabetes) [249, 250]. In addition, both of these agents appear to reduce the onset of Type 2 diabetes in susceptible populations. Thus, it appears that ACE inhibitors and ARBs improve vascular outcomes in patients with diabetes.

Aliskiren is a direct renin inhibitor that decreases plasma renin activity (PRA) and inhibits conversion of angiotensinogen to angiotensin I, the proximal rate-limiting step in the renin–angiotensin–aldosterone system (RAAS). Studies in our unit showed that aliskiren treatment was associated with improvement in endothelium-independent vasodilation at the skin microcirculation of T2DM patients [251]. Of interest, no changes were observed in subjects at risk of T2DM.

Lipid Lowering Medications

Statins have been shown to significantly lower the serum lipid levels and reduce cardiovascular morbidity and mortality in patients with and without coronary artery disease [252]. Because hypercholesterolemia and increased levels of oxidized LDL also impair endothelial function, it was initially thought that the beneficial effects of statins on cardiovascular disease (CVD) was solely related to their lipid lowering capacity but it was recognized that statins may act through mechanisms that are independent of LDL lowering [253]. More specifically, statins directly upregulate endothelial nitric oxide synthase (eNOS) and enhance NO production; these effects of statins are seen in normocholesterolemic cells [254–256]. By increasing NO production, statins may interfere with atherosclerotic lesion development, stabilize plaque, inhibit platelet aggregation, improve blood flow, exert anti-inflammatory actions, and protect against ischemia [257, 258].

Although statins reduce the risk of major vascular events, endothelial-dependent vasorelaxation, a surrogate marker of such macrovascular events, is not clearly improved with statins. In particular, vasoreactivity does not improve after statin treatment in patients with poorly controlled diabetes [259]. Endothelial-dependent vasodilation does improve independently of lipid lowering in patients with better glycemic and lipid control in both Type 1 and Type 2 diabetes [260–264]. Statin use was also reported to ameliorate postprandial hypertriglyceridemic- and hyperglycemia-induced endothelial dysfunction and reduced serum nitrotyrosine levels in Type 2 diabetes, suggesting that its short-term, lipid-independent vascular benefits are secondary to decreased oxidative and nitrosative stress [265].

Insulin Sensitizers

Abundant evidence has shown the association between insulin resistance and endothelial dysfunction. Prolonged hyperinsulinemia induced by a euglycemic insulin clamp has been shown to impair endothelial-dependent vasodilation [266]. Obese individuals without diabetes but with insulin resistance are found to have blunted endothelium-dependent, but normal endothelium-independent vasodilation [267]. Thus, treatments that can improve insulin sensitivity have been investigated.

Thiazolidinediones (TZDs) belong to a class of drugs known as peroxisome proliferator activating receptor-γ (PPARγ) agonists and enhance insulin sensitivity of peripheral tissues (fat and muscle). They are known as insulin sensitizers and are used for the treatment of Type 2 diabetes. Initial studies, in endothelia cell cultures employing troglitazone, which was subsequently removed due to serious hepatic side effects, reported the inhibited expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) E-selectin, plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor α (TNFα) [268–270].

An initial randomized placebo-controlled study from our unit that examined the effects of troglitazone on the endothelial function in early and late Type 2 diabetes showed that 12 weeks of troglitazone treatment improved the FMD and fasting insulin only in the group of patients with recently diagnosed Type 2 diabetes and no macrovascular complications. Furthermore, troglitazone resulted in no changes in nitroglycerin-induced dilation (NID), microcirculation activity or biochemical markers of endothelial dysfunction [e.g., von Willebrand factor (vWF), ICAM, VCAM] in all 3 groups [271].

Currently, there are two PPARγ agonists available, rosiglitazone and pioglitazone but their effects on endothelial function are not well understood. Rosiglitazone significantly increased skin nitric oxide (NO) production and blood flow in the foot of diabetic patients [272]. Pioglitazone has been shown to have pleiotropic effects that include improvement in lipid metabolism, increased serum adiponectin levels and reduction of cardiovascular events in high-risk T2DM patients [273]. Pioglitazone improved FMD in one study that included non-diabetic hypertensive patients but had no effect in another study that investigated a similar group of patients [274, 275].

DPP-4 Inhibitors and GLP-1 Receptor Agonists

Dipeptidyl peptidase-4 (DPP-4) is an enzyme that rapidly inactivates both GLP-1 and gastric inhibitory peptide, which are intestinal-derived incretin hormones that play a major gluco-regulatory role. DPP-4 inhibitors improve glycemic control and preclinical studies suggested that may also have beneficial cardiovascular effects through both incretin-dependent and -independent mechanisms [276]. A recent study in our unit did not find any effect of linagliptin, a DPP-4 inhibitor, in both micro- and microcirculation but reported an increase in axon reflex-dependent vasodilation, a marker of neurovascular function [277]. Additional studies that employed other DPP-4 inhibitors reported similar results [278].

GLP-1 receptor agonists (GLP RAs) are also widely employed to improve glycemic control in T2DM patients. Exenatide improved endothelial function to a similar degree as insulin glargine in insulin- and incretin-naïve T2DM patients, indicating no specific effect on endothelial function [279]. Similar results were found in a study that compared liraglutide versus insulin glargine in T2DM with the same characteristics [280].

SGLT-2 Inhibitors

Sodium–glucose cotransporter-2 (SGLT-2) inhibitors are a very recent class of antidiabetic drugs for T2DM patients. They mainly act in the proximal convoluted tubule of the kidney by reducing the renal threshold for reabsorption of glucose,

which leads to renal glycosuria and improvement of glycemic control. Their importance was greatly enhanced by studies that showed that they reduce risk for cardiovascular death, heart failure, and kidney failure [281–284]. However, despite these impressive therapeutic effects, there are no adequate data regarding the effects of SGLT-2 on endothelial function. Preliminary studies with dapagliflozin reported a beneficial effect, especially in early-stage T2DM with suboptimal glycemic control [285, 286].

Conclusions

The normal endothelium plays an important role in the prevention of atherosclerosis and microvascular disease, and nitric oxide is an essential molecule actively involved in physiologic and pathologic pathways in the body. Diabetes is associated with both a systemic inflammatory state and endothelial dysfunction. Numerous studies have demonstrated an important role of NO in the pathogenesis of diabetic vascular complications. The proposed mechanisms by which diabetes affects endothelial function include changes in glucose metabolism, alterations in insulin signaling pathways, impaired NO synthesis due to oxidative stress, decreased availability of substrates and cofactors of NO synthesis, eNOS modifications causing its uncoupling, presence of endogenous eNOS inhibitors, and increased NO breakdown. Knowing the mechanisms of impaired NO metabolism in diabetes is crucial because it creates the essential foundation for discovery of new potential therapeutic strategies to reverse endothelial dysfunction in diabetes, decrease the rate of micro- and macrovascular complications, and improve survival.

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Chapter 8
Adiponectin, Diabetes, and the Cardiovascular System

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Abbreviations

AgRP	Agouti-related peptide
AMPK	Adenosine monophosphate-activated protein kinase
APPL1	Adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif
apoA1	Apolipoprotein A1
apoE$^{-/-}$	Apolipoprotein-E knockout
BAT	Brown adipose tissue
BMI	Body mass index
CCK	Cholecystokinin
C/EBP	CCAAT/enhancer-binding protein
CNS	Central nervous system
CVD	Cardiovascular disease

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DPP-4	Dipeptidyl peptidase-4
DsbA-L	Disulfide-bond A oxidoreductase-like protein
eNOS	Endothelial nitric oxide synthase
EEC	Enteroendocrine cell
ER	Endoplasmic reticulum
FFA	Free fatty acid
Gcg	Preproglucagon gene
GIP	Glucose-dependent insulinotropic polypeptide
GIT	Gastrointestinal tract
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GLP-2R	Glucagon-like peptide-2 receptor
GRPP	Glicentin-related pancreatic polypeptide
HDL	High-density lipoprotein
HMW	High molecular weight
IL	Interleukin
IP1	Intervening peptide 1
IP2	Intervening peptide 2
IR	Insulin resistance
IRS1	Insulin receptor substrate 1
LDL	Low-density lipoprotein
MCP-1	Monocyte chemoattractant protein-1
MetS	Metabolic syndrome
NASH	non-alcoholic steatohepatitis
Na^+/H^+	Sodium–hydrogen
NO	Nitric oxide
NPY	Neuropeptide Y
OXM	Oxyntomodulin
PC	Prohormone convertase
POMC	Proopiomelanocortin
PP	Pancreatic polypeptide
PPAR	Peroxisome proliferator-activated receptor
ProG	Proglucagon
PYY	Peptide tyrosine-tyrosine
PVAT	Perivascular adipose tissue
SAT	Subcutaneous adipose tissue
SGLT-2	Sodium–glucose cotransporter-2
SGLT2i	SGLT2 inhibitors
T2DM	Type 2 diabetes mellitus
TNF	Tumor necrosis factor
TZD	Thiazolidinedione
VAT	Visceral adipose tissue
WAT	White adipose tissue

Introduction

Beyond a repository for energy via storage of triglycerides, adipose tissue also serves as an endocrine organ capable of synthesizing a number of biologically active compounds, the adipocytokines that regulate metabolic homeostasis. Adiponectin is the most abundant serum adipocytokine, principally secreted from adipose tissue. It acts as a key modulator of insulin sensitivity, and glucose and lipid metabolism [1]. Body fat distribution, insulin, sex hormones, tumor necrosis factor (TNF)-α, and peroxisome proliferator-activated receptor (PPAR)-α influence its regulation. This adipocytokine plays a crucial role in the endocrine functions of adipose tissue and in obesity-associated disorders [2]. Additionally, it exerts pleiotropic beneficial effects such as insulin-sensitizing, anti-inflammatory, anti-atherosclerotic, and cardioprotective properties [3]. Its serum concentrations decrease with obesity. Particularly, low plasma adiponectin levels are associated with the metabolic syndrome (MetS) and generally with an unfavorable cardio-metabolic risk profile, such as insulin resistance (IR), type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia [4]. Furthermore, the relationship between adiponectin, inflammation, and atherosclerosis makes this hormone an important factor that links obesity to cardiovascular disease (CVD). More recently, gut-proglucagon-derived peptides, which are briefly introduced herein, have also been identified as essential molecules in the regulation of glucose and energy metabolism.

The Role of Healthy White Adipose Tissue: An Active Endocrine Organ

Two types of adipose tissue can be identified, which have opposing functions: (1) white adipose tissue (WAT) stores dietary energy mainly in the form of triglycerides, and it releases free fatty acids (FFAs) into the circulation and (2) brown adipose tissue (BAT) is specialized in non-shivering thermogenesis through lipid oxidation. BAT in humans is restricted to neonates and is gradually replaced by WAT with aging. However, recent data designate that BAT is also viable and functional in human adults [5].

Particularly, WAT in humans is mainly located beneath the skin (subcutaneous adipose tissue [SAT]) and around the intra-abdominal organs (visceral adipose tissue [VAT]). Other common sites of adipose tissue accumulation include the bone marrow, the heart (epicardial adipose tissue), and the adventitia of blood vessels (i.e., periadventitial adipose tissue) [6, 7]. In addition to adipocytes, which represent the greatest percentage of cells within the adipose tissue, other cell types are also present, including adipocyte precursor cells, capillary endothelial cells, fibroblasts, and inflammatory cells, that are collectively termed the stromal vascular fraction [6, 7].

WAT functions as a key energy reservoir for other organs during the fasting state. The continuous storage and hydrolysis of triglycerides (i.e., a process known as lipolysis) is essential to maintain body weight homeostasis [6]. In addition, adipose tissue plays a role in thermo-insulation and mechanical protection of internal organs. Although WAT was traditionally perceived to act simply as a storage depot for excess energy, recently it has also been recognized to act as a highly dynamic endocrine organ that is involved in the regulation of insulin sensitivity, glucose, and lipid metabolism, as well as cardiovascular homeostasis. These functions are mediated by WAT's ability to produce and secrete bioactive substances termed adipokines, which exert pro- and anti-inflammatory functions and may act both locally (autocrine/paracrine interactions) and systemically (endocrine) [6, 8–10]. These adipokines include hormones implicated in energy balance (e.g., leptin, adiponectin), glucose tolerance and insulin sensitivity (adiponectin, resistin), conventional cytokines (e.g., TNF-α, interleukin-6), and proteins involved in lipid metabolism (e.g., lipoprotein lipase, retinol binding protein), hemostasis (e.g., plasminogen activator inhibitor-1 and angiotensinogen), and in inflammatory and stress responses (such as haptoglobin and metallothionein) [11].

Adipsin was the first adipokine to be discovered in 1987 [12]. However, it was the discovery of the cytokine-like factor, leptin, in 1994 that redefined WAT as an endocrine organ, implicated in the regulation of energy balance and other physiological processes [13, 14]. In the following decades, several hundreds of adipokines have been identified, such as adiponectin, chemerin, resistin, visfatin, and omentin. Adiponectin and leptin are the most abundantly produced adipokines. Leptin has been found to play an important role in the regulation of food intake and energy expenditure [13, 14], while adiponectin is an insulin sensitizer that possesses anti-inflammatory properties [10, 15]. Adipokines not only modulate the activity of adipocytes, but they also mediate the crosstalk between WAT and other organs to regulate their metabolism (i.e., the liver, the muscle, the pancreas, and the central nervous system [CNS]) [6, 16]. Since they possess both pro- and anti-inflammatory properties, adipokines play a critical role in integrating systemic metabolism with immune function [17]. Furthermore, WAT expresses numerous receptors that allow it to respond to various hormonal stimuli.

Adipose Tissue Dysfunction and Its Role in the Development of Cardio-metabolic Disorders

In response to excess energy supply, adipose tissue undergoes complete remodeling. This encompasses activation of various cell types, such as mural cells, macrophages, and preadipocytes. Failure to adequately remodel while expanding results in adipose tissue dysfunction, chronic inflammation, and metabolic dysfunction. Adipose tissue dysfunction is characterized by an increase in adipocyte size (adipocyte hypertrophy), infiltration of the adipose tissue by inflammatory cells, and dysregulation in the production and secretion of adipokines [6, 18].

Obesity in particular is associated with adipose tissue dysfunction and a state of chronic, low-grade inflammation. Several studies have pointed out an increase in the number of immune cells infiltrating the adipose tissue of obese individuals and mouse models of obesity [19, 20]. Particularly, macrophages play a significant role in augmenting the inflammatory response in adipose tissue. In fact, the accumulation of macrophages in adipose tissue has been shown to rise proportionally with increased body mass index (BMI), adipocyte hypertrophy, and IR [19]. Specifically, in obese patients, the macrophage content in WAT has been reported to be ~50% of the total number of cells, in comparison to ~5–10% in lean individuals [20]. According to bone marrow transplant studies performed in macrophage-deficient mice, macrophages that infiltrate the adipose tissue are proposed to derive from the bone marrow [20]. However, the signals that induce macrophage recruitment to the adipose tissue of obese individuals are not well understood. Macrophages are known as "professional phagocytes" as their main function is to clear apoptotic and necrotic cells, cellular debris, and foreign pathogens, through the use of scavenger receptors. Thus, macrophages are hypothesized to be recruited to the dysfunctional adipose tissue in response to hypertrophic adipocyte necrosis, in order to carry out their scavenger function. This theory has been partly proven in studies where mice were treated with an exogenous drug that induces apoptosis specifically in adipocytes [21]. Furthermore, hypertrophic adipocytes secrete large amounts of the chemoattractant, monocyte chemoattractant protein-1 (MCP-1), which can enhance macrophage infiltration in WAT of obese mice and humans [22, 23]. Upon recruitment to the adipose tissue, macrophages form aggregates surrounding the dead adipocytes, termed "crown-like structures." [24] Since macrophages are secretory cells, their crosstalk with adipocytes contributes significantly to the production of inflammatory factors, such as TNF-α, IL-6, and MCP-1 that act locally as well as systemically [25]. Thus, this vicious cycle of leukocyte recruitment and release of pro-inflammatory adipokine and cytokine synthesis by adipocytes and macrophages contributes not only to local inflammation, but also to a chronic systemic state of low-grade inflammation.

Under conditions of normal metabolic status, adipose tissue is known to produce an array of adipokines/cytokines including adiponectin, leptin, TNF-α, IL-6, resistin, omentin, visfatin, chemerin, and many others, resulting in a balance in the secretion of pro- and anti-inflammatory adipokines [17]. As adipose tissue expands, there is a shift in the secretory nature of adipocytes towards a predominant pro-inflammatory profile [17, 26]. In fact, IL-6 and TNF-α are among the pro-inflammatory cytokines that are consistently found to be increased in obesity, both at the local level (i.e., in the WAT) and systemically (i.e., in the bloodstream) [27]. On the other hand, anti-inflammatory mediators including adiponectin and IL-10 are found to be reduced in obese individuals [26]. Furthermore, most microarray studies in humans and animal models, comparing gene expression profiles of obese versus lean adipose tissue, noted an alteration in gene profiling with obesity, particularly an up-regulation in inflammatory-related genes [28–30]. Thus, an imbalance in the production of pro- and anti-inflammatory factors associated with obesity is believed to form the link between adipose tissue dysfunction and the development

of IR, CVD, and many other pathological conditions. It is noteworthy that there are certain circumstances also associated with adipose tissue dysfunction without, however, developing obesity. Lipodystrophy is one such condition, a group of clinically heterogeneous inherited or acquired disorders, characterized by selective but variable absence of body fat tissue, which results in ectopic accumulation of fat, leading to metabolic complications of IR, hyperglycemia, hypertriglyceridemia, and dyslipidemia [31]. In addition to lipid storage, the low levels of adiponectin and leptin may play a crucial role in triggering the IR and metabolic abnormalities.

Factors secreted by adipose tissue can influence vessel wall homeostasis either by working through the liver or directly at the vessel wall [32, 33]. At the liver, adipose tissue-derived factors can influence systemic lipid and lipoprotein metabolism, as well as changes in inflammatory and clotting system components, which can then impact the environment of the vessel wall [32, 33]. Furthermore, adipokines can directly affect the function of the major cell types present in the arterial wall, including endothelial cells, smooth muscle cells, and macrophages [32, 33]. Excess adipose tissue can adversely affect the vasculature by causing a dysregulation in the production of these adipose tissue-derived factors. In fact, obesity is known to accelerate atherosclerosis and has been associated with increased rates of cardiovascular death [34, 35].

The regional distribution of body fat is an important determinant of an individual's cardio-metabolic risk. Numerous epidemiological studies have pointed out that individuals with central obesity (i.e., accumulation of fat in visceral depots) are at higher risk for developing T2DM, CVD, and cancer than those with peripheral obesity (i.e., accumulation of fat in subcutaneous depots) [36–38]. Visceral adiposity, rather than subcutaneous adiposity, is believed to contribute to increased comorbidity risk due to (a) its anatomical site and (b) its adipokine/cytokine gene and secretory profile [39, 40]. Although both VAT and SAT express an identical series of inflammatory cytokines in obese individuals, the level of expression is different. In comparison to SAT, many pro-inflammatory adipokines/cytokines are predominantly secreted by VAT, under obese conditions, whereas adiponectin's (anti-inflammatory adipokine) expression is highly reduced [41, 42]. For instance, it has been shown that the mRNA levels of the inflammatory factor TNF-α are more highly expressed in VAT than SAT in obese individuals, while this factor is expressed equally in both fat depots in lean subjects [43]. Leptin levels are lower in VAT than SAT for both lean and obese [44, 45]. Relatively greater adiponectin mRNA levels have been found in SAT than VAT and these levels were much lower in the VAT of obese subjects, compared with lean subjects, suggesting there is a depot-specific down-regulation of adiponectin in obesity [46, 47]. These factors derived from VAT have favored access to the liver through the portal circulation and can accelerate atherosclerosis development by mechanisms related or not directly related to lipids [48]. For example, increased release of FFAs from adipose tissue can increase the synthesis and secretion of low-density lipoprotein (LDL), apolipoprotein B, and triglycerides from the liver, while they can also activate inflammatory processes, induce endothelial cell apoptosis, and impair nitric oxide (NO) production and endothelium-dependent vasodilation [49–52].

In addition to visceral fat, perivascular adipose tissue (PVAT) is also a crucial adipose tissue depot that can play a direct role in atherosclerosis development [32, 53]. Most major arteries that are typically affected by atherosclerosis such as the aorta, the coronary arteries, and the carotid arteries are surrounded by PVAT [54]. Originally, it was thought that PVAT was simply a structurally supportive tissue for the vasculature, however, more recently it has been shown that it can influence vascular homeostasis [54]. Adipokines secreted from PVAT have direct access to the adjacent arterial wall by diffusion. Due to this direct contact, factors derived from PVAT are believed to have more potent effects on the vasculature than factors released from other adipose tissue depots. PVAT also expands with obesity and displays a dysfunctional adipokine profile. In fact, PVAT surrounding atherosclerotic lesions or mechanically injured arteries displayed pro-inflammatory adipokine profiles and reduced adiponectin expression [55, 56]. On the other hand, removal of healthy PVAT enhanced neointimal formation [55]. Thus, under healthy conditions, PVAT has beneficial effects on vessel function. However, under conditions of obesity, in addition to having vasoconstrictive effects, PVAT becomes dysfunctional leading to the release of elevated levels of pro-inflammatory adipokines that can contribute to endothelial dysfunction, atherosclerotic plaque development, and plaque rupture [32, 53, 54, 57]. Aside from obesity, lipoatrophy, which is often combined with T2DM and hypertension [58], impairs not only metabolic homeostasis, but also the homeostasis of vessel function and blood pressure. In addition, in an animal model of hypertension without obesity, the ability of PVAT to attenuate vasoconstriction to agonists was decreased [59].

Many adipokines and cytokines mediate the crosstalk between adipose tissue and the vasculature in the "adipo-vascular axis." The altered release of these factors by dysfunctional visceral or PVAT can have direct effects on the vessel wall and promote atherosclerotic plaque development [60]. For instance, resistin and leptin levels are increased in obese individuals and are positively associated with coronary atherosclerosis and other cardiovascular complications in humans [61–64]. Resistin is also an independent predictor of major cardiovascular events including ischemic stroke and cardiovascular mortality [62, 65–68]. Resistin can affect the atherosclerotic process by promoting the upregulation of vascular endothelial adhesion molecules, increasing the production of pro-inflammatory cytokines by endothelial cells (i.e., endothelin-1, MCP-1) and macrophages (i.e., TNF-α), and inducing foam cell formation [69–71]. Similarly, leptin can increase the production of MCP-1 and endothelin-1 in endothelial cells [72, 73]. Furthermore, leptin plays a role in neointimal formation in response to endothelial damage by promoting the migration, proliferation, hypertrophy, and phenotypic transformation of vascular smooth muscle cells [60, 74]. Several studies have shown circulating levels of chemerin to be positively associated with inflammation, the MetS, and coronary artery disease [75–78], while an independent study demonstrated an inverse association between chemerin and carotid atherosclerotic plaque instability, as assessed by histological characterization, in subjects with severe carotid stenosis who underwent a carotid endarterectomy [68]. These contradictive findings may be partly explained by the dual action (pro-inflammatory and anti-inflammatory) of chemerin, which is highly

dependent on the type of cleavage it undergoes [79, 80]. It can serve as a chemoattractant promoting recruitment of immune cells to sites of injury [81]. However, beyond its pro-inflammatory role, chemerin also possesses anti-inflammatory properties, by inhibiting the production of inflammatory mediators and preventing monocyte adhesion to the vascular endothelium [79]. Elevated levels of IL-6 and TNF-α can promote endothelial dysfunction by decreasing the production of endothelial nitric oxide synthase (eNOS) and causing a decrease in the availability of NO [82, 83]. With increased plasma concentrations of adipocyte-derived cholesteryl ester transfer protein associated with obesity, the levels of small, dense atherogenic LDL particles also increase [84]. Small, dense LDL particles can easily enter the vascular wall, where they are susceptible to oxidative transformation and can promote endothelial damage and macrophage-to-foam cell transformation [85]. Furthermore, high levels of plasminogen activator inhibitor-1 produced by adipocytes under the influence of TNF-α and FFAs can contribute to atherosclerotic plaque progression by promoting atherothrombosis and inhibiting plasminogen-induced migration of vascular smooth muscle cells from the medial layer of the arterial wall to the intima [86]. This promotes the formation of unstable plaques with thin fibrous caps that are prone to rupture [87]. Other pro-inflammatory adipokines that may be associated with the progression of atherosclerotic plaques are visfatin and apelin, which are increased in response to obesity-induced elevation of IL-6 and TNF-α production [88]. On the other hand, some adipokines, like adiponectin, have a protective role in the vasculature and down-regulation of its levels can play a significant contribution to atherosclerosis development.

Adiponectin Biosynthesis and Structural Properties

In 1995–1996, both murine and human forms of adiponectin (also termed adipocyte complement-related protein of 30 kDa, AdipoQ, adipose most abundant gene transcript 1, and gelatin-binding protein of 28 kDa) were discovered and isolated by four independent groups [89–92]. Originally, adiponectin was thought to be exclusively synthesized and secreted by adipose tissue and fully differentiated adipocytes. However, it was later determined that various other cells or tissue can also produce adiponectin but to a lower degree than adipose tissue such as osteoblasts, myocytes, epithelial cells, liver parenchymal cells, and placental tissue [93–97]. Adiponectin is considered the most abundantly secreted adipokine, accounting for about 0.01% of total serum protein, with circulating levels ranging from 5 to 30 μg/mL [98]. Furthermore, high serum levels of adiponectin negatively correlate with cardiometabolic diseases and also vary between men and women [99, 100]. Women have significantly higher adiponectin levels than men, as testosterone is believed to have direct effects on modulating adiponectin production, complex formation, secretion, and clearance [101]. Slightly increasing with age, adiponectin levels have a diurnal variation with nadir at night and peak in the morning [102, 103].

Human adiponectin contains three exons and is a 30 kDa protein composed of 244 amino acids, which consists of four domains: an amino-terminal signal

sequence, a hypervariable domain, a collagenous domain comprising 22 Gly-XY repeats, and a carboxy-terminal complement 1q-like globular domain [104, 105]. In the circulation, it exists as oligomeric complexes with different molecular weights. Its full-length monomer form (which has not been observed in circulation and appears to be confined to the adipocyte) can establish interactions via the collagenous domain to generate three multimeric complexes: a low molecular weight multimer (trimer), a middle molecular weight multimer (hexamer), and a high molecular weight multimer (HMW, 12- to 18-mer) [106–108]. The trimer is the basic building block of oligomeric adiponectin. Disulfide bond formation is crucial for the assembly and stabilization of adiponectin oligomers. Two trimers self-associate via a disulfide-linkage (S–S) mediated by cysteine residue 39 at the hypervariable region to form a hexamer, which further assembles into a bouquet-like HMW multimeric complex that consists of 12–18 monomers. A smaller form of adiponectin also exists in the circulation (but in negligible amounts due to a very short half-life); it consists simply of its globular domain, which is generated from the full-length protein by proteolytic cleavage [109]. Leukocyte elastase secreted by activated monocytes and neutrophils may be responsible for the generation of globular adiponectin [110]. These isoforms are suggested to possess different biological activities, but evidence in this domain is still quite limited. However, it is strongly suggested that HMW adiponectin, which makes up 50% of the total adiponectin in the circulation is the major biologically active isoform [106, 108]. Mutations in human adiponectin, notably G84R and G90S mutants resulted in HMW distribution deficiency, while R112C and I164T mutants resulted in impaired multimerization and secretion [111]. These mutants were implicated in hypoadiponectinemia [111], stressing the importance of functional adiponectin multimer assembly. Post-translational modifications, such as hydroxylation and subsequent glycosylation, are important for the assembly, secretion, and bioactivity of the HMW isoform [106]. Wang et al. demonstrated that mutations in all four highly conserved lysine residues within adiponectin's collagenous domain that are crucial for hydroxylation and glycosylation completely abolish the assembly and secretion of HMW adiponectin [112]. Furthermore, several endoplasmic reticulum (ER)-associated proteins also play an important role in the oligomerization and secretion of higher-order adiponectin complexes [113]. Therefore, it is equally important to consider multimer distribution as well as plasma adiponectin levels in association with normal or pathological states [99].

Adiponectin Receptors

Adiponectin exerts its main biological effects via two transmembrane receptors, AdipoR1 and AdipoR2 (AdipoR), which were discovered in 2003 by expression cloning [114]. AdipoR1 and AdipoR2 are encoded by genes situated on chromosomes 1 and 12, respectively, and they display 66.7% homology at the protein level [114]. Despite containing seven transmembrane domains, these receptors are structurally and functionally distinct from the classical G protein-coupled receptors;

AdipoR1 and AdipoR2 have an inverted membrane topology with a cytoplasmic N-terminus and an extracellular C-terminal domain [114]. Furthermore, they are not coupled with G-proteins and activate their own unique set of signaling molecules, as detailed below. In fact, determination of the crystal structures of AdipoR1 and AdipoR2 revealed that the AdipoRs represent an entirely novel class of receptors [115]. Scatchard plot analyses demonstrated that AdipoR1 is a high-affinity receptor for globular adiponectin (but it can also bind full-length adiponectin with low-affinity), which mainly leads to the activation of adenosine monophosphate-activated protein kinase (AMPK) signaling, which is an energy-sensing enzyme [114]. On the other hand, AdipoR2 has intermediate affinity for both globular adiponectin and its full-length variants (i.e., HMW adiponectin), which mainly leads to the stimulation of PPAR-α signaling, which is a key transcription factor in metabolic regulation [114]. Other signaling pathways activated by the interaction between adiponectin and its receptors include insulin receptor substrate 1 (IRS1)/2, p38 MAPK, Rab5, Akt, and ceramide signaling [116, 117]. Specifically, cellular ceramides are reduced by adiponectin-induced AdipoR ceramidase activity and effectively decrease hepatic ceramide levels while improving insulin sensitivity [118–121]. Recent studies have shown that an adaptor protein called adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1), interacts with the intracellular domains of both receptors, in order to help mediate adiponectin's downstream signaling [122]. T-cadherin has been identified as an additional receptor (or binding protein) for adiponectin that specifically binds the higher-order complexes (hexameric and HMW adiponectin) but not globular adiponectin [123]. It is highly expressed in cardiac myocytes where it plays an important role in mediating the cardioprotective actions of adiponectin [124]. Its structure differs to that of AdipoR1 and AdipoR2 as it is attached to the plasma membrane via a glycosyl phosphatidylinositol anchor [123]. Since it has no intracellular domain it is believed to require the help of other co-receptors, which to date remain unidentified, to mediate its intracellular signaling. It has been recently reported that adiponectin enhances adiponectin/T-cadherin-mediated packaging in exosomes and increases ceramide efflux within these small vesicles [125]. Together, adiponectin acting on AdipoR and/or T-cadherin is essential for maintaining ceramide homeostasis [99, 120, 125].

Both AdipoR1 and AdipoR2 are ubiquitously expressed; in mice, AdipoR1 is predominantly expressed in skeletal muscle, while AdipoR2 is most abundantly present in the liver. In humans, however, both AdipoR1 and AdipoR2 are highly expressed in skeletal muscle and the liver. Similar to circulating adiponectin levels, AdipoR expression was significantly decreased particularly in the skeletal muscle, liver, and adipose tissue of mouse models (*ob/ob* or *db/db* mice) of IR and obesity, as well as of individuals with obesity and T2DM [126–129]. Several gain- and loss-of-function studies have been performed, which have demonstrated that AdipoR1 and AdipoR2 play fundamental roles in glucose and lipid metabolism, inflammation, and oxidative stress [114, 127]. Yamauchi et al. demonstrated that adenovirus-mediated overexpression of AdipoR1 in *db/db* mice caused a reduction in liver gluconeogenesis via AMPK activation, while overexpression of AdipoR2 enhanced glucose uptake, reduced oxidative stress, as well as decreased the expression of pro-inflammatory cytokines and chemokines, such as TNF-α and MCP-1, via

stimulation of the PPAR-α pathway [127]. Both receptors had a significant effect on increasing fatty acid oxidation. On the other hand, systemic disruption of both receptors resulted in abrogation of adiponectin binding and actions, leading to increased glucose production, impaired glucose tolerance, and IR [127]. Administration of adiponectin to AdipoR1 and AdipoR2 double knockout mice was not sufficient to offset these deleterious effects, suggesting that these two receptors are responsible for the majority of adiponectin's physiological actions [127].

The Role of Adiponectin in Physiology and Pathophysiology

Adiponectin acts as a "pleiotropic cytokine" linked not only to adipocyte metabolism and homeostasis, but also exhibits a wide range of diverse effects in many different organs and tissues, including WAT, the liver, the pancreas, the kidney, the skeletal muscle, the heart, the CNS, and the vasculature. Adiponectin appears as a key mediator of systemic insulin sensitivity and glucose homeostasis. Its major effect on the pancreas is to promote beta cell function and survival, since it reduces intracellular ceramide levels and, thus, it exerts anti-apoptotic activity [130]. This ceramide-lowering effect of adiponectin seems to promote also cardiomyocyte survival in the context of ischemia [118]. In regard to the kidney, adiponectin levels are positively correlated with proteinuria [131]. Interestingly, in the end stages of both CVD and chronic kidney disease, a compensatory upregulation of adiponectin levels is observed [132]. As it has been mentioned above, adiponectin decreases serum glucose levels by alleviating hepatic glucose output rather than glucose disposal. It also has major effects on hepatic triacylglycerol accumulation via AMPK-dependent and independent pathways, and sphingolipid pathway as well. Therefore, it leads to substantial protection from hepatic steatosis [133]. Moreover, adiponectin plays a paracrine role in adipose tissue. Overexpression of AdipoQ (the gene encoding adiponectin) causes adipose tissue augmentation due to increased number of adipocytes. It is noteworthy that genes involved with fat oxidation are upregulated, while inflammatory genes are suppressed [133–135]. Adiponectin not only inhibits local inflammation, but also exerts systemic anti-inflammatory effects [136, 137]. It stimulates different tissue macrophages towards an anti-inflammatory M2 phenotype. Simultaneously, adiponectin suppresses macrophage transformation into lipid-ladened foam cells in atherosclerotic plaque, leading to the suppression of atherosclerosis development [138]. Recently, adiponectin has even been suggested to slow the progression of various cancers as it can limit cellular proliferation and induce apoptosis [139].

Regulation of Adiponectin

The production and secretion of adiponectin are controlled by important factors that regulate adiponectin at the transcriptional mRNA expression and translational protein levels [140]. The adiponectin promoter containing the CCAAT box region is

key for basal transcriptional activity [141]. However, it has been demonstrated that enhancer elements found more upstream in the regulatory region may be also responsible for increasing adiponectin expression [142, 143]. To date, PPARs and CCAAT/enhancer-binding proteins (C/EBPs) remain the key transcriptional factors that activate adiponectin mRNA expression and activity in adipocytes [143, 144]. Specifically, nuclear receptors PPAR-γ and retinoid X receptor form a heterodimer that can bind directly to the adiponectin promoter at the PPAR-response element site [142]. Plasma adiponectin levels in mice decrease during adipose-tissue deletion of PPAR-γ, underlining the significance of functional PPAR-γ transcriptional activity [143]. In addition, this binding activity was shown to be enhanced in 3T3-L1 adipocytes when liver receptor homolog-1, a monomeric nuclear hormone receptor, was bound to its respective response element on the adiponectin promoter [142]. This was highlighted when mutated liver receptor homolog-response element resulted in a significant decrease in adiponectin promoter activity. C/EBP binding sites are similarly found in the promoter region, the CCAAT box, where notably C/EBPα can homo- or hereto-dimerize and upregulate adiponectin expression [145]. Of note, C/EBPα has been shown to be the preferred isoform to significantly increase adiponectin mRNA levels in both human Chub-S7 or 3T3-L1 terminally differentiated adipocytes, rather than C/EBPβ, and C/EBPδ which only possess prominent activity during early adipocyte differentiation [141]. The first intron of the human adiponectin gene has in fact been identified as an enhancer region containing C/EBPα-response elements that, together with PPAR-γ, produce a synergistic effect to regulate adiponectin transcription [141]. This is in line with the studies exhibiting only modest PPAR-γ-induced adiponectin levels in C/EBPα-deficient adipocytes, while a marked increase was observed during co-expression [143].

Many studies have shown that the regulatory function of adiponectin is largely influenced by caloric intake [145, 146]. Importantly, high-glucose-treated 3T3-L1 adipocytes exhibited increased C/EBP binding [146]. These findings were validated in mice that were fasted and then refed a standard diet, highlighting C/EBP as a major transcription factor crucial for adiponectin regulation in response to nutrients [146]. Furthermore, adipose tissue-specific C/EBPα expression levels were lower in obese subjects with MetS compared with diet-induced controls [145]. In line with this, obese subjects undergone bariatric surgery or caloric restriction regimens significantly augmented adipose tissue and plasma concentration levels of adiponectin [99]. However, some studies have reported that there is no difference in C/EBPα expression between insulin-resistant/MetS subjects and controls [145]. This may delineate the diet-controlled influence of C/EBPα on adiponectin-induced expression [145]. Notwithstanding this evidence, the mechanism underlying obesity-associated reductions in plasma adiponectin has not yet been fully clarified. Furthermore, a recent meta-analysis demonstrated that physical exercise in prediabetic and diabetic subjects was successful in increasing adiponectin levels [147]. In viscerally obese healthy men, maintaining a normal diet and active lifestyle over 3 years was also shown to decrease visceral adiposity burden and improve adiponectin [148]. Furthermore, when comparing the effect of physical activity on female and male obese subjects, circulating adiponectin levels were significantly increased

among the active participants, specifically the female group [149]. Maintaining a Mediterranean dietary pattern has also been postulated to elevate adiponectin concentrations [150]. Therefore, the physiological benefit of sustained lifestyle interventions seems to be mediated by promoting adiponectin transcription and secretion, even in an obese population.

Distinct post-translational modifications of adiponectin can lead to modified multimerization and secretion profiles essential for preserving its stability in the circulation [99, 140, 143, 151]. In 3T3-L1 preadipocytes, knockdown of ER-molecular chaperone, ER membrane-associated oxidoreductase-Lα (Ero1-Lα) resulted in reduced adiponectin secretion [143]. Ero1-Lα and its partner ER resident protein 44 work together to properly assemble higher-order adiponectin complexes [143, 144]. Similarly, in 3T3-L1 adipocytes, suppression of ER chaperone disulfide-bond A oxidoreductase-like protein (DsbA-L) markedly decreased adiponectin levels [152]. In line with obesity-induced ER stress, studies have shown an according downregulation in adiponectin translation [143, 151, 153]. In high-fat diet-treated mice, reduced ER stress and increased adiponectin multimerization and levels were observed during overexpression of DsbA-L [151]. PPAR-γ agonist rosiglitazone has not only been able to induce adiponectin expression, but also DsbA-L expression in 3T3-L1 adipocytes [152]. Correspondingly, Ero1-Lα expression levels were increased in mature adipocytes and in mice during PPAR-γ treatment [113, 154]. In agreement with this, studies using thiazolidinediones (TZDs) showed increased adiponectin biosynthesis and secretion of the HMW form, thereby targeting adiponectin at the translational and/or post-translational level [113, 143, 155]. While the precise mechanisms remain unclear, together these findings underline the importance of ER proteins in the multimerization and secretion of adiponectin towards reducing the development of obesity-related metabolic phenotypes.

Hormones (i.e., insulin, leptin, glucocorticoids, sex hormones, and catecholamines) also regulate adiponectin. As for insulin, while in vitro studies have shown contradictory results concerning whether it has inhibitory or stimulatory effects on adiponectin production and secretion [156–158], hyperinsulinemic–euglycemic clamp studies in lean subjects decreased total plasma adiponectin levels by 10–20% [159–161]. It has also been shown that insulin suppresses plasma adiponectin levels already at a plasma insulin concentration of 100 pmol/L and hyperglycemia diminishes the suppressive effect of insulin [159]. This finding suggests that insulin could be involved in the downregulation of plasma adiponectin in insulin-resistant patients.

Circulating adiponectin concentrations are higher in women than men, independent of the fact that women usually have more overall adiposity compared to men [103, 162–164]. The studies on the influence of estrogens on serum adiponectin have reported contradictory results. Two found no effect [162, 165] and the other found an inverse association between adiponectin and estradiol, and higher adiponectin levels in postmenopausal compared to premenopausal women after adjusting for age, fat mass, and fat distribution [166]. As for testosterone, it may inhibit adiponectin secretion. In mice, removal of the testes led to an increase in adiponectin, although administration of testosterone reduced adiponectin levels [162].

Hypogonadal men have higher adiponectin levels than eugonadal men, and they decrease to the levels of eugonadal men when they receive testosterone replacement therapy [167]. Similarly, experimental testosterone deficiency in eugonadal men increased adiponectin levels and supraphysiologic testosterone administration decreased them [168]. Also, women with high testosterone levels due to polycystic ovarian syndrome have been reported to have low adiponectin levels [169–171].

Regarding leptin, although a cross-sectional study reported a strong inverse relationship between serum adiponectin and leptin levels [172], leptin administered exogenously either to rodents or humans had no significant effect on the plasma levels of adiponectin [166, 173]. Catecholamines may also inhibit expression of adiponectin, since β-adrenergic agonists reduced adiponectin gene expression in cultured mouse fat cells and human adipose tissue and decreased plasma levels in mice [174]. Stimulation of cultured adipocytes by isoproterenol, a β1 and β2 agonist, caused reduced expression of adiponectin [175]. Another study in animals confirmed that peripheral injection of a β3-adrenergic agonist suppressed adiponectin mRNA expression in adipose tissue [173].

The dysregulation of adiponectin production is strongly believed to contribute to the onset of several obesity-related complications, as hypoadiponectinemia (i.e., low levels of circulating adiponectin) has been found to be associated with IR, T2DM, dyslipidemia, and atherosclerosis [98, 176–178]. Taken together, these findings equally stress the relevance of physiological (i.e., transcription factors) and environmental (i.e., diet, exercise) factors in controlling the regulation of adiponectin mRNA and protein levels and their clinical implications in obesity-linked diseases.

Adiponectin and Cardiometabolic Risk Factors

Insulin Resistance and Type 2 Diabetes Mellitus

In 2001, three independent groups identified for the first time the important physiological role of adiponectin as an endogenous insulin sensitizer, whereby it modulates glucose and lipid metabolism in insulin-sensitive tissues in both animals and humans [109, 179, 180]. Fruebis et al. first pointed out that acute administration of adiponectin in obese mice significantly decreased FFAs and glucose levels in the blood and increased fatty acid oxidation in skeletal muscle [109]. Berg et al. demonstrated effects directly at the level of the liver whereby adiponectin decreased basal glucose levels by inhibiting the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production [180]. The chronic effects of adiponectin on insulin sensitivity were also investigated in adiponectin transgenic mice and adiponectin knockout mice. Transgenic overexpression of adiponectin in mice leads to protection against obesity and insulin resistance due to enhanced energy

expenditure [179]. On the other hand, adiponectin knockout mice exhibit impaired insulin sensitivity in association with delayed clearance of FFAs in the circulation, reduced levels of surface membrane FFA transporters, and high levels of plasma TNF-α [181]. Furthermore, reduced IRS1-mediated insulin signaling was observed, since adiponectin can enhance the ability of insulin to stimulate IRS1 tyrosine phosphorylation [182, 183]. Viral-mediated adiponectin expression in these knockout mice can reverse the high-fat diet-induced IR [182]. Consistent with these findings, a recent study similarly reported reversal of high-fat diet-induced liver and muscle IR during adiponectin treatment in mice [184]. Importantly, adiponectin-induced IR reversal in vivo occurred by stimulating WAT lipoprotein lipase activity, thereby increasing adipose-specific triglyceride uptake and reducing ectopic lipid accumulation [184]. This resulted in increased AMPK activation in other insulin-sensitive tissues such as skeletal muscle, where increased fatty acid oxidation was observed upon adiponectin treatment [184].

Clinical observations also support the idea that plasma adiponectin levels are inversely associated with IR as well as type 2 diabetes. In several studies, adiponectin has a negative correlation with fasting glucose, insulin, and IR and a positive association with insulin sensitivity, independent of BMI [103, 176, 185]. In longitudinal studies, hypoadiponectinemia was able to predict the development of IR and T2DM, while increasing adiponectin has been connected with a lower risk of T2DM [186–190]. Interestingly, a meta-analysis of 19 studies and almost 40,000 participants indicated that T2DM risk was strongly associated with low levels of adiponectin [191]. Also, the authors highlighted that the ability of adiponectin to inhibit pro-inflammatory cytokine production may be an important feature in reversing metabolic dysfunction. Human genetic studies on single-nucleotide polymorphisms of the adiponectin gene (located on chromosome 3q27) have pointed out numerous genetic susceptibility loci for T2DM and the MetS [192–194]. Nevertheless, a recent mendelian randomization study suggested that adiponectin has no causal effect on T2DM and glucose homeostasis and that the associations among them in observational studies may be due to confounding factors [195].

Overall, adiponectin mainly exerts direct actions at the level of the skeletal muscle, the liver, and the adipose tissue in order to mediate its insulin-sensitizing effects through the activation of the AMPK and PPAR-α pathways. Activation of AdipoR1 by adiponectin in the liver and muscle tissues increases AMPK activity, leading to an inhibition of hepatic glucose production, increased uptake of glucose in muscle, and enhancement of fatty acid oxidation in both the liver and the muscle [196]. Increased fatty acid oxidation is the result of an increase in expression of proteins involved in fatty acid transport (i.e., CD36) and fatty acid oxidation (i.e., acyl-coenzyme A oxidase). Activation of the PPAR-α pathway via AdipoR2 also increases fatty acid oxidation. By increasing fatty acid oxidation, adiponectin can also lower circulating FFAs, which may improve insulin action [197, 198]. Thus, disruption of both AdipoR1 and AdipoR2 abolishes adiponectin's binding and actions, leading to exacerbation of IR and glucose intolerance [114].

Hypertension

There have been some contradicting studies surrounding the association between adiponectin and hypertension. In adiponectin-deficient mice, a high-fat and sucrose diet led to increased blood pressure [199]. Several human studies have reported that blood pressure has a negative correlation to adiponectin [200–202]. However, adjusting for insulin sensitivity did not show any significant correlation with hypertension and adiponectin, indicating that IR may mediate the potential association between adiponectin and blood pressure [203]. Interestingly, a 5-year prospective study showed that there is an inverse correlation between plasma adiponectin levels and future risk of developing hypertension [204]. In contrast, the Danish Copenhagen City Heart Study did not find such predictive role for adiponectin but suggested one for leptin [205]. A meta-analysis of 48 studies concluded that hypertensive patients have lower adiponectin concentration compared to normotensive subjects and an increase in adiponectin concentration of 1 mg/L induced the odds of hypertension by 6% [206]. Nevertheless, another research group did not find a difference in plasma adiponectin levels between normotensives and hypertensives with normal renal function [207]. Moreover, a recent study suggested that the ADIPOQ SNP T94G was associated with 2.8 times higher odds for resistant hypertension [208]. Arterial stiffness as measured by pulse wave velocity, which increases in hypertensive patients, was negatively associated with adiponectin in the hypertensive group [209–211]. These associations are consistent in patients with treated essential hypertension, where significantly lower plasma adiponectin levels were observed in patients with heart-to-femoral pulse wave velocity progression compared to the non-progression group [212]. It is suggested today that adiponectin and blood pressure may be related via three principal mechanisms, the NO system and endothelial dysfunction, the renin–angiotensin system, and central effect mediated via the sympathetic nervous system [213].

Dyslipidemia

Besides diabetes and IR, adiponectin is also related to dyslipidemia, another risk factor for CVD. Adiponectin is a strong independent positive predictor of high-density lipoprotein (HDL) levels and is negatively associated with serum triglycerides [176, 214–217]. In a recent study including non-diabetic male and female subjects, HDL concentrations were independently and significantly correlated with adiponectin levels [215]. Triglyceride levels seem to mediate the significant association between lower plasma adiponectin and higher plasma thrombin production in non-insulin-treated T2DM male subjects [218]. Moreover, an epidemiologic report on serum adiponectin levels and lipid profile, which compiled 25 studies, concluded that there is also an inverse correlation between very low-density lipoprotein (VLDL) and LDL levels and adiponectin [219].

Cigarette Smoking

Smoking has been associated with decreased levels of circulating adiponectin. Possible explanations for this decrease include smoke-induced increase in catecholamines that suppress adiponectin or consumption of adiponectin by endothelium injured by cigarette toxins [220, 221]. Among patients with heart disease, current and former smokers had lower adiponectin levels than non-smokers, after adjusting for BMI and IR [221]. Furthermore, when comparing smokers to non-smokers, both plasma adiponectin, and mRNA adiponectin levels measured in peripheral blood mononuclear cells were significantly lower in the smoker group [222]. This implies that atherosclerotic development in cigarette smokers may be mediated by the reduction of beneficial systemic and local effects of adiponectin [222]. The adiponectin-lowering impact of cigarette smoking was also observed in smokers who adhered to a Mediterranean diet vs. non-diet non-smokers, highlighting the inability of healthy diet interventions to rescue the negative effects of a smoking phenotype [223]. These findings strengthen smoking as an independent risk factor affecting adiponectin levels [222, 223]. A systematic review of 11 studies suggested that there is a decreased adiponectin level in current smokers and this reduction can be reversed by quitting smoking [224]. After smoking cessation, despite unaccompanied weight gain which could alter the adipokine profile, serum adiponectin levels were nonetheless not further reduced [225]. On the other hand, after 1 year, adiponectin levels actually increased among subjects with less abdominal obesity, therefore unmasking the benefit of smoking cessation in elevating adiponectin [225].

The Role of Adiponectin in Cardiovascular Disease

Adiponectin's Direct Vascular and Atheroprotective Effects

Animal studies have demonstrated that adiponectin protects against the development and progression of atherosclerosis. Adenoviral-mediated overexpression of adiponectin in apolipoprotein-E knockout (apoE$^{-/-}$) mice resulted in a reduction in atherosclerotic lesion formation in the aortic sinus by 30% compared with non-treated apoE$^{-/-}$ mice [226]. Interestingly, adenovirus-derived adiponectin accumulated in the fatty streak lesions of the apoE$^{-/-}$ mice, which are predominantly composed of macrophages and foam cells [226], suggesting that adiponectin mediates its beneficial effects directly at the level of the vasculature. Similarly, globular adiponectin transgenic apoE$^{-/-}$ mice had significantly smaller aortic lesions than control apoE$^{-/-}$ mice [227]. On the other hand, adiponectin deficiency in mice led to increased vascular smooth muscle cell proliferation and enhanced neointimal thickening of arteries in response to vascular injury [228, 229]. However, this injury-induced neointimal formation was attenuated upon supplementation of mice with adiponectin [229].

Adiponectin's atheroprotective effects have been established in vivo and in vitro in all stages of atherosclerotic plaque development, from endothelial dysfunction, plaque initiation, and progression, to plaque rupture and thrombosis [230]. Adiponectin has the ability to attenuate atherogenesis through its direct actions on all major cell types present in the vasculature, including vascular endothelial cells, macrophages, and smooth muscle cells [15]. Adiponectin maintains endothelial function by stimulating the activation of eNOS through AMPK-dependent phosphorylation of this enzyme and subsequent production of NO in the vascular endothelium [231]. In fact, adiponectin treatment of rat aortic segments protected the endothelium against hyperlipidemic injury, by promoting eNOS activity [232]. Adiponectin-related production of NO and eNOS phosphorylation are mediated through AdipoR1 and AdipoR2 receptors [233], both of which are expressed in human endothelial cells [234]. Furthermore, adiponectin suppresses the NF-κB inflammatory signaling pathway, decreasing the endothelial inflammatory reaction and reducing TNF-α-induced expression of adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin in endothelial cells [235–237]. This attenuates leukocyte attachment to the vascular wall and their migration into atherosclerotic plaques. The presence of adiponectin has also been shown to decrease TNF-α-induced plasminogen activator inhibitor-1 upregulation at the mRNA and protein level in endothelial cells [238]. Therefore, adiponectin not only regulates plaque inflammation but also the fibrinolytic system to reduce atherothrombosis. Interestingly, a study in older adults showed that higher endothelial cell adiponectin levels are associated with higher vascular endothelial function, evaluated by brachial artery flow-mediated dilation using ultrasonography, independent of circulating adiponectin levels [239].

Adiponectin can also modulate macrophage function by decreasing its accumulation of cholesterol, thereby suppressing macrophage-to-foam cell transformation [138]. Proposed anti-atherogenic mechanisms by which adiponectin achieves lower intracellular cholesterol levels are by (1) decreasing cholesterol uptake and (2) promoting an increase in cholesterol efflux capacity. Through suppression of macrophage SR-A (but not CD36), adiponectin has the ability to markedly decrease the uptake of oxidized LDL by macrophages [240]. In a recent study, adiponectin was shown to directly interact with the oxidized LDL epitope and inactivate it, suggesting adiponectin might carry opsonin-like properties [241]. Also, there has been recent interest in adiponectin's ability to enhance ABCA1-mediated cholesterol efflux [138]. It has been shown that low circulating adiponectin levels are significantly and independently related with reduced ABCA1 expression on monocytes of overweight and obese subjects, indicating that adiponectin may be an important regulator of ABCA1 expression [242]. Daily 4-week treatment of adiponectin knock-out mice with adiponectin led to increased HDL levels in the serum and increased ABCA-1 expression in the liver in a dose-dependent manner [243]. Accordingly, adiponectin-transgenic mice had significantly higher plasma HDL levels compared to wild-type mice and also had altered expression of key liver genes participating in lipid metabolism [244]. Since the liver plays a critical role in the regulation of cholesterol levels, reverse cholesterol transport from macrophages is recognized as the principal step in protecting against atherosclerotic plaque

development. In vitro studies suggest that adiponectin treatment might protect against atherosclerosis by significantly enhancing apolipoprotein A1 (apoA1)-mediated cholesterol efflux from macrophages through an ABCA1-dependent pathway [245]. Also, adiponectin has been shown to modulate cellular cholesterol efflux in murine and human macrophages by positively affecting ABCA1 mRNA and protein expression [245, 246]. In THP-1 human macrophages, apoA-I-lipid affinity was increased in the presence of adiponectin, thereby accelerating cholesterol efflux [247]. This highlights that the combination of adiponectin and apoA1 is more efficient in promoting efflux than apoA1 alone.

Adiponectin also promotes the polarization of human monocytes into alternative anti-inflammatory M2 macrophages as opposed to the classically activated M1 phenotype, leading to a decrease in M1 markers and a suppression in the release of pro-inflammatory cytokines, such as TNF-α and MCP-1 [135, 248]. Upregulation of adiponectin levels can restore AdipoR expression in M1-polarized mouse macrophages [249]. Furthermore, adiponectin has also been shown in mice to regulate peritoneal macrophage polarization towards a M2 phenotype [135]. An increase in the ratio of M1:M2 macrophages present in the plaque is believed to influence and promote atherogenesis. Interestingly, a study in mouse bone marrow and peritoneal macrophages showed that macrophage polarization is a key determinant regulating AdipoR expression and differential APN-mediated macrophage inflammatory responses [249]. Also, in a macrophage AdipoR1 transgenic mouse model, AdipoR1 molecules were overexpressed in macrophages and the modified macrophages infiltrated, circulated or resided in metabolically active tissues such as adipose tissue, vascular artery, liver, and skeletal muscle through blood vessels [250]. The macrophage AdipoR1-transgenic mice exhibited enhanced whole-body glucose tolerance and insulin sensitivity with reduced pro-inflammatory cytokines, MCP-1 and TNF-α, both in the serum and in the insulin target metabolic tissues. Lastly, adiponectin can also prevent fibrous cap rupture by inducing the expression of tissue inhibitor of metalloproteinase-1 by macrophages [251]. In addition to its effects on endothelial cells and macrophages, adiponectin inhibits growth factor-induced smooth muscle cell proliferation and migration as well as platelet aggregation and thrombus formation [252–255]. The inflammatory environment in atherosclerotic plaques makes HDL vulnerable to oxidation, where incubation of adiponectin in human vascular smooth muscle cells has been shown to attenuate vascular calcification and reduce pro-inflammatory cytokine production caused by oxidized HDL [256].

Adiponectin protein has been detected in the vasculature of normal and atherosclerotic mice [257] as well as in healthy human carotid arteries and carotid atherosclerotic plaques [258]. On the other hand, there was a lack of adiponectin mRNA in these tissues [258], suggesting that adiponectin protein in the plaque area and the healthy vasculature is not due to de novo cellular expression. Instead, adiponectin must enter these layers from outside sources [98, 257, 259]. Indeed, one murine study assessing the ultrastructural localization of adiponectin within endothelial cells of the aortic wall observed the presence of adiponectin in endocytic vesicles, suggesting that adiponectin undergoes endocytosis from the circulation into the endothelial cells; however, the mechanism through which adiponectin

endocytosis occurs is currently unknown [257]. Others have suggested that adiponectin in the vascular wall is derived from the adventitia and surrounding PVAT, an abundant producer and secretor of adiponectin [98, 259]. Lending support to the latter hypothesis, one study demonstrated higher adiponectin expression in the PVAT of neurologically symptomatic patients versus asymptomatic patients who underwent a carotid endarterectomy [259]. This study complements later observations of higher adiponectin protein expression among unstable versus stable plaques [258].

Adiponectin receptors have also been noted to be expressed in human carotid atherosclerotic plaques as well as in the healthy vascular wall [260, 261]. While both AdipoR1 and R2 expression levels were observed to be higher in the lesion area compared to the non-diseased carotid zone, lower AdipoR2 expression and activity was observed in atherosclerotic plaques with greater instability [258]. Analysis of AdipoR expression levels in specific cells of the vasculature determined that both AdipoR1 and AdipoR2 are expressed in macrophages, along with smooth muscle cells, and endothelial cells [261]. Moreover, AdipoR are expressed abundantly (93%) in circulating monocytes, as opposed to other circulating cells [262]. Interestingly, AdipoR1 expression decreased upon monocyte differentiation into macrophages, while AdipoR2 expression was not affected during the differentiation process [261]. Nonetheless, AdipoR1 expression remained higher than AdipoR2 expression in monocytes as well as in fully differentiated macrophages [261]. Ultrastructural localization of adiponectin protein by immunoelectron microscopy in the healthy and diseased vasculature of mice was revealed to be similar to that of the AdipoRs, further suggesting that adiponectin mediates its atheroprotective actions via these receptors [257].

While AdipoR1 and AdipoR2 are well known for their involvement in the metabolic action of adiponectin, their role in the vasculature still remains unclear. Several studies have determined that AdipoR1 and AdipoR2 are crucial for mediating adiponectin's actions to suppress lipid accumulation and inhibit macrophage-to-foam cell transformation [258, 263]. On the other hand, these receptors exhibited differential effects in regulating genes that are important for lipid metabolism and inflammation in human macrophages, where AdipoR1 showed greater potency in reducing the levels of pro-inflammatory cytokines, while AdipoR2 had greater potency in suppressing the expression of the scavenger receptor, SR-AI [263]. It was recently noted that AdipoR2 deficiency (but not AdipoR1) led to a reduction in the size of brachiocephalic atherosclerotic plaques in apoE$^{-/-}$ mice; however, these plaques contained a higher degree of macrophages, less collagen content, and no clear fibrous cap, compared with AdipoR2$^{+/+}$apoE$^{-/-}$ mice [264]. This evidence suggests that AdipoR2 may be protective against atherosclerotic plaque instability. Further investigation is needed to determine the contribution of each receptor in mediating adiponectin's actions in the vasculature, in order to highlight their true potential as therapeutic targets for the treatment and/or prevention of atherosclerotic disease.

Adiponectin's Direct Cardioprotective Effects

Adiponectin exhibits direct cardioprotective effects; it is particularly involved in cardiac metabolism, cell survival, and hypertrophy. These effects are mediated through AdipoR1 and AdipoR2, which are expressed in human cardiomyocytes [265], and T-cadherin, which is found abundantly in the myocardium [124]. Interestingly, adiponectin is produced and secreted by human cardiomyocytes [266]. While its levels are relatively low in comparison to amounts produced by adipose tissue and contribute minimally to circulating levels of adiponectin, cardiac-derived adiponectin acts via an autocrine/paracrine mechanism [267].

Cardiac Metabolism and Function

Adiponectin can regulate cardiac energy metabolism and function leading to more efficient utilization of glucose and fatty acids. It can increase neonatal and adult cardiomyocyte glucose uptake via activation of the AMPK, IRS1, and Akt1 pathways, as well as enhance fatty acid β-oxidation via AdipoR1–APPL1 signaling [268–270]. Increased fatty acid uptake upon adiponectin treatment is a result of increased expression of fatty acid transporter protein-1 and induced translocation of CD36 to the outer cell membrane of the cardiomyocyte [268, 270].

Myocardial Ischemia–Reperfusion Injury and Cell Survival

Several studies have also demonstrated that adiponectin can reduce myocardial oxidative stress and promote cell survival, protecting against ischemia–reperfusion injury. Specifically, adiponectin has been reported to accumulate in myocardial tissue following ischemia–reperfusion injury, where it is capable of reducing reactive oxygen species, maintain the integrity of the cardiomyocytes surrounding the infarcted region, and attenuate apoptosis. This has been hypothesized to occur through several different signaling pathways, including down-regulation of myocardial NADPH-oxidase activity, activation of AdipoR1–APPL1 and Akt-dependent signaling, as well as stimulation of ceramidase activity, among others [118, 271–276].

Cardiac Hypertrophy

Both in vitro and in vivo experiments have demonstrated that adiponectin can protect against pathological cardiac hypertrophy. Studies involving adiponectin knockout mice have reported enhanced pathological cardiac hypertrophy and increased

mortality, under experimental models of pressure overload and angiotensin II infusion, compared with wild-type mice [277–279]. These effects were reversed upon supplementation with adiponectin [277, 278]. An important anti-hypertrophic mechanism of adiponectin is suggested to occur through AdipoR1–APPL1–AMPK activation, leading to the suppression of nuclear factor kappa-B-induced cardiac hypertrophic growth signaling [280, 281]. Interestingly, T-cadherin disruption also exacerbates cardiac hypertrophy under pressure overload to a level comparable to adiponectin knock-out mice. Therefore, it is concluded that T-cadherin also plays a critical role in carrying out the cardioprotective effects of adiponectin against pressure-induced hypertrophy [124].

Adiponectin and Cardiovascular-Related Outcomes and Mortality

Although adiponectin has attracted much attention because of its anti-inflammatory, vasculo- and cardioprotective, and anti-atherogenic properties both in vivo and in vitro, there is contradictory data surrounding the usefulness of circulating adiponectin as a biomarker or predictor of cardiovascular-related outcomes or mortality. While low levels of adiponectin have been associated with increased prevalence of obesity-related cardiovascular disorders, higher levels have also been linked with worse cardiovascular outcomes and mortality. Many refer to these contrasting associations as the "adiponectin paradox." These discrepancies among clinical studies may reflect differences in disease stage and in the populations included.

Two systematic reviews and meta-analyses were performed evaluating existing evidence with regard to the association between circulating adiponectin levels and the full spectrum of carotid artery disease, from subclinical atherosclerosis (i.e., carotid intima-media thickness) to plaque presence, to ischemic stroke risk [282, 283]. Interestingly, depending on the population studied, either a negative or positive association was noted between adiponectin and carotid intima-media thickness and carotid plaque presence. A negative association was observed in obesity-associated inflammatory conditions (i.e., subjects with T2DM, MetS, or CVD), while a positive association was noted in classic chronic inflammatory/autoimmune conditions (i.e., subjects with rheumatoid arthritis or systemic lupus erythematosus) [282, 283]. However, these associations were either weak or non-significant. Furthermore, adiponectin was found to be an independent and direct predictor of ischemic stroke risk in subjects without clinically manifest CVD. Increased levels of adiponectin were associated with an 8% increase in the risk for ischemic stroke, with a more sizable association observed among men compared to women [147]. However, adiponectin levels were noted to be suppressed in the acute stage following an ischemic stroke and remained suppressed up to 6 months post-ischemic stroke.

Several studies have demonstrated an association between high circulating levels of adiponectin and lower incidence of coronary heart disease and related adverse non-fatal events [284–288]. Individuals with adiponectin levels in the highest quintile have been shown to have a reduced risk for myocardial infarction [284]. In the Framingham Offspring Study, elevated plasma adiponectin levels were highly protective of future coronary heart events in men [287]. In contrast, adiponectin has been independently and directly linked to higher CVD events and mortality among individuals with prevalent CVD, heart failure, or advanced age [289–293]. It is noteworthy that a recent systematic review and meta-analysis, which included 28 studies and 43,979 subjects, pointed strongly to a paradoxical positive association between circulating adiponectin levels and cardiovascular mortality rates (pooled HR 1.28, 95% CI 1.19–1.37) [294]. One study in particular, assessed the relationship of total and HMW adiponectin with all-cause and cardiovascular mortality in a large cohort of older adults [295]. The cohort was stratified into three groups: (1) individuals without prevalent CVD, heart failure, or atrial fibrillation; (2) those with prevalent CVD; and (3) those with prevalent heart failure or atrial fibrillation. Interestingly, different associations were observed depending on the group studied, with the associations becoming progressively more adverse across subgroups with increasing cardiovascular risk. In group 1, a U-shaped relationship was observed; in group 2, no association; and in group 3, a direct relationship. Adjustments for various metabolic and inflammatory factors had a major influence on the associations observed; in group 1, they abolished the inverse association with mortality at the lower range of adiponectin concentrations, suggested a direct association in the second group, and strengthened the association in the third group. As a result, these findings highlight the importance of taking into account the underlying CVD state, the age of the population, as well as inflammatory and metabolic factors, in the interpretation of the relationship between adiponectin and cardiovascular risk and mortality.

Contradictory data also exists in regard to atherosclerotic plaque instability. One study identified circulating adiponectin as the strongest predictive factor of the presence of thin-cap fibroatheroma, as assessed by virtual histology intravascular ultrasound in men with stable coronary artery disease [296]. In contrast, another study observed no correlation between adiponectin levels and carotid atherosclerotic plaque instability, as assessed by gold-standard histological characterization [68].

Although the basis for this “adiponectin paradox” remains undefined, several plausible explanations have been proposed [297, 298]. First is the concept that increasing adiponectin levels is a failing attempt to protect individuals with greater risk of CVD and mortality. This may reflect the phenomenon of adiponectin resistance (decreased signaling efficacy) in metabolically active organs, such as the adipose tissue, liver, skeletal muscle, heart, and the vessel wall [299]. Second is that there is a strong correlation between adiponectin and natriuretic peptides, which are established risk factors of cardiovascular mortality rate, and thus, adiponectin works just as a marker of increased natriuretic peptides [300]. The myocardium in response to elevated cardiac strain produces an increase in circulating natriuretic peptides,

which in turn directly stimulate adiponectin production in human adipose tissue and consequently raise plasma adiponectin levels. Lastly, impaired kidney function is often associated with an advanced CVD state. Therefore, it could be hypothesized that increased levels of adiponectin may be attributed to reduced excretion and clearance via the kidney. These plausible mechanisms suggest that elevated levels of adiponectin may in fact be a secondary consequence, as opposed to a primary contributor of cardiovascular dysfunction and mortality.

Therapeutic Modulation of Adiponectin and Its Receptors

Modulation of adiponectin and its receptors is a promising therapeutic strategy for the prevention and/or treatment of cardiometabolic disorders.

Adiponectin

Adiponectin levels are reduced in subjects who suffer from obesity and cardiometabolic disorders. Thus, an important therapeutic approach would be to pharmacologically restore the capacity of adipose tissue to produce and secrete adiponectin, as well as raise circulating adiponectin levels.

Various currently used therapeutic interventions can modulate and increase circulating adiponectin levels. TZDs, such as rosiglitazone and pioglitazone, are antidiabetic therapeutic agents that improve systemic insulin sensitivity and glucose tolerance in obese individuals, T2DM patients, and in animal models of IR and diabetes [301–303]. Since adiponectin is an insulin-sensitizing adipokine, it is believed that TZDs partly mediate their anti-diabetic properties via upregulation of plasma adiponectin levels [161]. A low-dose treatment of pioglitazone led to an amelioration of IR in *ob/ob* mice but not in *ob/ob* mice that were adiponectin deficient, suggesting that pioglitazone-mediated reduction in the severity of IR is partly due to an adiponectin-dependent pathway [304]. A meta-analysis confirmed that administration of TZDs led to an increase in endogenous adiponectin levels in patients with IR and T2DM [305]. Interestingly, HMW adiponectin is the predominant form of adiponectin that is upregulated by TZDs [306]. TZDs are synthetic agonists of PPAR-γ, a transcription factor that acts as a master regulator of adipocyte differentiation and adipocyte gene transcription. Thus, TZDs are known to raise circulating adiponectin levels by inducing the adiponectin promoter activity and stimulating the transcription of the adiponectin gene in adipocytes via activation of PPAR-γ [307, 308]. These effects of TZDs can be blocked by the selective PPAR-γ antagonist GW9662 [309]. Combs et al. reported circulating levels of adiponectin to be reduced by fivefold in patients with dominant-negative PPAR-γ mutations, highlighting the importance of PPAR-γ in the regulation of adiponectin synthesis [308]. In addition to improving insulin sensitivity, TZD therapy in association with enhanced adiponectin levels has also been shown to ameliorate the

stability of atherosclerotic plaques in patients with T2DM by reducing the necrotic core component of coronary plaques [310]. In patients with non-alcoholic steatohepatitis (NASH), TZD treatment (6–12 months) was associated with parallel increases in circulating adiponectin levels and histological improvements in steatosis [311]. However, these improvements were accompanied with a significant gain in weight. A non-TZD, selective PPAR-γ modulator, called CHS-131 (also known previously as INT131), may also serve as a promising candidate for NASH patients, as it increases adiponectin levels without the significant weight gain and adverse effects associated with PPAR-γ full agonists [311, 312]. In a mouse model of NASH, treatment with CHS-131 led to improved insulin sensitivity and lipid metabolism, increased plasma adiponectin levels, improved liver histology and markers of hepatic fibrosis, as well as reduced inflammation in adipose tissue [313].

Other therapeutic agents have also been identified to raise adiponectin levels, such as anti-hypertensive drugs (i.e., angiotensin II receptor antagonists, angiotensin-converting-enzyme inhibitors, and β_1 receptor blockers), PPAR-α agonists (i.e., fibrates), and statins (i.e., pitavastatin) [203, 314–316]. Specifically, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers seem to increase adiponectin through PPAR-γ-activated adiponectin gene transcription and enhanced adipogenesis [317]. Particularly, temocapril, an angiotensin-converting enzyme inhibitor, increased adiponectin expression in patients with essential hypertension [203]. A study on omental and subcutaneous preadipocytes from pre-menopausal women showed that plasma adiponectin was increased dramatically with renin–angiotensin system blockers [317]. Glucagon-like peptide 1 (GLP-1) analogs and dipeptidyl peptidase 4 (DPP4) inhibitors can also cause a significant increase in adiponectin expression in 3T3-L1 adipocytes [318], as well as in clinical trials [319]. Another anti-diabetic agent, a sodium–glucose cotransporter-2 inhibitor (SGLT-2i), empagliflozin, has been reported to increase adiponectin levels in mice [320]. A meta-analysis of nine randomized placebo-controlled trials revealed a significant effect for fibrate therapy in increasing circulating adiponectin levels (weighed mean difference: 0.38 μg/mL; 95% confidence interval: 0.13–0.63 μg/mL; $p = 0.003$) [321]. The effect size remained significant when the analysis was restricted to fenofibrate trials [321]. Interestingly, an association between statins and adiponectin levels was observed to be dependent on statin type. A meta-analysis reported a significant elevation in circulating adiponectin levels following treatment with pitavastatin, particularly, in cases where statins were used for a duration ≥12 weeks [316]. However, atorvastatin and rosuvastatin treatments either showed no effect or significantly reduced adiponectin levels, respectively [316, 322].

Adiponectin Receptors

Under conditions of adiponectin resistance, more useful therapeutic strategies would be to enhance the action of adiponectin by increasing the expression and/or activation of the adiponectin receptors, rather than upregulate circulating adiponectin levels.

AdipoR expression has been reported, particularly, in adipose tissue and in monocytes/macrophages, to be transcriptionally induced by nuclear hormone receptors; PPAR-α/γ can positively regulate AdipoR2 expression, while liver X receptors can stimulate both AdipoR1 and AdipoR2 [261, 323]. Thus, AdipoR expression can be modulated therapeutically by various nuclear hormone receptor agonists to enhance the actions of adiponectin. In fact, induction of AdipoR2 via PPAR-α activation was capable of potentiating adiponectin's actions in macrophages by having an additive effect on reducing intracellular cholesterol ester content [261]. Furthermore, dual activation of PPAR-α and PPAR-γ had a greater effect on improving IR in obese diabetic KKAy mice than single drug treatment due to increases in both adiponectin levels and AdipoR expression [323]. Interestingly, a recent study demonstrated that higher doses of statins and/or longer treatment duration can compromise the expression and function of adiponectin receptors, particularly on the monocyte–macrophage lineage, which in turn can produce a more pro-inflammatory phenotype [322]. Although undoubtedly statins remain the mainstay of lipid management for cardiovascular prevention, they do not fully abolish the cardiovascular risk. Besides statins, telmisartan, an angiotensin receptor blocker with selective PPAR-γ activity, enhanced the reduced ventricular cardiomyocyte AdipoR2 and aortic AdipoR1 expression in diabetic rats to comparable levels as in control animals [324]. Similarly, metformin upregulated AdipoR1 and AdipoR2 receptor expression levels in muscle and AdipoR1 in WAT of Zucker diabetic rats [325]. In addition, lifestyle interventions can also positively affect AdipoR1 and AdipoR2 expression [326]. Osmotin, a novel phytohormone structurally similar to adiponectin, is proposed to act as an agonist for AdipoR1 [327]. Studies with in vivo or in vitro treatment of osmotin revealed its adiponectin's memetic effect towards CVDs. A study on injury by oxygen and glucose deprivation of cardiac myoblast cells followed by reperfusion showed that osmotin reduced the release of pro-inflammatory factors and increased the release of anti-inflammatory factors [328]. Also, there is evidence that osmotin can suppress the development of aortic atherosclerotic lesions in apoE$^{-/-}$ mice [329].

Activation of AdipoRs using peptide and small molecule-based agonists that can mimic the effects of adiponectin may also act as important therapeutic approaches for the prevention/treatment of cardiometabolic disorders. Several peptidic agonists of AdipoRs were designed following the identification of the active site within the C-terminal globular domain of the native adiponectin protein. The most commonly studied are ADP355, ADP399, Pep70, and BHD1028, which exhibit adiponectin-like activities in both in vitro and in vivo assays [330]. It has been shown that ADP355 peptide restores the subcutaneous tissue and reverses hyperinsulinemia, hypertriglyceridemia, and hypoadiponectinemia [331]. Moreover, it activates hepatic LDL receptor expression and ameliorates lipid metabolism in both wild type and apoE$^{-/-}$ mice and inhibits atherosclerosis in apoE$^{-/-}$mice [332]. Finally, this adiponectin-based peptide can restore the liver from dysfunction and inhibit macrophage-mediated inflammation [333]. Through screening of compound chemical libraries, Okada-Iwabu et al. identified the first orally active synthetic small molecule agonist (named AdipoRon) to bind and activate both AdipoR1 and

AdipoR2 [334]. It is currently the most extensively studied non-peptidic adiponectin replacement therapy drug candidate. Its effects are similar to those of adiponectin, where AdipoRon was reported to activate AMPK and PPAR-α signaling pathways to ameliorate IR, dyslipidemia, and glucose intolerance in obese diabetic mice [334]. Furthermore, in vitro and in vivo studies have demonstrated that AdipoRon possesses anti-inflammatory, anti-oxidative, and anti-apoptotic properties, in addition to attenuating vascular smooth muscle cell proliferation [335, 336]. A recent study also demonstrated that AdipoRon exerts beneficial effects in muscle via human AdipoR by increasing insulin sensitivity in AdipoR-humanized mice [337]. Overall, these findings suggest that AdipoRon may be a promising new therapeutic agent for the treatment of obesity-related disorders, but its efficacy and safety have yet to be tested in humans. Unfortunately, a major limitation of AdipoRon is its low cellular activity. With the recent crystallization of the AdipoR structure, this can help optimize the interaction between AdipoRon and AdipoR as well as identify novel agonists of the AdipoR pathway [115].

Proglucagon Family of Molecules and Gut-Derived Peptide Physiology

Impaired insulin secretion from declining β-cell function, increased hepatic gluconeogenesis from the liver, and decreased peripheral glucose utilization by the muscle tissues constitute the traditional core defects, known as the triumvirate, responsible for the development of T2DM [338, 339]. Insulin secretion may be paradoxically increased early in the course ofT2DM, as the pancreas is trying to compensate for the elevated fasting plasma glucose concentration. However, at some point the β-cells can no longer sustain this increased insulin secretion rate, and impaired glucose tolerance and T2DM ultimately become clinically apparent [340, 341]. The San Antonio Metabolism Study [342] demonstrated clearly that β-cell failure occurs early in the natural course of T2DM and in fact plays a pivotal role in the pathophysiology of IR. IR may be the best predictor of T2DM development [343, 344] as well as a strong indicator of CVD [345]. The San Antonio Heart Study showed a progressive increase of CVD events with progressive severity of IR [346]. There has been increasing evidence that compromised adipocyte metabolism plays an important role in the pathogenesis of T2DM [347]. Adipose tissue becomes resistant to the antilipolytic effect of insulin in T2DM, which results in elevated FFA concentrations and lipotoxicity [348], that further increases IR and promotes β-cell failure. In such a state of chronic inflammation and stress, fat cells fail to secrete normal amounts of adiponectin, which inadvertently potentiates IR [1]. People with diabetes have diminished incretin effect as a result of incretin hormone deficiency and/or resistance [349], setting the gastrointestinal tract (GIT) as a key part of a quintet [350], contributing to the pathogenesis of T2DM, alongside the β-cells, the liver, the muscle tissue, and the adipose tissue. Hyperglucagonemia, resulting from increased pancreatic α-cell secretion, enhances hepatic glucose production and

increases hepatic IR, with a key role in the pathogenesis of diabetes [351]. The diabetic kidney is another tissue with a crucial role in the pathogenesis of the disease; namely the diabetic kidney, instead of excreting the excessive glucose load, enhances glucose reabsorption primarily via increased sodium–glucose cotransporter (SGLT)-2 in the convoluted segment of the proximal tubule [352]. The "Ominous Octet" involved in the pathophysiology of diabetes, as classically described by DeFronzo [338], is completed by perhaps the most important contributor, the CNS. Obese people with and without diabetes are insulin resistant but despite marked hyperinsulinemia, their appetite is not suppressed as expected. This indicates that appetite centers may also be resistant to insulin, which has been demonstrated using functional magnetic resonance imaging [353, 354].

Energy homeostasis and eating patterns are tightly regulated by a complex interplay of CNS networks and cognitive centers with peripheral signals originating largely from the GIT, adipose tissue, and external food cues [355]. Enteroendocrine cells (EECs), distributed along the entire GIT mucosa, are the specialized cells capable of sensing luminal content and producing polypeptide hormones, collectively termed as gut-derived hormones [356]. Vagal afferent fibers innervate the wall of the GIT and are closely embedded to the mucosal epithelium. Gut-derived hormones secreted by the EECs act on their respective receptors on those vagal pathways, instrumenting effects on the bidirectional communication between the GIT and the brain, establishing the Brain-Gut Axis [357]. Those gut peptides act also directly on the nuclei of hypothalamus, brainstem, and higher brain reward centers with control on the hedonic aspects of eating behavior. The most important hypothalamic region regulating appetite control is the arcuate nucleus, with distinct neuron types that have opposing effects on food intake. Aberrant gut hormone responses have been implicated in the pathophysiology of obesity, while alterations of hormone levels have been observed following dietary interventions and/or bariatric surgery [358]. Modulating gut hormone levels or targeting their receptors has been a promising therapeutic approach in patients with obesity and MetS [359]; several clinical trials demonstrate the cumulative benefit in the metabolic profile of patients on dual or even triple gut peptide receptor agonist therapy [360], aiming to mirror as closely as possible the "hormonal aftermath" after bariatric surgery [361]. Therefore, it is of paramount importance to shed light and review the physiological properties of those gut peptides. The major hormones implicated in the regulation of energy homeostasis are ghrelin, GLP-1, peptide tyrosine-tyrosine (PYY), oxyntomodulin (OXM), glicentin, amylin, glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide (PP), glucagon and cholecystokinin (CCK) [362, 363]. Understanding the signaling pathway of peptides deriving from the preproglucagon gene (*Gcg*) has been instrumental in diabetes and obesity pharmacology. *Gcg* is expressed in a specific population of the EECs of the GIT (L type), the pancreatic islet α-cells, and neurons along the nucleus of the solitary tract [364]. The *Gcg* encodes Proglucagon (ProG), a peptide which undergoes post-translational processing mediated by prohormone convertase (PC) enzymes in a tissue-specific fashion.

In the α-pancreatic islet cells, PC2 is primarily dominant with glucagon, glicentin-related pancreatic polypeptide (GRPP), intervening peptide 1 (IP1), and major proglucagon fragment being the more prevalent products. PC1/3 appears more dominant in the intestinal L cells and specific neurons and, as a result, ProG is primarily cleaved to GLP-1, glucagon-like peptide 2 (GLP-2), OXM, glicentin, and intervening peptide 2 (IP2) [365]. *Gcg* expression and therefore ProG synthesis is highest in the colon.

Ghrelin

Ghrelin is predominantly secreted by P/D1-type (X/A like) EECs in the gastric fundus, with the duodenum producing approximately ten times less ghrelin than the stomach and progressively lower concentrations found distally [366]. It is a 28-amino acid peptide cleaved from a precursor, preproghrelin, which subsequently requires post-translational acylation of its serine-3 residue with attachment of a medium-chain fatty acid, typically octanoic acid. The enzyme ghrelin-*O*-acyltransferase catalyzes this reaction, which is necessary for ghrelin to become biologically active and bind to its growth hormone secretagogue receptor (GHS-R1a) [367]. Uniquely among other known gut peptides, ghrelin has a potent orexigenic effect. Circulating ghrelin levels peak pre-prandially in humans, an effect that appears to be consistent irrespective of particular fixed or voluntary food- or time-cues, and decrease rapidly in the postprandial state [368–370]. Ghrelin acts via receptors in the hypothalamus and vagal nerves. The primary site of action is in the arcuate nucleus of the hypothalamus, where it activates the orexigenic neuropeptide Y (NPY) and Agouti-related peptide (AgRP) neurons, as supported by attenuation of those effects in NPY-knockout or AgRP-knockout mice [371]. The binding of ghrelin on GHS-R1a of those NPY/AgRP neurons also induces a GABA-mediated inactivation of anorexigenic proopiomelanocortin neurons [372]. Additional sites of action include other hypothalamic nuclei, dorsal complex of the brainstem, and midbrain dopaminergic areas, while ghrelin has also been shown to have activating modulatory effects on brain areas that control appetite behavior such as amygdala, orbitofrontal cortex (OFC), and anterior insula [373, 374]. Physiologically, biologically active (acylated) ghrelin increases appetite and food intake, stimulates hepatic gluconeogenesis, delays gastric emptying, and reduces IR [366]. Plasma ghrelin levels are normally increased during prolonged fasting states and are immediately suppressed after food intake. On the contrary, in obesity, lower than expected fasting ghrelin levels have been observed, paired with a dysregulated postprandial suppression [375, 376]. Circulating ghrelin levels appear to be inversely correlated with BMI, implicating ghrelin's role in the long-term energy homeostasis beyond just the short-term post meal initiation effects. A well-established exception to this pattern is Prader–Willi syndrome, where elevated ghrelin levels are observed along hyperphagia [377].

PYY

PYY is a 36-amino acid peptide, member of the PP-fold family containing several tyrosine residues with two of them located at each terminus of the peptide. PYY occurs in two forms, $PYY_{1–36}$ and $PYY_{3–36}$. The biologically active form is the $PYY_{3–36}$, which is a truncated 34-amino acid peptide created by cleavage of the N terminal tyrosine and proline residues from $PYY_{1–36}$ by the enzyme dipeptidyl peptidase-4 (DPP-4) [378]. PYY is postprandially secreted by L type EECs primarily in the distal gut, in a proportional fashion to the caloric intake but also affected by the macronutrient composition of the meal, with protein being a more potent stimulus compared to fat or carbohydrates [379]. The initial rise in PYY levels is observed within 15–30 min from food intake and somewhat surprisingly rise to reach a plateau between 1 and 2 h after a meal, despite highest expression levels of PYY in the distal gut. This physiologic mechanism along with the fact that PYY secretion is reduced following a vagotomy [380], suggesting that neural reflexes along with luminal contact with nutrients, control its secretion. The PP-fold peptide family exert their effect via the Y family of G-protein coupled receptors. PYY has the highest affinity for the Y2 receptor, which functions as an autoinhibitory presynaptic receptor in NPY/AgRP neurons of the arcuate nucleus in the hypothalamus and mediates the anorexigenic effects [381]; PYY also seems to have additional modulatory effect within both corticolimbic and higher-cortical areas [382]. In the periphery, PYY serves as a satiety signal reducing appetite, promotes insulin sensitivity, induces lipolysis, and delays gastric emptying [383]. Exogenous administration of $PYY_{3–36}$ to lean and obese individuals leads to appetite suppression and reduced food intake, an effect that also appears to be dose dependent [384, 385]. Notably, obese individuals have lower fasting PYY levels compared to normal-weight individuals as well as a more attenuated postprandial PYY secretion [386].

GLP-1

GLP-1 is primarily secreted by L cells of the distal jejunum and ileum in response to ingested nutrients in a biphasic fashion; an early phase about 15 min after food ingestion and then a second peak at about 1-h postprandial [387]. A neuro-hormonal interplay with input from the enteric nervous system, other gut peptides as well as direct nutrient contact with the L cells, regulates the secretion of GLP-1. Shortly after release, GLP-1 is rapidly inactivated by DPP-4, with a plasma half-life of less than 2 min. The major circulating form of GLP-1 is $GLP\text{-}1_{(7–36)amide}$, although $GLP\text{-}1_{(7–37)}$ is also equally potent [388]. GLP-1 binds to a specific GLP-1 receptor, which is widely expressed in various tissues centrally, such as hypothalamus, and peripherally, including pancreatic β-cells, the liver, the kidneys, and muscle tissues [389]. GLP-1 acts as a potent incretin hormone, promoting glucose-dependent

insulin secretion, suppressing appetite, delaying gastric emptying, enhancing β-cell proliferation, and inhibiting glucagon secretion [386, 390]. These properties have rendered GLP-1 a key target for the development of diabetes therapies, namely GLP-1 receptor agonists and DPP-4 inhibitors, as we will describe in detail later in this chapter.

OXM

OXM is a 37-amino acid peptide chain, homologous to glucagon with a C-terminal extension of IP1 and secreted by L cells in response to food intake [391]. OXM and GLP-1 possess similar anorexigenic potency, despite lower affinity of OXM to the GLP-1 receptor [392], implicating perhaps a separate receptor yet to be identified. Studies utilizing manganese-enhanced magnetic resonance imaging techniques in mice have suggested that there might be different patterns of neuronal activation in the brainstem and hypothalamus between OXM and GLP-1 [393]. The constant crosstalk between gut hormones in the regulation of energy homeostasis is also supported by the ability of OXM to inhibit the pre-prandial ghrelin peak by about 44% in humans, which likely also contributes to the appetite suppressant effects of the molecule [394]. In normal-weight individuals, exogenous OXM anorexigenic effect upon food intake typically lasts for several hours. In a randomized, double-blind controlled cross-over trial in obese and overweight individuals, after subcutaneous self-administration of pre-prandial OXM, the peptide was shown to reduce energy intake while increasing activity-related energy expenditure, creating a net negative energy balance [395].

Glicentin

Glicentin is a 69-amino acid peptide chain that incorporates the sequences of OXM and GRPP and is another product of the ProG cleavage by PC1/3 in the L EECs [396]. The exact biologic role of glicentin remains yet to be fully identified, primarily secondary to lack of reliable quantitative methods of this particular hormone. Lower fasting glicentin levels have been observed in adolescents with obesity or T2DM and adults with morbid obesity [397], but no causal effect has been established to date. Notably, in morbidly obese individuals following bariatric surgery, postprandial glicentin levels are elevated early in the postoperative course, and this rise is maintained for at least 1 year with positive correlation to weight loss, possibly mediated through regulation of satiety [398]. Additionally, increased fasting glicentin levels have been reported in obese individuals following sleeve gastrectomy or bariatric surgery, peaking at 1 year postoperatively, but those changes were not correlated with weight loss or improvement in glycemic control [399].

PP

PP belongs in the PP-fold family along with PYY and NPY. It is a 36-amino acid peptide with anorexigenic effect, primarily secreted by the F cells of the pancreatic islets of Langerhans [400] in response to nutrient ingestion and in proportion to the caloric load. PP binds with most affinity to the Y4 receptor, modulating mostly appetite-regulating regions within the brainstem and hypothalamus. Besides nutrient load, other gastrointestinal and pancreatic hormones can regulate circulating PP levels, namely somatostatin and its analogs can reduce PP levels, while ghrelin, secretin, and CCK can rapidly stimulate PP release [401, 402]. Peripheral administration of PP in lean and obese mice has shown to reduce their food intake with reduction in gastric emptying and increased vagal tone [403]. In turn, PP increased oxygen consumption and stimulated sympathetic activity, with a possible increase in overall energy expenditure. Similar decrease in appetite and food intake has also been observed in normal-weight individuals as an effect of PP [404]. Obese patients with Prader–Willi Syndrome have suppressed basal and postprandial PP levels, but exogenous PP administration to those subjects does reduce food intake, possibly implying that PP relative deficiency may be associated with hyperphagia [405, 406]. However, PP variations in non-syndrome-related obese phenotypes have not been clearly identified to date with mixed available data.

CCK

CCK is one of the first identified satiety hormones [407]. It is secreted by I cells (also called inclusion cells) of the duodenum and jejunum as well as certain central neurons as a response to nutrients in the gut; most particularly fat- and protein-rich meals [408]. In addition to the anorexigenic effects, CCK's main actions include stimulating gallbladder contraction and pancreatic enzyme secretion, relaxing the sphincter of Oddi and delaying gastric emptying, essentially coordinating the digestive process [409]. CCK receptors have been identified in the brain and on peripheral nerves, pancreas, stomach, gallbladder, lower esophageal sphincter, ileum, and the rectum [410]. There are two types of CCK receptors that have been identified so far, namely CCK-1 and CCK-2 receptors. Low CCK levels have been reported in patients with bulimia nervosa, with no clear pathophysiologic explanation, while obesity has been linked to a blunted effect of the CCK-related satiety properties [411, 412].

Amylin

Amylin is a 37-amino acid peptide which is stored in pancreatic β-cells and co-secreted with insulin [413], in response to consumed nutrients and neural signals. Amylin and insulin increase and decrease in a similar fashion and act

complementary. Amylin reduces food intake, increases energy expenditure, slows gastric emptying and suppresses postprandial glucagon secretion [414]; downregulation of amylin receptors and therefore an attenuated postprandial amylin secretion effect have been reported in obesity. Amylin acts on specific receptors in rewards centers of the brain, such as the area postrema, an interaction that may be key at regulating satiety [415, 416]. In a randomized, blinded, placebo-controlled multicenter study of 88 non-diabetic obese subjects, administration of pramlintide—an amylin analog—was tested for 6 weeks and resulted in reduced food intake and meal proportions along with healthier eating habits (reduced fast food intake and lowered binge eating tendencies) [417].

GIP

GIP is a 42-amino acid peptide secreted mostly from the K-EECs in the duodenum and proximal jejunum in response to a meal, particularly, dietary lipids and acts on GIP receptors, found in pancreatic islet cells, hypothalamus, and adipose tissue [418]. GIP demonstrates an incretin effect but unlike GLP-1, it has no effect on gastric emptying or induction of satiety [419]. Under hyperglycemic conditions, GIP potentiates insulin release, while not affecting glucagon secretion, but under hypo- or normo-glycemic conditions, it increases glucagon release but does not affect insulin [420]. GIP appears to have strong anabolic effects on adipose tissue, promoting fat accumulation via enhanced lipoprotein lipase activity and reduced release of FFAs [421, 422].

Glucagon

Glucagon is a 29-amino acid peptide secreted from pancreatic islet α-cells in response to hypoglycemia and is the main hormone to counteract the effects of insulin [423]. PC2 is responsible for post-translational processing of ProG in the pancreas to generate glucagon. PC2 knockout mice demonstrate reduced levels of circulating glucagon, and as a result improved glucose tolerance profile [424]. Glucagon exerts its effects through a membrane bound G-protein-coupled receptor, which stimulates adenylate cyclase, inducing cyclic adenosine monophosphate levels and activation of the protein kinase A pathway. The glucagon receptor gene encodes for the receptor and is primarily expressed in the liver and kidneys [425]. Glucagon levels are low in the postprandial state and increase with fasting or in hypoglycemic states, primarily to activate hepatic gluconeogenesis and glycogenolysis and inhibit glycogenesis [426]. Repeated hypoglycemic events may ultimately lead to an impaired response, namely either deficient or absent glucagon secretion. T2DM is a prime example of dysregulated glucagon secretion, perhaps secondary to defective suppression of glucagon by insulin or other β-cell products, resulting in a state of IR along with inappropriately elevated glucagon levels [427].

GLP-2

GLP-2 is a 33-amino acid peptide hormone secreted by L cells of the distal gut, acts on the glucagon-like peptide-2 receptor (GLP-2R), and primarily induces proliferation and inhibits apoptosis of enterocytes [428, 429]. Peripheral administration of GLP-2 in rodent studies reduced energy intake in lean and diet-induced obese mice but had no effect on gastric emptying or on satiety in humans [430, 431].

SGLT2/SGLT2i Physiology

Glucose is primarily reabsorbed in the proximal convoluted tubule by membrane bound carrier proteins, the sodium–glucose cotransporter-2 (SGLT-2) receptors [432]. Glycosuria typically occurs when the threshold for renal reabsorption is met at around 180 mg/dL of blood glucose. As the blood glucose decreases, urinary glucose excretion is also reduced [433]. Inversely, in hyperglycemic conditions such as diabetes mellitus, the ability of renal glucose reabsorption is pathologically raised by upregulating the SGLT-2 receptors [352]. SGLT2 inhibitors (SGLT2i) are medications designed to block those receptors, decreasing renal glucose reabsorption and enhancing glycosuria, a phenomenon that is further enhanced in hyperglycemic states [434]. Enhanced glycosuria in return is associated with caloric loss and osmotic diuresis, which likely account for the resulting weight loss, blood pressure improvement, and reduced plasma volume. Inzucchi et al. in 2018 demonstrated improved erythropoietin production by SGLT2i, with improvement in plasma volume markers and further optimization of cardiovascular hemodynamics in those patients [435]. SGLT2i may also beneficially affect cardiac remodeling, improve endothelial function and reduce arterial stiffness, and improve cardiac contractility via higher calcium mitochondrial concentrations [436, 437]. The pertinent benefits of this class of medications, particularly in heart failure, may also likely be related and mediated by the inhibition of the sodium–hydrogen (Na^+/H^+) exchange. In a failing myocardium, the Na^+/H^+ potentiates via the upregulation of the Na^+/H^+ exchanger 1, which has been shown to be inhibited by SGLT2i [438]. Furthermore, shifting of cardiac substrate from FFAs and glucose to the more energy-efficient ketone bodies is another proposed mechanism of action for the SGLT2i [439]. Features of non-alcoholic fatty liver disease seem to be improved while on SGLT2i therapy [440, 441], portraying another possible mechanism of action and effect of this class of medications. Finally, several studies of SGLT2i have demonstrated their anti-inflammatory, anti-apoptotic, and antioxidant properties, via modulating mediators such as IL-6 and IL-10, TNF-α, and cyclo-oxygenase-2 [442].

Conclusion

Obesity has long been associated with IR, hypertension, and CAD, but the mechanism has remained largely unknown. Adiponectin may be one of the factors that explain these associations. Since a deficiency in adiponectin or a dysregulation in its receptor pathway may result in the development of these processes, increased endogenous production of adiponectin or exogenously administered peptide and small molecule-based agonists of the adiponectin receptor pathway may contribute to restoring insulin sensitivity and preventing atherosclerosis and CVD by increasing fatty acid oxidation and insulin-mediated glucose uptake, and decreasing the endothelial and macrophage inflammatory process associated with atherosclerotic plaque development. Although animal studies have demonstrated benefits, clinical trials are needed to determine whether the beneficial effects of adiponectin can also be observed in humans and whether either adiponectin or adiponectin receptor agonists represent a novel treatment option for type II diabetes and CAD. Moreover, modulating the hormone levels of gut-derived peptides may also serve as a promising therapeutic approach for obesity and cardiometabolic disorders.

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Chapter 9
Diabetes and Atherosclerosis

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Introduction

Macrovascular disease, followed by renal disease, is the main cause of mortality and morbidity in diabetes. The development of cardiovascular complications in diabetes is mainly associated to the progression of atherosclerosis. Therefore, studying factors that may uniquely contribute to the accelerated development of atherosclerosis in diabetes has been ongoing for several decades. Regardless of years and years of research in this complex and multidimensional process, new genetic and metabolic pathways linked to the development of atherosclerosis and cardiovascular disease (CVD) in diabetes recently emerged, and they are being actively studied.

It is fully accepted that arteriosclerosis is a chronic inflammatory process, not a degenerative process that starts with endothelial dysfunction/injury, which facilitates deposition of lipids/lipoproteins in the vessel wall, foam cell formation, and SMC proliferation and frequently leads to a vascular thrombotic event secondary to plaque erosion and/or rupture. Numerous studies have been conducted examining how diabetes, mediated by hyperglycemia or dyslipidemia, elicited or enhanced dysfunction of the endothelium, chronic vascular inflammation, foam cell formation, and SMC proliferation and led to alterations in the clotting/fibrinolytic system, thus facilitating thrombi formation and acute CVD events. Endothelial dysfunction leads to dysregulation of the vascular tone, as described below, and increased vascular permeability facilitating increased transport of LDL across the endothelium. LDL becomes trapped

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by the extracellular matrix of the subendothelial space [1] and, due to the microenvironment conditions of this space, which excludes plasma soluble anti-oxidants, becomes oxidized. With oxidation of LDL, endothelial cells are stimulated to release potent chemoattractants, such as monocyte-chemoattractant protein 1 and others [2, 3] and monocyte-colony-stimulating factor [4] which promote the recruitment of monocytes into the subendothelial space. Recruitment of monocytes into the vascular wall is also facilitated by endothelial dysfunction due to the release and activation of several inflammatory mediators such as TNF, IL1, and IL6, which stimulate the expression of adhesion molecules, thus, mediating monocyte and T-cell adhesion to the endothelium. Some of the adhesion molecules are only expressed into sites with chronic inflammation, but some are expressed both in inflamed and normal vessel wall [5]. Migration of monocytes into the vessel wall further promotes oxidation of LDL.

Heavily modified LDL is cytotoxic to endothelial and smooth muscle cells [6], and it is no longer recognized by the LDL receptor. Heavily modified LDL is taken up by macrophage scavenger receptors leading to massive accumulation of cholesterol in macrophages and to their transformation into foam cells, the hallmark of the atherosclerotic process [7]. Besides promoting the transformation of macrophages into foam cells, oxidized LDL is a potent inducer of inflammatory molecules and growth factors and stimulates the immune system. Stimulation of the immune system leads to the formation of antibodies and, as a consequence, to the formation of immune complexes that may play a crucial role in macrophage activation and, therefore, contribute not only to SMC proliferation but also to perpetuate chronic inflammation in the vessel wall and contributing to the rupture of atheromatous plaques [8, 9]. In recent years, phospholipids and sphingolipids were identified as important players in diabetes and atherosclerosis. Sphingolipids, which, together with free cholesterol, are an integral part of every cell membrane and are the main regulators of cell function and signaling [10, 11]. Changes in sphingolipid concentration and distribution in cell membranes will alter receptor- and non-receptor-mediated binding and cell signaling leading to marked alterations in cell function [12]. The impact of this new class of lipids in the development of atherosclerosis and diabetic complications will be quite likely crucial to fully understand their complex pathogenesis [13–15].

In 1993, microRNAs were discovered, and since then, they have considered as critical modulators of endothelial homeostasis and their dysregulation is closely linked to endothelial dysfunction [16]. The endothelium was designed to detect and respond to changes in hemodynamic forces. A pulsatile one-way flow favors an atheroprotective endothelium and a disturbed flow (low oscillatory) favors an atheroprone endothelium [17–19]. Interestingly, epigenetic and environmental factors are quite similar in diabetes and atherosclerosis, thus, explaining in part the accelerated development of atherosclerosis in diabetes [20]. Under the influence of a "diabetic milieu," microRNA patterns suffer changes that will affect the levels of proteins that regulate endothelial function, thus, enhancing endothelial dysfunction and creating the necessary conditions to trigger pathways favoring the development and progression of atherosclerosis [20]. To explain the accelerated development of atherosclerosis in diabetes, several studies describing the inactivation of anti-oxidant defensive mechanisms in diabetes as a possible mechanism have been published [21–24].

In this chapter, we will update knowledge concerning factors associated or enhanced by the diabetic state that may accelerate the development of atherosclerosis, enhance

plaque rupture, and contribute to increased thrombi formation. Special emphasis will be placed on endothelial dysfunction, including dysregulation of vascular tone, oxidative stress and inflammation, foam cell formation including lipid and lipoprotein modification, and immune responses associated with these modifications, and on the abnormalities of the clotting/fibrinolytic system including platelet hyperreactivity. Also new areas of research on the association of diabetes and atherosclerosis such as changes on phospholipids and sphingolipids will be discussed as well as the changes in microRNA patterns induced by hyperglycemia, hyperlipidemia, and drugs which will alter the regulation of their target genes, affecting the levels of specific proteins, and leading to dysregulation of endothelial functions and promotion/acceleration of atherosclerosis. An overall representation of the possible pathogenic mechanisms involved in the development of atherosclerosis in diabetes is depicted in Fig. 9.1.

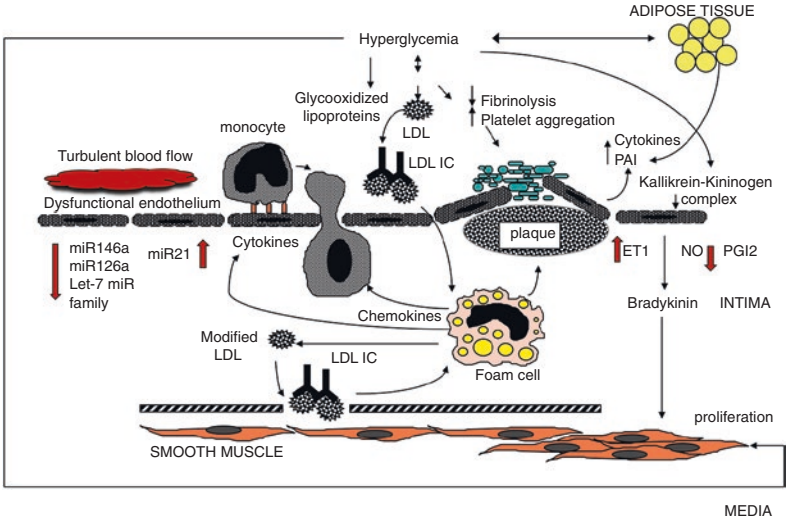

Fig. 9.1 Diagrammatic representation of the possible pathogenic mechanisms for the development of atherosclerosis in diabetes. Turbulent blood flow, as well as increased levels of glucose, ROS and other pro-atherogenic factors contribute to endothelial dysfunction. Hyperglycemia contributes to multiple changes in lipoprotein metabolism and in cell-lipoprotein interactions and induces decreased production of prostacyclin (PGI2), reduced NO activity and increased endothelin and bradykinin release. These changes contribute to the accelerated development of atherosclerosis in diabetic patients by reducing vasodilatation of the endothelium and, in the case of bradykinin promoting vasoconstriction and SMC proliferation. Increased plasma glucose levels promote also non-enzymatic glycation of lipoproteins and enhance their susceptibility to oxidative modification. These modified lipoproteins decrease fibrinolysis and increase platelet aggregation, which contributes to increased thrombosis. Modified lipoproteins may also reduce NO release and stimulate the expression of cell adhesion molecules (CAMs) leading to monocyte adhesion to the endothelial cell layer and migration of these cells to the subendothelial space. Glycated/oxidized lipoproteins in the intimal layer may be further modified by oxidative processes that result in the formation of glycoxidized lipoproteins that, in turn, stimulate the immune system to form antibodies. The resulting immune complexes are taken up by macrophages, and they stimulate the formation of cholesteryl ester-laden cells (foam cells) and the release of cytokines. Cytokines released during these processes will elicit release of reactive proteins by the liver (C-reactive protein) besides further injuring the endothelium and, thus, exacerbate the cycle. MicroRNAs so far described as important players in diabetic atherosclerosis are depicted

Endothelial Dysfunction

The endothelium controls local vascular tone and permeability, leukocyte adhesion, platelet reactivity, acute and chronic inflammatory reactions, redox balance, hemostasis, and thrombosis. Dysfunction of the vascular endothelium is a hallmark of both diabetes and atherosclerosis. Loss of functional integrity of the endothelium can be induced by multiple factors including fluid shear stress, pro-inflammatory cytokines, infectious agents, hyperlipidemia, hyperglycemia, multifactorial oxidative stress, advanced glycation end-products, adipokines, environmental toxins, such as air pollutants, cigarette smoking, and sex hormonal imbalance among others. Due to its ability to influence the behavior of other type of cells in the circulating blood (leukocytes and platelets) and within the vessel wall (smooth muscle cells and pericytes), a dysfunctional endothelium is responsible not only for the initial steps in the atherosclerotic process but also for its progression and for its final step: the formation of thrombi which leads to vessel occlusion and to acute ischemic events.

Nitric Oxide

A basal production of nitric oxide (NO) by endothelial cells contributes to the regulation of the vasomotor tone of the endothelium, and for preserving its non-atherogenic, non-thrombotic behavior is synthesized from L-arginine by nitric oxide synthase (eNOS) and its synthesis can be stimulated by receptor-dependent agonists (bradykinin and acetylcholine), by fluctuations in blood flow, and by non-receptor-dependent agonists (calcium ionophores) [25, 26]. Fluid mechanical forces differentially regulated transcription of eNOS gene by endothelial cells. When the endothelium is exposed to a regular laminar flow, NO formation is enhanced [27]. NO produced by the endothelium can rapidly diffuse across cell membranes to act as a potent mediator influencing the behavior of SMC, circulating blood platelets and leukocytes or react with superoxide and be inactivated. Some of the NO physiologic actions are mediated via activation of a soluble guanylate cyclase, which converts GTP to cGMP [19] in platelets, leukocytes, and SMC. eNOS mediates reduced platelet activation [28], inhibition of platelet aggregation, and reduction or prevention of monocyte adherence to the endothelium [29]. Other eNOS physiologic actions are mediated by S-nitrosylation [30] of various target proteins that modulate several cellular processes such as cell proliferation [31], apoptosis [32, 33], exocytosis [34], ion channel activity [35], as well as blood flow and systemic oxygen delivery [36, 37]. Nitrosylation is a reversible process similar to phosphorylation [38]. NO also reacts with hemoglobin in erythrocytes enhancing oxygen delivery to tissues [19], prolongs bleeding time [39], and reduces plasma fibrinogen levels [40]. Reduced NO bioavailability leads to impaired vasodilation, abnormalities in the normal function of the vascular endothelium and, as a consequence,

participates in the development of atherosclerosis and thrombotic events in humans [19, 41].

Impairment of NO-mediated vasodilation has been shown in both type 1 and type 2 diabetes [42, 43] and contributes to the accelerated development of macrovascular disease in diabetes. Besides hyperglycemia, insulin resistance, and hyperlipidemia, oxidized lipoproteins, which are present in increased levels in diabetes, may also be behind the reduced NO activity in diabetes, as shown by several studies [44–46].

Hyperglycemia disrupts endothelium homeostasis by compromising insulin receptor binding. Stimulation of the insulin receptor may lead either to NO-mediated vasodilation via activation of PI3K, AKt, and eNOS phosphorylation of the serine residue 1177 [47] or to vasoconstriction by activation of the Ras/MAPK-signaling pathways [48, 49]. Activation of the MAPK pathway enhances insulin resistance and is linked with the development of diabetic retinopathy and nephropathy. In a normal endothelium and normal metabolic conditions, insulin is a vasodilator and stimulates endothelial NO production [50]. However, several clinical trials [51, 52] clearly showed that increased levels of insulin were frequently associated with increased risk for macrovascular disease and that led to the concept that administration of exogenous insulin would contribute to or enhance the development of macrovascular complications in diabetes. This concept has been clearly discredited by the data of two major trials [53, 54], one performed in type 1 and another in type 2 diabetic subjects (the DCCT and UKPDS trials). Both of them clearly show a reduction in cardiovascular events with intensive glycemic control, and although the reduction did not reach statistical significance, it completely excluded the hypothesis that insulin administration would contribute to an increase in cardiovascular events.

However, although it is clear that administration of exogenous insulin do not lead to an increase in macrovascular disease, hyperinsulinemia syndromes like the insulin resistance syndrome, particularly when associated with central obesity, are definitively associated with accelerated development of macrovascular disease and although glucose intolerance and hyperinsulinemia are associated with several cardiovascular risk factors including dyslipidemia, hypertension, and dysfibrinolysis, the increased risk for macrovascular disease cannot be fully explained by the hypertension and dyslipidemia. Baron et al. [55] have shown that insulin causes endothelial-derived nitric oxide-dependent vasodilatation and that inhibition of nitric oxide production with L-NMMA causes complete abrogation of the insulin-induced vasodilatation. Interestingly, the same authors [55] have also shown that insulin is unable to modulate endothelial-dependent vasodilatation in obese insulin-resistant subjects or in type 2 diabetic subjects. Therefore, although insulin levels are markedly elevated in these subjects, insulin action is reduced, nitric-oxide-dependent vasodilatation usually induced by insulin is impaired, and endothelial dysfunction occurs. A possible explanation is insulin's capacity to paradoxically activate endothelin secretion. It has been shown that administration of insulin bolus on subjects with metabolic syndrome results in higher plasma endothelin 1 levels likely due to increased endothelin secretion [56]. Endothelin once released activates two G-protein-coupled receptors (GPCRs), ETA and ETB, on vascular SMC [57].

In conclusion, insulin-resistant states of obesity, hypertension, and NIDDM exhibit blunted insulin-mediated vasodilatation and impaired endothelium-dependent vasodilatation, regardless of high levels of endogenous insulin. Endothelial dysfunction is, therefore, an integral part of diabetes and of the syndrome of insulin resistance, independently of the absolute levels of glucose or insulin and strongly contributes to worsen insulin resistance and promote macrovascular disease.

NO may also influence lipoprotein oxidation. If cells are stimulated to express active NO synthase, their oxidative capability is lost [58]. On the other hand, if conditions in the vessel wall favor the release of superoxide, NO can be converted into peroxynitrate, which is a powerful oxidant [59]. A study measuring anti-oxidant status, lipid peroxidation, and nitric oxide end products in a group of Asian-Indian patients with type 2 diabetes with and without nephropathy [60] confirmed that oxidative stress was increased and the anti-oxidant defenses compromised in these patients and that these derangements were more severe in patients with diabetic nephropathy. Finally, abnormalities in the kallikrein/kinin pathway and dysregulation of microRNAs in the endothelium and in other cells associated with the atherosclerotic process may also affect NO. Many of these factors may have dual actions in different conditions.

Prostacyclin

As nitric oxide, prostacyclin (PGI_2) is synthesized mainly by vascular endothelial cells and smooth muscle cells, and it is a potent vasodilator and an inhibitor of platelet adhesion and aggregation [61]. Several studies [62, 63] have shown that the synthesis of PGI_2 by the vasculature of diabetic patients and by the coronary SMC in diabetic and atherosclerotic patients is reduced. Expression of prostacyclin is significantly lower in the presence of high glucose levels [64].

Formation of prostacyclin by a non-disturbed endothelium is mainly mediated by COX-1 and prostacyclin synthase that are constitutively expressed in endothelial cells. COX-2, which is inducible in endothelial cells only takes over as a major source of prostacyclin when severe systemic inflammation is present. The actions of PGI_2 are mediated primarily by two receptors, the cell surface GPCR IP receptor and the cytosolic nuclear receptor PPAR beta [65, 66], but knowledge concerning all the responses elicited by the engagement of these receptors by prostacyclin and the signaling pathways they activate is far from being completely understood [66].

The reason why inhibition of COX-2 derived prostacyclin elicited by the use of non-steroidal anti-inflammatory medications, resulted in hypertension, atherosclerosis and thrombosis was difficult to explain until recently when a link between inhibition of COX-2 and endothelial NOS was described [67, 68]. These investigators showed that when COX-2 was blocked by NSAIDs, the inhibition of the methylarginine pathway was equally removed resulting in increased levels of an endogenous eNOS inhibitor, asymmetric dimethylarginine and, therefore, both nitric oxide and COX-2-induced prostacyclin were reduced. This side effect of

NSAIDs should likely be prevented by the addition of L-arginine, a nutritional supplement.

The synthesis and release of both NO and prostacyclin by a non-injured endothelium can be mediated by bradykinin leading to relaxation of VSMC and arterioles [69]. In contrast, when the integrity of the endothelium is compromised, bradykinin will act directly in vascular smooth cells promoting vasoconstriction [70] and fibrosis via activation of prostaglandin F2. Bradykinin is generated by kallikreins from their precursor kininogens, and it is a potent vasodilator that increases vascular permeability and plays a primary role in inflammation. The direct action of bradykinin in vascular smooth muscle cells is mediated by its binding to its β2 receptors and subsequent activation and nuclear translocation of p42 and p44 MAPK leading to the generation of reactive oxygen species [71, 72]. Activation of the MAPK pathway leads to an increase in extracellular matrix proteins, such as collagen I and fibronectin [72, 73]. Douillet et al. [73] have shown that combined activation of TGF-β and MAPK mediates the increased production of collagen and TIMP-1 that contributes to the extracellular matrix accumulation induced by bradykinin.

Abnormalities in the kallikrein/kinin pathway have been found in the DCCT/EDIC cohort of type 1 diabetes [74] and increased expression of B2-kinin receptors has been described in the vessel wall of diabetic animals [75]. Interestingly, hyperglycemia, which is known to induce endothelial dysfunction due to its ability to promote endothelial cell toxicity, has also been shown to up-regulate the expression of kinin receptors in vascular smooth muscle cells [76]. Thus, in diabetes, the abnormalities in the kallikrein/kinin system, by modulating vascular fibrosis, play an important role in the development of atherosclerosis.

Endothelin 1

The endothelium secrets not only vasorelaxing agents, but also vasoconstricting agents, and normal endothelial function reflects a balance between the vasorelaxing agents previously described (NO and Prostacyclin) and vasoconstrictor agents such as endothelin 1 and thromboxane A2. Endothelin-1 was first described in 1988, and since then, a considerable amount of research has been performed to study this potent vasoconstrictor. Early studies suggested that endothelin-1 concentrations in the plasma of type 1 and type 2 diabetic patients were significantly elevated (approximately 3.5-fold) compared to levels in non-diabetic patients [77]. Both insulin and glucose have been shown to stimulate the release of endothelin by cultured cells including human endothelial cells [78] porcine and bovine aortic endothelial cells [79], and vascular smooth muscle cells [80]. The insulin receptor, besides activating NO production in the endothelium via the PI3K/AKT pathway, can also activate the phosphorylation of the Src-homology 2 domain containing (SHC) transforming protein leading to activation of the MAPK pathway and increased endothelin expression in the endothelium [81]. The endothelin receptors are G protein-coupled receptors. ET_A and ET_{B2} mediate vasoconstriction and ET_{B1} mediates vasodilation.

When endothelin binds to ET_{B1}, release of NO and resulting vasodilation takes place suggesting that this type of receptor is vasculoprotective [82, 83]. Therefore, the deleterious or protective effect of endothelin 1 depends on the type of receptor engaged.

Endothelin signaling is associated with the development of atherosclerosis via stimulation of growth factor expression that leads to VSMC growth, migration, and matrix remodeling [84, 85]. Endothelin has been found to be up-regulated in atherosclerotic lesions both in experimental animal models and in humans [86, 87].

The role of endothelin on the vasoconstriction of the coronary circulation and on heart function in a model of type 1 diabetes is exaggerated likely due to alterations in the voltage-gated calcium channels [88, 89] and can be normalized by treatment with bosentan, a dual-receptor (ET_A and ET_B) antagonist but with higher affinity to ET_A [90]. Studies in animal models of insulin resistance like the Zucker rats show a decrease in vasoconstriction mainly due to the fact that ET_B is stimulated and that leads to NO generation and uncoupling of calcium signaling [91].

Despite the rapid accumulating evidence showing that endothelin contributes to the development of various cardiovascular disorders and related complications, clinical trials with endothelin receptor antagonists in cardiovascular diseases have been rather disappointing [92] due to side effects and that has prevented further development. Part of these negative results may stem from the complexity of ET receptor expression and interaction in various tissues under physiological and pathological conditions. Interestingly, it has been shown that statin treatment can inhibit ET upregulation and signaling [93]. Better understanding of the interactions between endothelin receptors and other therapeutic targets is, therefore, essential to open possible alternative therapeutic strategies.

Thromboxane

Thromboxane A_2 is one of the best studied vasoconstrictors, and it is the physiologic counteracting mediator for NO. Thromboxane A2 is an eicosanoid and one of the metabolites of arachidonic acid generated by the action of three enzymes—phospholipase A2, COX-1/COX-2, and TxA2 Synthase (TXAS). Thromboxane A_2 is released by platelets, macrophages, neutrophils, and endothelial cells during times of cell injury and inflammation. Thus, it is obvious that the increased activation of platelets in diabetics [94] contributes to the formation of thromboxane A_2 (TxA_2) as well as prostaglandin H_2 in this disease state [95]. Other factors that may equally contribute to the increased platelet biosynthesis of thromboxane A_2 include cigarette smoking [96], hypercholesterolemia [97], and homozygous homocystinuria [98]. The rate of thromboxane (Tx) A_2 biosynthesis appears to reflect the influence of coexisting disorders like diabetes, hypertension, and dyslipidemia on platelet biochemistry and function [99]. TxA_2 and other isoprostanes are known to initiate and contribute to the progression of atherosclerosis by the regulation of platelet aggregation and leukocyte-endothelium interactions [100]. Interestingly, high levels

of TxA_2 seem to be associated with hypersensitivity of coronary arteries to ergonovine maleate in patients with variant angina, suggesting that TxA_2 is associated with increased vascular spasticity [101]. Enhanced TxA_2 biosynthesis may represent a common link between cardiovascular risk factors, atherosclerosis, and the thrombotic complication associated with macrovascular disease in diabetes. Irreversible inhibition of COX-1-derived TxA_2 with low-dose aspirin is prophylactic against both primary and secondary vascular thrombotic events, underscoring the central role of TxA_2 as a platelet agonist. COX-1 inhibitors have adverse effects such as GI toxicity and bleeding [102], and they may cause cardiotoxicity.

Inflammation

The inflammatory process associated with atherosclerosis is quite complex and multidimensional since it involves multiple cells that play a role in the development of atherosclerosis. The joint efforts of activated endothelial cells, smooth muscle cells, monocytes and monocyte-derived macrophages, as well as various types of T-lymphocytes result in a complex milieu of reactive oxygen species, modified lipoproteins, cytokines and chemokines, adhesion molecules, and growth factors which leads to a sustained chronic inflammatory process within the vessel wall. This chronic inflammatory process is one of the main contributors to progression of atherosclerosis and to the final CVD event: plaque rupture and thrombosis.

One of the first links between the endothelium and inflammation was the discovery of the vascular cell adhesion molecule-1 (VCAM-1) that was found to selectively promote the adhesion of leukocytes and lymphocytes via the expression of its counter-receptor very late antigen-4 (VLA-4), which is known to precede the recruitment of monocytes to nascent lesions [103].

Oxidative Stress

It has been clearly shown that hyperglycemia and hyperlipidemia in diabetes directly lead to overproduction of reactive oxygen species (ROS) in endothelial cells via mitochondrial respiratory chain enzymes, xanthine oxidases, lipoxygenases, cyclooxygenases, nitric oxide synthases, and peroxidases [104–107]. Hyperglycemia, besides leading to increased ROS production, will also induce increased formation of advanced glycation end-products (AGEs) and methylglyoxal (MGO) which result in an increased oxidative stress and quite likely cellular death. All that leads to the development of diabetic complications and specifically to the accelerated development of atherosclerosis in diabetes. Among the glucose-induced pathways leading to the formation of ROS, the lipoxygenase pathway should be mentioned since high glucose induces the expression of the 12/15 lipoxygenase (12/15LO), and the analysis of the lipid oxidation products in human arteriosclerotic lesions

clearly shows that oxidation of polyunsaturated fatty acids therein was mainly mediated by lipoxygenases [108]. Also, several studies have shown that the 12/15LO pathway is also able to mediate oxidative modification of LDL [109, 110] as well as to mediate the increase of hydroxy-eicosatetraenoic acid (12(S)HETE) in aortic endothelial cells [111] which has been found to stimulate monocyte adhesion to endothelial cells [112]. Therefore, it is quite interesting that increased glucose levels can cause LDL oxidation and that reactive carbonyl species, advanced glycation end-products (AGEs) and advanced lipoxidation end-products (ALEs) all can contribute to the modification of lipids in lipoproteins leading to the formation of modified lipoproteins [113] able to stimulate the immune system and induce the formation of antibodies and immune complexes as described later on.

The hyperglycemia- and hyperlipidemia-induced oxidative stress leads to endothelial dysfunction and that elicits increased expression of adhesion molecules in the endothelium, adhesion, and migration of monocytes to the subendothelial space where once activated secrete and release cytokines and chemokines like MCP-1 as well as procoagulant molecules such as tissue factor [114, 115]. All these processes have important pathophysiologic implications since they create a pro-inflammatory endothelial phenotype which is the keystone to understand the role that endothelial cells play in chronic inflammatory processes like atherosclerosis. In the lesion-prone regions of the vessel wall, all these pro-inflammatory agonists as well as the biomechanical stimulation by a disturbed blood flow leads to endothelial activation by stimulating several signaling pathways predominantly via the pleiotropic transcription factor, nuclear factor-kB (NF-kB) [57, 116] and results in a coordinated program of genetic regulation within the endothelium. ROS drive NF-kB mainly via oxidation of a cysteine residue, Cys-62, which is part of the NF-kB p-50 subunit [117]. Some studies, however, suggest that NF-kB can also be regulated by peroxynitrite [118]. As discussed later in this chapter, recent studies suggest that NF-kB activation may lead to changes in the chromatin structure of endothelial cells and confer an epigenetic level of regulation to the pro-inflammatory endothelial phenotype during atherogenesis.

The endothelium has a plethora of anti-oxidant defense mechanisms carried out by genes guided by nuclear factor erythroid-derived 2-like activation (Nrf2) as well as by heme-monoxygenase-1 (HMOX-1). Interestingly, it was recently described that enzymes known to protect against the damage of oxidative stress, such as catalase, superoxide dismutases, and glutathione peroxidases, are inactivated in diabetes [21], as demonstrated by several cellular and animal studies [22–24].

Adhesion Molecules

Endothelial cells elaborate leukocyte-specific adhesion molecules, both constitutively and in response to ROS, cytokines, and other mediators [119, 120]. Circulating monocytes display receptors for these cell adhesion molecules. Vascular cell adhesion molecule-1 (VCAM-1) [121, 122], intercellular adhesion molecule-1 (ICAM-1)

[122, 123], E-selectin [122], and platelet endothelial cell adhesion molecule (PECAM, CD31) [122] are expressed in atherosclerotic lesions. Soluble forms of these adhesion molecules are present in endothelial cell culture supernatants and human sera [124–126]. In diabetes, increased levels of soluble cell adhesion molecules were found in plasma of type 1 and type 2 diabetic patients who were reported as early as 1994 [127–129]. Recently increased levels of VCAM-1 and E-selectin were found not only in patients with type 2 diabetes but also in patients with impaired glucose tolerance [130]. The levels of these adhesion molecules were correlated with the levels of glucose and insulin obtained after a glucose tolerance test [130]. Similar results were obtained by another recent study by Matsumoto et al. [131] that shows that increased levels of VCAM-1 and E-selectin, but not ICAM-1, are significantly increased in a group of type 2 diabetic patients with macroangiopathy and that the increase persisted after adjustment for age, sex, duration of diabetes, blood pressure, HbA1c, HDL-cholesterol, and smoking status. In contrast, in a very small study of 28 diabetic patients without any complications at entry into the study and followed prospectively for 5 years, high baseline ICAM-1 levels were able to predict the development of macrovascular disease after adjusting for age, systolic blood pressure, creatinine, and glycemic control [132]. In addition, a positive correlation between plasma concentration of VCAM-1 and the thickness of the intimal plus medial layer of the carotid arteries was observed in type 2 diabetic patients [129], suggesting that circulating VCAM-1 levels may be a marker of atherosclerotic lesions in type 2 patients with symptomatic and asymptomatic atherosclerosis. Increased levels of P-selectin [133] were also found to be significantly correlated in a group of 517 subjects (187 with type 2 diabetes) with arterial stiffness and arterial wall thickness, and the later association was independent of other clinical factors.

Another important link between atherosclerosis and inflammation is the fact that activated endothelium and smooth muscle cells release chemokines such as monocyte-chemoattractant protein 1, granulocyte-monocyte-stimulating factor, IL1 and IL8 [116, 134] which further contribute to the progression of the disease, leading to further recruitment of T-lymphocytes and monocytes/macrophages [135]. The balance of pro- and anti-inflammatory mediators together with resolvins [136], which are agents that promote the resolution of inflammation, is responsible to lesion progression or regression.

Increased expression of adhesion molecules can also be induced in vitro using cultured endothelial cells by exposure to either modified lipoproteins (oxidized, glycated, and AGE-LDL) or cytokines [137, 138]. Some lipoproteins, like AGE-LDL, upon occupancy of macrophage receptors, induce the release of tumor necrosis factor, interleukin 1, and platelet-derived growth factor, and these mediators in turn promote the expression of adhesion molecules [137]. Infusion of AGE products in rabbits produced a variety of vascular changes. In endothelial cells, these included increased expression of VCAM-1 and ICAM-1 mainly in areas affected by atheroma [137]. Further supporting the significance of these interactions, it has been shown that blockade of RAGE can inhibit AGE-product-induced impairment of endothelial barrier function and consequent hyper-permeability. Inhibition of AGE-product formation using anti-oxidants has a similar effect. More recently, it has

been shown that lesions from human coronary arteries from patients with diabetes when compared to lesions from non-diabetic patients exhibit increased levels of an immunoreactive chemokine, fractalkine, which mediates firm adhesion of leukocytes [139].

Modified lipoproteins also have the potential to induce the release of cytokines by yet another mechanism. They are immunogenic and, therefore, elicit production of antibodies and, as a consequence, the formation of immune complexes. These immune complexes containing modified LDL are able to stimulate macrophages and release increased amounts of TNF and IL1β [138]. The release of these cytokines leads to increased expression of adhesion molecules [140, 141].

In type 1 diabetes, we examined the potential of adhesions molecules (VCAM-1, ICAM-1, and E-selectin) and other endothelial dysfunction factors like cytokines (IL1, TNF, and IL6) and clotting and fibrinolytic factors [fibrinogen and plasminogen activator inhibitor 1 (PAI-1)] to predict over the period of 16–19 years the development and progression of atherosclerosis, using sequential measurements of carotid intima medial thickness (IMT) and these biomarkers. Our results were not conclusive but strongly suggested that both mild and severe degrees of inflammation are associated with the development of atherosclerosis. It also suggests that measurements performed, time-wise, near IMT assessment reflect best the association between subclinical atherosclerosis and increased levels of these parameters and that early in the atherosclerotic process, these measurements may not have great clinical relevance [124].

Later in this chapter, the different modified lipoproteins as well as pro-inflammatory mediators released by activated macrophages as well as alterations in the clotting and fibrinolytic pathways will be described in more detail.

Influence of Altered Hemodynamics on Endothelial Dysfunction

Endothelial cells are able to sense hemodynamic forces generated by blood flow, and mechano-activated transcription factors play a role in regulating endothelial functions associated with atheroprotective or atherogenic blood flow. Therefore, the expression of several endothelial genes important for thrombosis, growth regulation, and pro-inflammatory activation seems to be transcriptionally regulated by fluid mechanical stimuli. Shear stress response elements in the promoters of these genes are able to up or down-regulate gene transcription [142–144]. Some genes like COX-2, eNOS, and superoxide dismutase are up-regulated by laminar shear stress. One of the most hemodynamic-responsive transcription factors is the zinc finger transcription factor and Kruppel-like factor 2 (KLF2) [142]. Expression of KLF2s has been demonstrated in the endothelium of athero-resistant regions of human arteries using in situ hybridization [145]. The expression of KLF2 in the endothelium promotes an anti-inflammatory and anti-thrombotic endothelial phenotype, mainly due to its antagonism of the NF-kB pathway [146]. KLF2 is

up-regulated by pulsatile, unidirectional laminar flow and orchestrates a multifunctional atheroprotective endothelial phenotype. In contrast, oscillatory or disturbed flow results in enhanced expression of NF-kB resulting in a pro-inflammatory atherogenic phenotype [18]. KLF2 expression depends on the activation of mitogen-activated protein kinase/extracellular-regulated kinase [147–149]. Nuclear factor erythroid 2-related factor 2 (Nrf2), another flow-mediated transcription factor, is also activated by the atheroprotective flow via the phosphoinositol 3-kinase/AKT and extracellular-regulated protein kinase 5 (ERK5) and plays a role in the regulation of intracellular redox balance as well as in the resistance to extracellular oxidant stresses [150–152]. KLF2 and Nrf2 act independently in the activation of flow-mediated gene expression, but KLF2 is required to full activation of Nrf2 and is required for anti-oxidant vasoprotection [153, 154]. Together, these two factors account for approximately 70% of the atheroprotective flow-induced transcriptome [154] in the endothelium, and therefore, they are the main regulators of the vasoprotective endothelial phenotype. KLF2 also regulates the release of microRNAs via the shedding of endothelial microvesicles and promotes the release of NO and C-type natriuretic peptide shown to be deficient in dysfunctional endothelium. One of the pleiotropic effects of statins is the upregulation of KLF2 expression in endothelial cells when used at pharmacologic dosages [155, 156]. Mice genetically deficient in KLF2, when compared with wild-type mice, displayed increased atheroma formation [157].

The atheroprotective and atherogenic flow influences the endothelium not only by the effects at transcriptional level, described above, but also by two additional mechanisms; epigenetic modifications, and microRNAs.

Epigenetic Modifications and Micro RNAs

Recently, epigenetic factors have emerged as possible major contributors to the accelerated development of atherosclerosis in diabetes. Environment and lifestyle modifications closely influence changes in gene expression (on–off switch) by altering DNA methylations and histone modifications. These epigenetic modifications can be reversible and short lived, but they can sometimes persist even after the signal that induced them disappears. In addition, there is another way to process epigenetic modifications via a set of mobile small regulatory elements, the microRNAs (miRNAs), which are small endogenous non-coding RNA molecules that regulate post-transcriptional gene expression. MicroRNAs are able to silence gene expression via binding to complementary miRNA recognition elements (MREs) in the 3′ and 5′ unstranslated regions of their target mRNAs. Gene polymorphism, either in the miRNA target site or in the miRNA itself, can disrupt binding or contribute to disease development. Atheroprotective and atherogenic flow influences endothelial gene expression via miRNAs and epigenetic modifications [158, 159]. Multiple miRNAs have been identified, which regulate the several steps of atherosclerosis development in diabetes; we will discuss a few of them with the understanding that

the field is still in flux and many of the effects observed can be dependent on the tissue and conditions/stimuli present; and potential clinical translation still needs to be verified and confirmed. To better assess the role of miRNAs in the development of atherosclerosis in diabetes miRNAs that regulate cholesterol homeostasis, endothelial cell homeostasis and the inflammatory response need to be carefully studied.

Several miRNAs identified as being associated with atherosclerosis in "in vitro" studies have been found to be expressed in atherosclerotic plaques similarly with those found expressed in the experimental diabetic milieu. These include miR-10a, miR-21, miR126, miR145, miR46a/b, miRNA185, and miRNA-326 among others. Some of these miRNAs are associated with endothelial dysfunction; some are involved in more than one process such as endothelial cell dysfunction and inflammation; and others are involved in cell proliferation and apoptosis. We will mention a few that seem promising. Some are highly expressed in the atheroprotective regions of the endothelium such as mi10a RNA [160] whose main action is to down-regulate the NF-KB pro-inflammatory pathway. miR19-a [161], miR-23b [162], and miR101 [163] in cultured endothelial cells are also up-regulated by atheroprotective blood flow, leading to the suppression of endothelial cell proliferation. In contrast, expression of miR92a [164] and miR34a [165], for instance, is downregulated by atheroprotective flow and up-regulated by atherogenic flow in cultured endothelial cells. Suppression of miR92a expression results in KLF2 and KLF4 upregulation and some of their downstream transcriptional targets in vitro and in vivo [164, 166]. Studies in LDL-receptor-deficient mice showed that inhibition of miRNA-92a limits the development of atherosclerosis in this animal model at least in part by increasing the expression of KLF2 and KLF4 [167]. Downregulation of miR34a leads to downregulation of NFκB signaling [165] therefore contributing to the atheroprotective flow-mediated suppression of endothelial inflammation. The atheroprotective flow also induces the secretion of the miR143–145 cluster via a KLF2-dependent pathway. These secreted miRNAs act on vascular smooth muscle cells to regulate their turnover and phenotype and reduce atherosclerotic lesion size in ApoE-deficient mice [168].

From the miRNAs found in atherosclerotic regions, two emerged as being markedly important. One miR-126 is considered necessary for the maintenance of vascular structure in vivo [169]. It is highly expressed in endothelial cells regulating endothelial cell migration, cytoskeleton reorganization, capillary network stability, cell survival, and apoptosis [170]. Interestingly loss of miR-126 is associated with diabetes, and it is reduced by a glucose-dependent mechanism. Low-plasma miR-126 caused VEGF resistance and endothelial dysfunction, and it is related with diabetic complications [171]. Similar to miRNA-126, miR-21 and miR-146a-5p seem also influenced by hyperglycemia. In a recent study, the presence of high glucose and a disturbed blood flow in HUVEC cells led to upregulation of miR-21 which resulted on overproduction of ROS and a defective anti-oxidant response due to downregulation of SOD2. These effects were reversed by a miR-21 inhibitor [172]. MicroRNA-146a has been identified as a negative regulator of NF-kB [173]. Thus, when this microRNA is decreased by hyperglycemia, the pro-inflammatory state is considerably enhanced [173]. Also downregulation of miR-146a by hyperglycemia in aortic EC led to upregulation of NADPH oxidase 4 and therefore to an increase in the generation of ROS [174].

Finally, recent studies show that levels of the let-7 miRNA family are decreased in diabetes [175], but they can be restored to normal levels after therapies used to lower cholesterol like statins and to lower glucose levels like metformin, or DPP4 inhibitors.

In conclusion, microRNAs regulate many cellular processes and more than 2000 miRNAs have been discovered so far. If the role of polymorphisms in disease is properly validated, then the future of pharmacogenomics will certainly have a bright future and may be extremely useful.

Mechanisms of Foam Cell Formation

Foam cells are the hallmark of the arteriosclerotic process. Diabetes appears to enhance foam cell formation in experimental animals and in humans. In animal models, type 1 diabetes induced by autoimmune-mediated beta cell destruction or by toxins (alloxan or streptozotocin) increases fatty streak formation [176–178]. Similarly, in human postmortem studies, it has been shown that diabetes accelerates the formation of fatty streaks. A study in 3000 youths, ages 15–34 years of age, who died of trauma were included on the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. That study showed that youths over 25 years of age with elevated levels of glycated hemoglobin (>8%) had significantly more fatty streaks in the right coronary artery than controls even when their lipid profiles were normal [179, 180]. A recent high-resolution ultrasound in vivo study of common carotid arteries of 11-year-old children with type 1 diabetes showed that these children had an increased intima-media thickness compared to a matched control group [181]. The increased intima-media thickness in children with type 1 diabetes did not appear to be due to conventional risk factors, such as increased blood pressure, increased total and LDL cholesterol, and triglycerides or low HDL levels [181]. Thus, these results indicate that diabetes, in the absence of conventional risk factors, accelerates arteriosclerosis in humans. The mechanisms behind such increase are not known, but they may be related with the presence in diabetes of increased levels of modified lipoproteins or of lipoproteins of abnormal composition since the best-known mechanism leading to the transformation of macrophages into foam cells is the uptake of lipoproteins of abnormal composition by macrophages [7]. Another mechanism, even more efficient in inducing foam cell formation, is the uptake of oxidized LDL immune complexes by Fcγ Receptor I [182, 183].

Quantitative/Qualitative Abnormalities of Lipoproteins

In poorly controlled diabetic patients, plasma LDL, intermediate density lipoproteins (IDLs), and VLDL levels are elevated [184–186]. The increase in VLDL levels has been attributed to increased hepatic production or decreased clearance of VLDL [187] and may be very significant in the development of arteriosclerosis in diabetes

and in women [188]. High-density lipoprotein (HDL) levels in diabetes vary with the type of diabetes and with glycemic control. In type 2 diabetic patients, HDL levels are usually low and do not always increase with improved metabolic control [185, 189]. In type 1 diabetic patients, HDL-cholesterol levels are low during poor glycemic control and increase to normal or above normal when adequate control is attained [186, 190]. Changes with improved glycemic control are less marked in women than in men [186]. In type 1 black diabetic women, little association is observed between plasma lipid levels and glycemic control [190].

Quantitative abnormalities, which can be located either in the protein or in the lipid moiety of the lipoproteins can lead to intracellular accumulation of cholesterol in the vessel wall. In diabetic patients with a decreased ratio of apoC/apoE [191, 192], although enhanced hepatic clearance of remnants was observed, there was also increased uptake of remnants by macrophages that lead to CE accumulation. We have also demonstrated, several years ago that triglyceride-enriched LDL isolated from IDDM patients in poor metabolic control was taken up and degraded less efficiently than normal LDL by human fibroblasts [193]. Hiramatsu et al. [194] confirmed our studies and demonstrated that triglyceride-enriched LDL isolated from both diabetic and non-diabetic subjects with hypertriglyceridemia was poorly recognized by fibroblasts. Bagdade et al. [195] demonstrated altered-free cholesterol/lecithin ratios in LDL and VLDL fractions in males with type 1 diabetes, resulting in altered lipoprotein metabolism. Similar observations were made in type 2 diabetic patients, and, like in type 1 diabetic patients, the abnormalities did not respond to improved glycemic control [196]. Finally, high levels of small, dense LDL, and increased levels of apolipoprotein B have been described in patients with type 1 and type 2 diabetes, when in poor metabolic control [197, 198] contributing to the enhanced atherogenicity of diabetic plasma.

HDL composition can also be markedly affected by diabetes, and this may impair reverse cholesterol transport [199]. Fielding et al. [200, 201] observed that cholesterol efflux from normal fibroblasts was inhibited when the cells were incubated with plasma from poorly controlled type 2 diabetic patients compared with normal plasma. An increase in the triglyceride content of HDL has also been noted in type 2 diabetic patients with hypertriglyceridemia and low levels of HDL-cholesterol [196, 202, 203] and cannot be fully corrected by improved glycemic control [196]. As with VLDL and LDL, the composition of surface lipids in HDL is abnormal in diabetes and, at least in HDL_3, remains so despite improvements in glycemic control [196]. Alterations in the apoprotein content of HDL in diabetes have also been described [204], diminishing the anti-atherogenic potential of the particle.

Lipoprotein Modification

In diabetes, increased non-enzymatic glycosylation affects any protein exposed to elevated levels of glucose. Glucose is covalently bound, mainly to lysine residues in protein molecules forming fructose lysine. Subsequently, further reactions occur,

mainly in long-lived proteins, leading to the development of unreactive end-products, many of which are cross linked, brown, or fluorescent [205]. The most common description for these end-products is advanced glycation end-products (AGEs). The formation of these end-products and the accompanying increase in protein fluorescence are mediated by free radical oxidation [206]. Thus, since glycation and oxidation are involved, the products are also called "glycoxidation products." It has been recognized that some of the advanced glycation end-products are derived from oxidation of lipids [207]. Oxidation of unsaturated fatty acid side chains yields reactive carbonyl-containing fragments (glyoxal, 4-HNE, MDA), which in turn may react with aminogroups, mainly lysine residues [208]. Some of the lipoxidation products are similar to glycoxidation products [207].

It has been postulated that enhanced glycation, oxidation, and glycoxidation of lipoproteins may underlie the development of macrovascular disease in diabetes. This is quite an attractive hypothesis since it would explain the individual variation in the development of complications in diabetes. Regardless of the similarity in glycemic control and cardiovascular risk factors, the development of complications would depend upon differences in oxidative stress and variations in the anti-oxidant defenses as well as in differences in the immune response to the modified lipoproteins. A short summary of the large body of evidence showing that modified lipoproteins may be relevant to the accelerated development of atherosclerosis in diabetes is presented below.

Lipoprotein Glycation

Schleicher et al. [209] were the first investigators to demonstrate that human lipoproteins (LDL and HDL) undergo increased glycation when exposed to elevated glucose concentrations and to postulate that increased glycation of lipoproteins in vivo might have significant metabolic consequences. Their initial studies showed that the extent of incorporation of glucose into HDL and LDL apolipoproteins (apo A-I, A-II, B, C, and E) was directly proportional to the time of incubation and to the concentration of glucose. Subsequent studies by our group built upon these observations. We demonstrated that the extent of glycation of LDL correlates well with other short- and medium-term indicators of glycemic control (mean plasma glucose, plasma protein glycation, and HbA1c) and that increased LDL glycation is present even in normolipidemic diabetic patients in satisfactory glycemic control [210].

Studies performed by a number of investigators who have prospectively treated IDDM patients with intensive insulin regimens to achieve euglycemia have reported decreases in LDL levels of 5–27% [211, 212]. That decrease in LDL levels may be related to the decrease in LDL glycation induced by the intensive insulin therapy and subsequent increase in LDL clearance. LDL clearance is mediated primarily by the LDL-receptor-mediated pathway. Studies [213, 214] aimed at investigating the metabolism of glycated LDL in cultured human fibroblasts have shown that in normal human fibroblasts, which possess the classical LDL receptor, there was impaired

binding and degradation of glycated compared to control LDL, the impairment being proportional to the extent of glycation. Modification of as few as 2–5% lysine residues of LDL led to a 5–25% decrease in LDL catabolism by human fibroblasts [214].

The above studies were confirmed by our laboratory, using LDL isolated from diabetic subjects and sex, age, and race-matched control subjects. We have shown that recognition by human fibroblasts of LDL isolated from diabetic patients is markedly impaired [193]. Interestingly, unlike fibroblasts, human monocyte-derived macrophages recognized LDL glycated in vitro to a greater extent than native LDL, fourfold over control LDL values [215]. A separate, low-affinity, high-capacity receptor pathway by which glycated LDL gains entry into the macrophage was identified. Other studies performed in our laboratory [216] further support the enhanced atherogenicity of glycated LDL in diabetes. In these studies, we isolated, using a boronate affinity chromatography column that binds fructose-lysine adducts, two fractions of LDL from type 1 diabetic patients and compared their metabolic behavior. The glycated LDL fraction was poorly taken up and degraded by fibroblasts. In human monocyte-macrophages, uptake of the bound (glycated) LDL was twofold greater than that of the non-bound (non-glycated) LDL fraction. The uptake, however, was not mediated by the LDL-receptor pathway, but by a high-capacity, low-affinity receptor pathway. From these studies, we concluded that "in vitro" glycated LDL and LDL from diabetic patients are poorly recognized by the classical LDL receptor, but they are preferentially recognized by a distinct receptor pathway present in human macrophages leading to increased intracellular accumulation of cholesteryl esters thereby contributing to the accelerated development of atherosclerosis in diabetes.

Glycated LDL also affects platelet aggregation. Compared to LDL from control subjects, LDL from type 1 diabetic patients is a more potent stimulator of thromboxane B2 release and thrombin-induced platelet aggregation [217].

Lipoprotein Oxidation and Glycoxidation

Glycation of LDL under hyperglycemic conditions is likely to result in increased formation of oxidized LDL [218]. Several mechanisms have been proposed to explain the increased oxidation of lipoproteins in diabetes. One of them involves the auto-oxidation of simple monosaccharides [219], such as glucose, and of fructose lysine [219, 220], the first Amadori rearrangement product, under physiologic conditions and in the presence of trace amounts of metal ions. Auto-oxidation of these compounds generates superoxide radicals, and lipid peroxidation occurs. Another mechanism possibly responsible for increased oxidation of LDL in diabetes is the impaired clearance of glycated LDL that leads to an increase in the lipoprotein circulation time and facilitates its exposure to oxidative stress. In damaged vessel walls, trapping of LDL due to covalent glucose-derived cross-linking of LDL to glycated structural proteins may be yet another mechanism contributing to increased LDL oxidation in diabetes.

Several studies support the above mechanisms. Brownlee et al. [221] have reported an increase in LDL-collagen cross-linking when the lipoprotein is exposed to modified collagen (containing browning products), compared to control collagen. Glycated LDL is more susceptible to oxidation than non-glycated LDL and increased oxidative modification of LDL occur in the presence of high glucose levels [220]. Tsai et al. [222] showed that in poorly controlled insulin-dependent diabetic patients without macrovascular disease, the lag phase of conjugated diene formation after initiation of LDL oxidation by the addition of copper was shorter than in normal control subjects. That increase in susceptibility to oxidation was not associated with an increase of small dense LDL in the diabetic population, but with a decrease in the total peroxyl radical trapping potential of plasma (TRAP) which was significantly decreased in the IDDM patients. Our laboratory showed that, in contrast to what happens in poorly controlled diabetics, in IDDM without preexistent complications and with normal lipid levels and good glycemic control, the susceptibility of LDL to oxidation is not enhanced [223].

That oxidized or glycoxidized LDL indeed plays an important role in the pathogenesis of atherosclerosis that has been confirmed by the presence of oxidized lipoproteins in the vessel wall [224, 225] and regression of lesions in animals treated with anti-oxidants [226]. Recently, AGE epitopes have also been described in atherosclerotic lesions of euglycemic rabbits [227]. Interestingly, the AGE epitopes were found in similar locations as the epitopes generated during the modification of lipoproteins by oxidation. Other studies show that blockade of the receptor for advanced glycation end-products (RAGE) results in decreased inflammation in pre-formed lesions in streptozotocin-induced diabetic apoE-deficient mice [228]. Because RAGE binds to a number of interesting ligands in addition to AGEs, it is not known if the effects of RAGE blockade in this study were due to preventing the uptake of AGEs. In fact, RAGE blockade reduced atherosclerotic lesion size [228] and intimal thickening after arterial injury [229] in non-diabetic mice, suggesting that the role for RAGE ligands is not dependent on the diabetic state.

Several clinical studies further strengthened the morphologic findings described above. Regnstrom et al. [230] have shown that the degree of susceptibility to oxidation of LDL isolated from 35 male survivors of myocardial infarction was positively correlated with the severity of coronary atherosclerosis. Several other investigators described increased susceptibility to oxidation of LDL in patients with coronary heart disease [231], as well as patients with carotid or femoral atherosclerosis [232].

In contrast with the pletora of information that exists concerning LDL oxidation, very little is known concerning oxidation of other lipoproteins. Oxidation of VLDL has been shown to be cytotoxic [233], but very little is known concerning possible metabolic alterations that may result from the oxidation of this lipoprotein. Oxidative modification of HDL in vitro has been shown to impair the ability of HDL to stimulate cholesterol efflux from foam cells [234]. Recently, Bowry et al. [235] reported that HDL is the major carrier of oxidized lipids in plasma and may be responsible for the hepatic clearance of oxidized lipids from plasma.

Phospholipid Oxidation

The carbonyl-containing intermediates resulting from oxidative stress-induced damage of carbohydrates, may not only modify proteins but also phospholipids [236] and nucleotides [237]. Oxidized phospholipids (OxPL) can be detected in inflammatory tissues, including atherosclerotic plaques, and have been found to promote inflammation as well as the development and progression of atherosclerosis [238]. In humans, OxPL circulate in association with ApoB-100 particles, such as LDL and LP(a) protein, and the levels of OxPL/ApoB are predictors of future cardiovascular events [239, 240]. Oxidized LDL contains oxidized phospholipids that can mediate the uptake of oxLDL by scavenger receptors [239] but are also likely to be taken up by oxLDL-IC opsonized after interaction with Fc receptors. The observed differences when macrophages are incubated with copper-oxidized oxidized LDL vs. highly oxidized MDA-LDL [241] could be a consequence of differences in the content of oxidized phospholipids in those two forms of oxidized LDL.

Additionally, recent studies carried out in animal models show that the uptake of oxidized and other forms of modified LDL is regulated by the CD36 scavenger receptor, and the interaction of CD36 with oxLDL has been proposed as a key factor determining macrophage retention in plaques, therefore, creating conditions favorable of chronic inflammation [242]. This has led to studies in animal models that suggest that compounds able to down-regulate the expression of CD36 may have a protective effect by reducing the uptake of modified LDL and the resulting foam cell formation [242, 243].

Modified LDL Antibodies and LDL-Containing Immunocomplexes

In addition to the interactions described above, modification of proteins, such as oxidation and AGE modification, may alter their structure sufficiently to render them immunogenic. The levels of oxLDL antibodies have been repeatedly reported to correlate with different end-points considered as evidence of atherosclerotic vascular disease, progression of carotid atherosclerosis, or risk for the future development of myocardial infarction [244–248]. Salonen et al. [245] reported a direct relationship between the titer of autoantibodies to MDA-LDL and the rate of progression of carotid atherosclerosis. Lehtimaki et al. reported higher levels of oxidized LDL antibodies in patients with angiographically verified coronary disease [246]. According to Erkkilä and co-workers, oxLDL antibody levels were significantly elevated in men with myocardial infarction [247]. In type 2 diabetic patients, Bellomo et al. found higher levels of oxLDL and MDA-LDL antibodies compared to healthy controls [248]. It must be noted that several studies have yielded contradictory data, showing either no correlation between modified LDL antibodies and end-points of atherosclerotic disease, or even showing inverse correlations [249–254].

Initially, in animal models, IgM antibodies to modified LDL seem to predominate over IgG antibodies [255, 256] and have a protective effect in relation to the development of atherosclerosis. The possibility of "vaccination" was even considered [257]. This concept was completely abandoned by data showing that the proposed protective murine IgM antibodies are predominantly reactive with oxidized phospholipids [256] but mainly because human antibodies were extensively characterized and when the isotype distribution of modified LDL antibodies was studied under stringent conditions, using affinity chromatography-purified antibodies, the predominant isotypes were IgG1 and IgG3, distantly followed by IgM [258, 259]. The balance between IgG and IgM LDL antibodies may have, however, some pathogenic relevance, as suggested by reports showing that common carotid and femoral intima-media thickness are directly related to the levels of IgG oxLDL antibodies and inversely related to the levels of IgM oxLDL antibodies [260].

A clearer perspective about the pathogenic role of modified LDL antibodies seems to emerge when the levels of circulating antigen-antibody complexes (immune complexes, IC) containing modified forms of LDL (LDL-IC) are measured [8, 253, 261–263]. LDL-IC have been reported to be increased in patients with coronary artery disease [253] and in diabetic patients with nephropathy [263]. The composition of IC isolated from the sera of diabetic patients by precipitation with polyethlylene-glycol (PEG) has demonstrated a significant enrichment in carboxymethyl lysine (CML) and MDA-lysine [259], suggesting that oxLDL and AGE-LDL are involved in IC formation. This is supported by the detection in the IC of significantly elevated concentrations of oxLDL and AGE-LDL IgG antibodies of higher affinity than those that remain free in the supernatant [8, 259, 263].

The advantages and disadvantages of the measurement of LDL-IC, their proinflammatory potential, and their role in the development and progression of atherosclerosis have been summarized in previous publications [264, 265]. The transformation of human monocyte-derived macrophages into foam cells can be induced either by insoluble LDL–IC or as LDL–IC adsorbed to red blood cells (RBC). Both types of LDL–IC may be formed in vivo. Subendothelial LDL deposits are likely to include LDL-IC formed in situ, and these are probably large insoluble aggregates. Soluble LDL-IC circulates in blood adsorbed to RBC via C3b receptors and other non-specific interactions. In vitro, both insoluble and soluble (RBC adsorbed) LDL–IC prepared with rabbit apoB antibodies induce profound alterations in lipoprotein metabolism and in the cholesterol homeostasis of monocyte-derived macrophages [182, 266]. These observations were reproduced using LDL-IC prepared with human copper-oxidized LDL and purified human oxLDL antibodies [9]. The increased accumulation of CE in human macrophages exposed to LDL-IC is secondary to an increased uptake of the LDL complexed with antibody, followed by altered intracellular metabolism of the particle [183]. LDL-IC were taken up by macrophages as a consequence of their interaction with the FcγI receptor [267]. Surprisingly, while inducing foam cell formation, the LDL-IC also stimulates a considerable increase in LDL-receptor activity [266, 268]. The increase in LDL-receptor activity seems to be specifically induced by LDL-IC and not by other types of immune complexes [183, 268]. However, the most important data that

completely validate the pro-inflammatory role of LDL-IC were human studies performed by our group and others that clearly show that increased levels of oxLDL-IC, MDA-LDL-IC, and AGE-LDL-IC were associated in vivo with development and progression of atherosclerosis and CVD (stroke, MI and CVD death) in large cohorts of type 1 diabetes (DCCT/EDIC cohort) and type 2 diabetes (the VADT cohort) [269–273].

Sphingolipids

Sphingolipids are synthesized de novo in the endoplasmic reticulum (ER) and derive from catabolism of other sphingolipids via the salvage or hydrolytic pathways. Ceramide is the central piece of the sphingolipid metabolism and is the key precursor in the biosynthesis of different sphingolipids. Alterations in the distribution and concentration of sphingolipids together with traditional risk factors contribute to the pathogenesis of atherosclerosis and cardiovascular disease [274]. Abnormalities in sphingomyelin (SM), ceramide, and glycosphingolipids have been associated with increased atherosclerosis, and higher plasma levels of sphingomyelin have been proposed as independent risk factors for coronary heart disease in humans [275]. Recent studies not yet published (personal communication) strongly suggest, however that in type 2 diabetes, plasma levels of sphingolipids, including sphingomyelin, do not adequately translate the major deviations of diabetes-induced sphingolipids and that the content of sphingolipids in individual lipoproteins not in plasma are more indicative of disease and likely more representative of the development of complications.

Interestingly, the LDL present in atherosclerotic plaques has a higher content of SM compared to plasma LDL, mainly arising from de novo synthesis in the aorta [276]. SMases may hydrolyze LDL-sphingomyelin in the arterial wall increasing LDL-ceramide and resulting in aggregation of lipoproteins, which like LDL-IC, leads to initiation and progression of atherosclerosis [277].

When experimental myocardial infarction is induced in male Wistar rats [278], significant alterations in plasma, erythrocytes, and platelets sphingomyelin levels were observed. Also increased plasma sphingomyelin levels have been reported in ApoE mice compared to wild-type mice [279]. Overexpression of SMS2 in mice exaggerates the inflammatory process in atherosclerosis [280], whereas inhibition of sphingolipid synthesis by myriocin reduces atherosclerosis [281].

Like sphingomyelin, increased plasma and aortic ceramide levels are also associated with increased risk of cardiovascular disease [281]. The ratio of certain ceramide species may be predictive of cardiovascular death in coronary artery disease independent of lipid markers [282], perhaps because it promotes lipoprotein aggregation, inflammation and apoptosis leading to plaque instability. In vitro ischemia/reperfusion of rat hearts was associated with decreased sphingomyelin levels and significantly increased ceramide concentrations [283]. Increased ceramide concentrations may induce apoptosis in cardiomyocytes and, therefore, contribute to

increased cardiomyopathy, mortality, and morbidity in diabetic patients [274]. It has been shown that ceramide-induced apoptosis of cardiomyocytes may result from TNF-α-induced synthesis of ceramide [284].

Plasma glycosphingolipid concentrations are also elevated in patients at increased risk of atherosclerosis [285] and accumulate in atherosclerotic lesions in human and ApoE knockout mice [286, 287]. However, inhibition of glycosphingolipid synthesis has no effect in decreasing atherosclerosis [288, 289]. Recent studies in diabetic nephropathy which is closely associated with the development of atherosclerosis in diabetes, demonstrated that lower plasma levels of very long-chain lactoceramides are predictive of diabetic nephropathy in type 1 diabetes [290]. It is, therefore, likely that the same can be observed in atherosclerosis. It remains to be determined whether elevated or decreased levels of glycosphingolipids are pro-atherogenic in humans.

In contrast to ceramide and sphingomyelin, plasma S1P [274] is believed to be cardioprotective. Plasma levels of S1P do significantly decrease after myocardial infarction [291] and increase in patients after percutaneous coronary intervention [292]. Low S1P levels are associated with impaired cell signaling and vasodilation, but these defects can be corrected by loading HDL with S1P [293] indicating that low S1P could be a contributing factor of HDL dysfunction in atherosclerosis.

Adiponectin, by stimulating its receptor's inherent ceramidase activity, leads to the formation of sphingosine which is then phosphorylated via sphingosine kinase to produce S1p [294]. Since adiponectin levels are low in diabetes that leads to an increase in tissue ceramides and a decrease in S1p levels due to the decreased ceramidase activity [284].

There is an overlap between inflammation and ceramide production converging on the TLR4 pathway [295]. A subset of fatty acids that induce ceramide synthesis [296] are similar to those that activate TLRs. Saturated fat induces ceramide production and an inflammatory response via a TLR4-dependent pathway [297]. Lipopolysaccharide (LPS), which activates TLR4, induces accumulation of ceramide in serum, liver, kidney, and spleen. The combination of LPS and palmitate synergistically activates ceramide production via TLR4-dependent and independent signaling pathways [298]. A TLR-4-mediated pathway to ceramide production is induced by activation of SMase [299].

Pathogenesis of Diabetic Vasculopathy

Macrophage Activation by Modified Lipoproteins and LDL-Immune Complexes

AGE-product/receptor interactions in macrophages may induce release of cytokines, TNF, and IL1 among others [300], and these cytokines may mediate growth and remodeling and accelerate the atherosclerotic process. AGE-products in vessel walls have been localized immunologically to intracellular locations in

macrophages, smooth muscle cells, and in foam cells. Vlassara et al. [301] identified initially a receptor for AGE-products on monocyte/macrophages. Schmidt et al. [302] cloned and characterize the RAGE receptor and have demonstrated its involvement in oxidative stress, endothelial dysfunction, inflammation, and development of atherosclerosis and diabetic complications in general [303–305] as well as the ability to minimize these effects once the RAGE receptor is blocked [306, 307]. Interestingly, the role of simvastatin in stabilizing atheroma plaques is mediated by the inhibition of RAGE expression [308]. Other modified lipoproteins, such as oxLDL, have also been described as stimulating the release of cytokines and inducing foam cell formation, but it only occurs after an epigenetic reprogramming of monocytes. Exposure of monocytes to oxLDL will induce reprogramming of monocytes leading to an enhanced response to TLR 2 and 4 as well as to upregulation of CD36 and SRA [309].

Also oxidized phospholipids generated during LDL oxidation may activate inflammatory cells through their interaction with TLR4 [310]. Furthermore, oxLDL can also activate Th1 cells leading to the release of interferon-gamma, which in turn activates macrophages, inducing release of chemokines that attract more T-cells to the area [311].

Release of cytokines by macrophages can also be induced by exposure of the cells to IC-containing modified forms of LDL. This results on upregulation of LDL and scavenger receptor expression, and leads to the release of IL1, TNF, IL6, IL12, ROS, as well as to complement activation [9, 312, 313]. Actually, in a large number of experiments carried out in our laboratory, incubation of human macrophages with LDL-IC in concentrations known to induce foam cell formation stimulated both cytokine release and the respiratory burst more efficiently than any other type of IC or oxLDL [313–315]. That can be explained by a different pattern of trafficking of oxLDL and oxLDL-IC in macrophages. OxLDL-IC are taken up by FcγR and oxLDL by scavenger receptors and while oxLDL-IC consistently activate acid sphingomyelinase (ASmase), oxLDL induces a rapid and transient increase in ASmase if the exposure is short. With chronic exposure of macrophages to oxLDL, the levels of ASmase are sustained instead of transient, but they are considerably lower than those induced by oxLDL-IC. Since the functionality of ASmase is critical for the macrophage inflammatory response to stimuli, it explains why the activation of macrophages by IC is frankly higher than that of oxLDL [315]. Immunecomplexes containing malondialdehyde (MDA) were the most effective in releasing cytokines, MCP-1, and metalloproteinases, and in inducing cell apoptosis [316].

Cytokines and chemokines together with ROS contribute to the increased expression of adhesion molecules in the endothelium and to the recruitment of monocytes into the subendothelial space. Once macrophages are activated either by stimulatory signals or by interacting with endothelial cells, they will cause or aggravate endothelial cell damage. OxLDL-IC priming of the Nlrp3 inflammasome, a critical component of the innate immune system that mediates both the secretion of pro-inflammatory cytokines and caspase 1 activation to promote apoptosis, involves the cooperation of TLR and FcγR [317]. Both blocking uptake of oxLDL-IC by

FcγRI using Fab(2) fragments or using TLR4 antagonists will attenuate vascular inflammation and atherogenesis [318, 319],

Activation of macrophages leads not only to the release of cytokines and other growth factors but also to the release of growth factors such as PDGF [320–322], transforming growth factor [323], and collagenases [324]. The pro-inflammatory factors released during macrophage activation contribute to the development of atherosclerosis by enhancing smooth muscle cell (SMC) proliferation and matrix production by SMC, by causing plaque destabilization by inducing endothelial cell procoagulant activity [325], releasing platelet activating factor [326], and by enhancing endocytosis, cholesterol synthesis, and LDL-receptor expression in monocytes/macrophages. Activated macrophages will overexpress CD40, an important modulator of the inflammatory response in the vessel wall, upon interaction with CD40 ligand. It is well known that in acute coronary syndromes, the levels of CD40 ligand are elevated, and increased levels of CD40 ligand were also found in a group of 39 patients with diabetes and angiographically documented CAD [327]. Treatment with rosiglitazone but not with placebo was able to significantly decrease the levels of CD40 ligand in the same patients [327].

In conclusion, macrophage activation is a key step in the pathway leading to endothelial dysfunction, inflammation, and atherosclerosis development and progression [264, 265]. Considering the importance of inflammation in atherosclerosis, many of the CVD treatments like statins have been evaluated for its positive effect on inflammation and this field in still being actively pursued [328].

Expression of Metalloproteinases Induced by Modified Lipoproteins and Modified LDL-Immune Complexes: Role in Plaque Rupture

Angiographic studies on patients with acute myocardial infarction led to the surprising finding that, frequently, the atherosclerotic lesion that gave rise to the occlusive thrombus did not have high-grade stenosis [329, 330]. These studies led to the concept that the composition of atherosclerotic plaques is more important than their size in triggering plaque rupture and acute vascular events.

The thickness and collagen content of the fibrous cap as well as the size of the lipid core are the most important elements in determining plaque vulnerability. Vulnerable plaques that are prone to rupture have a thin fibrous cap, due to a marked decrease in collagen content, and their lipid core usually occupies more than 40% of the plaque area. Thus, mechanisms that contribute to decrease the collagen content of plaques have been the focus of considerable attention in recent years. Collagens are synthesized and assembled by vascular smooth muscle cells and degraded by collagenases. Thus, both decreased production of collagen by smooth muscle cells, as well as enhanced degradation of collagen by collagenases, can contribute to plaque vulnerability [331]. It has been shown that the expression of collagens in smooth muscle cells is regulated by cytokines and growth factors [332].

Transforming growth factor-β (TGF-β) and PDGF stimulate the synthesis of collagen type I and III whereas IFN-γ markedly decreases collagen biosynthesis [332]. Studies examining the pathology of atherosclerotic lesions and studies with cell culture systems indicated that IFN-γ, which is released by activated T-cells, inhibits smooth muscle cell proliferation and collagen expression in smooth muscle cells [333]. IFN-γ also promotes apoptosis of smooth muscle cells [334]. Decreased synthesis of collagen is not, however, the only mechanism leading to the decreased collagen content in vulnerable atherosclerotic plaques. As mentioned before, increased degradation of collagen by collagenases is also an important factor. Most of the collagen (50–75%) in a normal artery is type I collagen [335]. Interstitial collagenase, or metalloproteinase (MMP-1), is an important proteinase specialized in the initial cleavage of collagens, mainly type I. Other metalloproteinases, such as MMP-2 and -9, catalyze further the breakdown of collagen fragments or activate MMP-3 and -10 and other members of MMP family, promoting the degradation of a broad spectrum of matrix constituents, such as proteoglycans and elastin. MMP activity is regulated not only by the production but also by tissue inhibitors of MMPs (TIMPs) [336]. MMP-1 has been found in vulnerable regions of atherosclerotic plaques, suggesting that this collagenase plays a role in plaque destabilization [337]. We have shown that oxidized LDL and oxLDL-IC stimulate the expression of MMP-1 in human vascular endothelial cells at transcriptional level. That increased expression is associated with a marked increase in collagenase activity [338–340]. Similar results were observed in human macrophages stimulated with oxLDL-IC for MMP-1 and 9 [316]. We have also demonstrated that marked glucose-induced upregulation of MMP-1 was observed in macrophages when IL6 was added to the experiment. This upregulation was mediated through activation of Erk1/2, JNK, and c-Jun [341]. Interestingly, when the experiments were performed using co-cultures of fibroblasts and monocytes, upregulation of MMP-1 was induced by the release of IL6 by fibroblasts. In conclusion, this study demonstrates that IL6 derived from fibroblasts is essential for MMP-1 upregulation by cross-talking between fibroblasts and U937 macrophages exposed to high glucose, revealing an IL6-dependent mechanism in MMP-1 upregulation [342].

A few studies have investigated MMP levels and activities in diabetes. Serum levels of MMP-2, MMP-8, and MMP-9 are increased in patients with type 2 diabetes [343]. In a group of 377 subjects with type 1 diabetes, plasma levels of MMP-2 were significantly associated with higher incidence of CVD events and high levels of MMP-1, MMP-2, and MMP-3 were significantly associated with all-cause mortality, during a 12-year follow-up. The associations were attenuated after adjustment by eGFR both for MMP-2 and CVD and MMP-3 and mortality [344].

Another possible mechanism of plaque rupture is increased cell death. Contrarily to what it was conventionally accepted it has been shown recently that “apoptotic” cells can release cytokines and that, following apoptosis, an inflammatory response in the arterial wall induced by the overexpression of Fas-associating death domain protein (FADD), one of the signaling molecules in the apoptotic pathway may occur [345]. Furthermore, apoptotic cells have a potent procoagulant activity due to the redistribution of phosphatidylserine on the cell surface during apoptosis, which

leads to increased tissue factor activity, a key element in the initiation of coagulation. During cell apoptosis, shedding to the lipid core of membrane apoptotic microparticles rich in PS, which carry almost all tissue factor activity, is responsible for the procoagulant activity of the plaque [346]. The increased expression of tissue factor is not limited however to the plaque but it is also found in circulating monocytes in patients with acute coronary syndromes [347]. Recently, a study was performed in which human monocyte-derived macrophages were incubated with cytokines and oxLDL to assess the production of these microparticles vesicles. Oxidized LDL through binding to the CD36 receptor was able to significantly increase the production of tissue factors expressing prothrombotic microparticles via a caspase 3/7-dependent manner [348]. The increase observed in tissue factor was 78%, and the production was inhibited by treatment of the cells with mevastatin. Whether or not diabetes enhances the expression of tissue factor in circulating monocytes or in plaques is not known.

Thrombus Formation

Thrombi may form in atherosclerotic vessels leading to tissue ischemia, tissue death or both. Formation of thrombi starts with adhesion of platelets to areas of endothelial damage and, as a consequence, to local accumulation of platelets at sites of vascular injury. Platelet aggregation follows platelet adhesion due to the release of intraplatelet materials that may affect not only the clotting/fibrinolytic system but also lead to the formation of microemboli. Thus, to understand the formation of thrombi, the final step of an acute vascular event, it is essential to understand the functional abnormalities of platelets.

Abnormalities in Platelet Function

Many alterations in platelet function are seen in diabetes mellitus. Several studies have shown that platelets from diabetic subjects are more sensitive to platelet aggregating agents and that synthesis of thromboxane B_2 is increased [349, 350]. These findings have been shown both in diabetic patients immediately after the onset of the disease as well as in patients with vascular disease, suggesting that platelet damage may occur as a result of diabetic vascular disease as well as possibly contributing to the development of the process.

A defense mechanism against thrombosis is mediated by natural anti-coagulants like protein C, which inhibits factor V and VIII and by the tissue factor pathway inhibitor that blocks the binding of partially activated factor VII to exposed tissue factor (TF), the complex that initiates coagulation. The next step is fibrinolysis and that depends on clot structure, which is altered in diabetes and on fibrinolytic factors, the tissue-plasminogen activator (tPA) and the plasminogen activator inhibitor 1 (PAI-1) which blocks the conversion of plasminogen into active plasmin [351].

Abnormalities in Coagulation

Activation of the coagulation system leads to formation of fibrin clots, which in turn may lead to vessel occlusion and an acute cardiovascular event, therefore, representing the final step in the atherosclerotic process. Most of the individual factors in both the intrinsic and the extrinsic coagulation pathways, as well as the inhibitors of coagulation, may be altered in diabetes. Both in type 1 and type 2 diabetes, there are an increase in procoagulant factors and decreased fibrinolysis activity. The mechanisms leading to these alterations in diabetes are mainly secondary to hyperglycemia and insulin resistance but other factors like increased release of ROS by cells involved in the atherosclerotic process, and increased levels of AGEs are also able to enhance coagulation by modulating tissue factor expression via activation of the NF-kB activation [352]. Furthermore, exposure of human macrophages and smooth muscle cells to oxidized LDL enhances their ability to support activity of two major complexes of the intrinsic pathway, Xase, a membrane bound complex formed by factor VIIIa and IXa, and prothrombinase (factor Xa and cofactor Va) leading to a 10- to 20-fold increase in thrombin formation [353]. The increase in the intrinsic procoagulant activity was related to formation of additional fVIII-binding sites due to increased translocation of phosphatidylserine to the outer membrane of oxLDL-treated cells and a fivefold higher affinity of interaction between components of the Xase complex [353]. Since oxLDL is present in high levels in diabetes, this may be also an important prothrombotic mechanism in this disease.

Interestingly, acute hypoglycemia may create also clotting problems by altering the structure of the fibrin clot, increasing factor VIII activity, accelerating thrombin generation, and inducing alterations in fibrinolytic activity [354, 355]. That likely explains some of the negative results of the ACCORD trial [356] concerning intensive insulin therapy. Due to the possible negative impact in clotting and fibrinolysis of some of the glucose and/or lipid-lowering medications, the study of drug effects on CVD events is now a requirement for diabetes-related drugs.

High circulating levels of tissue factor (TF) and Factor VII, the complex that initiates the thrombotic process, are increased in patients with type 2 diabetes, and they are directly modulated by glucose and insulin [357]. The increase in factor VII is related to hypertriglyceridemia, a common finding mainly in poorly controlled diabetes and lowering of triglycerides may attenuate the problem [358].

High levels of plasma fibrinogen levels have been considered as an independent risk factor for thrombotic events in population-based studies [359] and have been found to be markers of subclinical atherosclerosis and peripheral vascular disease in diabetes [360–362]. Besides glucose control, exercise may also affect plasma fibrinogen, and it has been shown that exercise conditioning will lower plasma fibrinogen levels in type 2 diabetes [363].

Thrombin generation is increased in type 1 and type 2 diabetes and high thrombin levels lead to denser, less permeable clots which are resistant to lysis [357, 364]. Hyperglycemia mediates high thrombin production and, therefore, adequate control of diabetes will lead to a reduction in thrombin levels [364, 365].

Because fibrinogen-to-fibrin formation is catalyzed by thrombin, investigations have centered on the regulation of thrombin activity in diabetes and on an in vivo index of thrombin activity, the fibrinopeptide A (FPA). FPA is cleaved from the alpha-chain of fibrinogen by the action of thrombin. This is the first step in the conversion of fibrinogen to fibrin. FPA levels tend to be elevated in diabetes, especially when control is poor or vascular problems exist [366].

Another factor contributing to activation of the coagulation system in diabetes is a decrease in antithrombin III (ATIII). ATIII is the most important inhibitor of the coagulation system, and its activity may be modulated by glucose both in vitro and in vivo.

Ceriello et al. [367] described an inverse correlation between antithrombin III activity and both HbA1c and plasma glucose, independent of plasma concentrations of antithrombin III. Brownlee et al. [368] have shown that increased glycation of antithrombin III impairs its thrombin-inhibiting activity and could contribute to the accumulation of fibrin in diabetic tissues.

An additional potent inhibitor of coagulation that is altered in diabetes is protein C. Activated protein C is a vitamin K-dependent plasma protein that acts at the level of factor V and VIII in the intrinsic coagulation scheme. Several investigators have reported decreased levels of protein C antigen and activity levels in type 1 diabetes [367, 369], and a return to normal with treatment [367].

Abnormalities in the Fibrinolytic System

The fibrinolytic system is a critical regulator of thrombosis. Theoretically, small amounts of fibrin are constantly deposited on the endothelium and are continually dissolved, resulting in a dynamic balance between clotting and fibrinolysis. The generation and activity of plasmin, the enzyme responsible for the degradation of fibrin deposits and thrombi, are regulated mainly by the production of two critical proteins by the vascular endothelium, tissue-plasminogen activator (tPA), and the main inhibitor of tPA, plasminogen activator inhibitor-1 (PAI-1). tPA converts inactive plasminogen into plasmin at the site of fibrin formation.

Lipoproteins are able to regulate tPA and PAI-1 release, as demonstrated by in vitro studies using cultured endothelial cells. Very low-density lipoprotein (VLDL) isolated from normal individuals induces the release of tPA from cultured endothelial cells, whereas VLDL from hypertriglyceridemic individuals is unable to do so [370]. Endothelial production of PAI-1 was, however, increased by incubation with VLDL obtained from hyperglycemic patients [371]. PAI-1 can be released not only by endothelial cells but also from the adipose tissue, particularly visceral fat, and that may account, in part, for the association between obesity, diabetes, and impaired fibrinolysis [372, 373]. Cytokines such as interleukins 1 and 6, transforming growth factor-β, and TNF may contribute to augment PAI-1 expression in adipocytes [374, 375]. Another factor likely to influence PAI-1-circulating levels in diabetes is the renin–angiotensin system since binding of angiotensin II to the AT1

receptor augments PAI-1 synthesis [376] and the renin–angiotensin system (RAS) is activated in patients with type 2 diabetes.

Human studies performed in large cohorts have shown that low tPA antigen levels have a higher predictive value for mortality in patients with established CVD than cholesterol, triglycerides, fibrinogen, blood pressure, diabetes, or smoking [377]. In diabetes, impaired fibrinolysis (low tPA and/or high PAI-1) is an independent risk factor for myocardial infarction in diabetic subjects [378–380]. Decreased fibrinolytic function in type 2 diabetes correlates with the presence of CVD [381].

PAI-1 expression and release are increased in patients with type 2 diabetes [382–384], and the increase is more marked in patients with hypertriglyceridemia [385]. Increased concentrations of free fatty acids in diabetes may account for the insulin and VLDL-mediated augmentation of PAI-1 synthesis, and therefore, normalization of elevated concentrations of free fatty acids by improvement of glycemic control near-normalizes the fibrinolytic system activity in type 2 diabetes as shown by Juhan-Vague et al. [386]. The use of ACE inhibitors is known to attenuate hypofibrinolysis both in the circulation and in tissues [387], which renders the recommendations of the American Diabetes Association to use angiotensin receptor blockers or angiotensin-converting enzyme inhibitors extremely useful not only for the treatment of hypertension and reduction of albuminuria but also to attenuate the impairment of fibrinolysis in diabetes.

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Chapter 10
The Effects and Treatment of Inflammation on Diabetes Mellitus and Cardiovascular Disease

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Introduction

Diabetes Mellitus (DM), one of the most common chronic disorders, is a frequent cause of mortality and morbidity worldwide. DM has a strong association with coronary artery disease and has been described as a "Coronary disease equivalent." It has been shown that the risk of cardiovascular disease progression in diabetics is similar to non-diabetics with previous coronary artery disease [1–3]. Over 95% of patients with diabetes have Type 2 DM which will be the focus of this chapter.

Inflammation has been suggested as the potential driver of this disease process. It has also been suggested that DM itself leads to inflammation which drives some of the complications associated with it, most importantly atherosclerosis. A better understanding of the molecular pathways of inflammation leading to diabetes mellitus and its complications can potentially lead to novel treatment options. Identifying the triggers for this inflammatory process can aid in the prevention of the development of diabetes mellitus.

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A Basic Review of the Immune System and Inflammation

The immune system relies on two different mechanisms to defend against foreign bodies: the innate and the adaptive immune systems.

The innate immune system is the first line of immune defense and includes multiple mechanisms to protect against foreign bodies. This system includes (a) physical barriers such as the skin and the cellular wall of the gastrointestinal and urinary tract as well as (b) secretions that form mucous membranes and cover a large part of the respiratory and gastrointestinal tract. The innate immune system mounts a response to foreign antigens through the germline-encoded *Pattern Recognition Receptors* (*PRRs*) which leads to the release of cytokines, resulting in cellular signaling. This leads to both the further recruitment of the innate immunological response and the triggering of the adaptive immune system. The signaling pathway that leads to an amplified response is described as inflammation. Microbial structures known as *Pathogen-Associated Molecular Patterns* (*PAMPs*) [3, 4] as well as various endogenous signals activated during tissue or cell damage, known as *Danger-associated molecular patterns* (*DAMPs*), are able to trigger those PRRs [5, 6].

Toll-like receptors (*TLRs*) are the most studied and well-identified PRRs [7]. TLR activation leads to an intracellular signaling pathway, resulting in the nuclear translocation of transcription factors such as activator protein-1 (AP-1), NF-κB, or interferon regulatory factor 3 (IRF3) [8]. This response leads to the cellular production of inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) [9]. TLRs are expressed on both the cell surface and the intracellular membranes.

Another set of receptors, the *Nucleotide-Binding Domain*, *Leucine-Rich Repeat* (*NLR*) *Proteins* do not reside on membranes but in the cellular cytoplasm. These receptors can detect endogenous signals of intracellular damage and initiate programed cell death or *apoptosis*. A specific NLR of interest is the NLRP3 which, via signaling pathways, leads to the formation of a pro-inflammatory complex termed the *inflammasome*. The inflammasome in turn activates pro-inflammatory cytokines including IL1, IL18, and IL33 [10].

Another important PRR is a group of proteins called the *pentraxins*, homopentameric proteins that can detect certain foreign molecular patterns, the best known of which is the *C-reactive protein* (*CRP*). CRP is able to detect bacterial low-density lipoproteins and leads to the activation of the complement system which eventually leads to neutralization of the host bacteria [11].

Ultimately, cell death and microbial killing are driven by enzymatic degradation using oxidative reactions. *Myeloperoxidase* is one of the enzymes that not only catalyzes oxidative reactions, but it has also been implicated in the modulation of inflammation [12, 13].

Until recently, the innate immune system was perceived as a first line of defense that relies on recognizing antigens different from the host without any memory of previous exposures. However, recent reports suggest that the innate immune system

can mount a more robust response after being exposed to certain antigens. This type of response, termed *trained innate immunity*, is mediated by epigenetic changes rendering certain genetic sequences more accessible for transcription [14]. A chronic inflammatory state, as seen in diabetes, can be further amplified by this phenomena as the trained innate immune system is able to ramp up the production of myelocytes that can further increase inflammation via a positive feedback loop [15]. This was first witnessed in animal models as a quantitative increase in neutrophils and monocytes was observed in obese and western diet-fed mice, an excellent model for both metabolic syndrome and diabetes mellitus [16, 17].

On the other hand, the adaptive immune system can form specific antigen receptors that can attach to foreign bodies using somatic rearrangement of the basic element. These basic elements can develop into millions of receptors and trigger inflammatory cascades whenever an antigen attaches to its corresponding antibody receptor. The adaptive immune system relies on two different cell lines, T and B cells. These cell lines can be differentiated based upon the specific antigens present on their surfaces and are known as clusters of differentiation (CD). Each cell line has subtypes of cells with different CD. T cells differentiate primarily into CD4 and CD8 cells. CD4 cells are involved in the detection of foreign bodies and the subsequent activation of other cell types including CD8 cells and B Cells. CD8 cells act primarily by lysing cells that are infected with intracellular microbes. B cells produce specific antibodies in response to a foreign antigen or *immunoglobulins*. T and B cell signaling is achieved through the release of *interleukins* amplifying the immune response when a foreign antigen is detected.

An Intertwining Relationship Between Diabetes and Inflammation

Inflammation as a Risk Factor for Type II DM

The Innate immune system plays a role in the development of insulin resistance related to type 2 DM. A subtype of macrophages named “M1” produce a proinflammatory response compared with the second subtype of macrophages “M2” which produce an anti-inflammatory response. Obese humans are noted to have a polarization towards M1 macrophages with a downstream release of pro-inflammatory cytokines and chemokines [18] (Fig. 10.1).

Multiple studies throughout the past few decades have found association between inflammation and the development of Type II DM [19, 20]. Elevated inflammatory makers such as CRP and IL-6 were associated with higher incidence of development of diabetes mellitus [21]. Tumor necrosis factor-alpha (TNF-α), an inflammatory signaling cascade factor, was found to be higher in rodent models with obesity and diabetes. Neutralization of TNF-α caused a significant increase in glucose uptake in response to insulin [22]. The administration of the anti-inflammatory

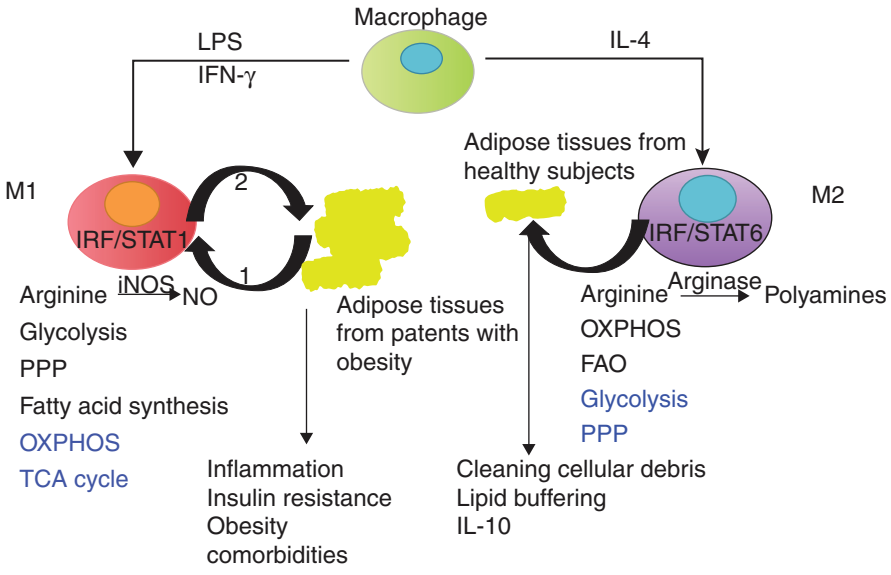

Fig. 10.1 The relation between macrophages and obesity or diabetes. (Source: Ren W et al. Glutamine Metabolism in Macrophages: A Novel Target for Obesity/Type 2 Diabetes. Adv Nutr. 2019 Mar 1;10(2):321–330)

sodium salicylate in high doses to patients with suspected or known diabetes mellitus, resulted in a significant reduction in glucosuria [23, 24].

One third of interleukin-6 originates from the subcutaneous adipose tissue [25]. IL-6 is the primary interleukin that is involved in the hepatic synthesis of CRP as well as other pro-inflammatory markers. This pro-inflammatory response is associated with further leukocyte recruitment and activation of the adaptive immunity. Those inflammatory markers increase the risk of development of diabetes, and they also lead to accelerated atherogenesis (Fig. 10.2). The Women's Health Study (WHS) demonstrated an association between higher level of CRP and the increased risk of development of type 2 DM [26].

Metabolic syndrome with its different components including obesity and hypertension has been strongly associated with the risk of developing Type II diabetes mellitus [27–30]. Multiple studies have demonstrated that several inflammatory markers including CRP and IL-6 were elevated in metabolic syndrome [31–33]. These same markers have also been associated with insulin resistance. This suggests that an underlying inflammatory process may be driving insulin resistance in patients with the metabolic syndrome.

Activation of TLRs' specific subtypes has been implicated in metabolic syndrome and the development of insulin resistance. Inhibition of TLR2 and TLR4 has been associated with a decrease in insulin resistance [34, 35]. The main function of these receptors is the recognition of lipopolysaccharide (LPS) present on gram-negative bacteria. High-saturated fat diet may possibly activate TLR pathways [36,

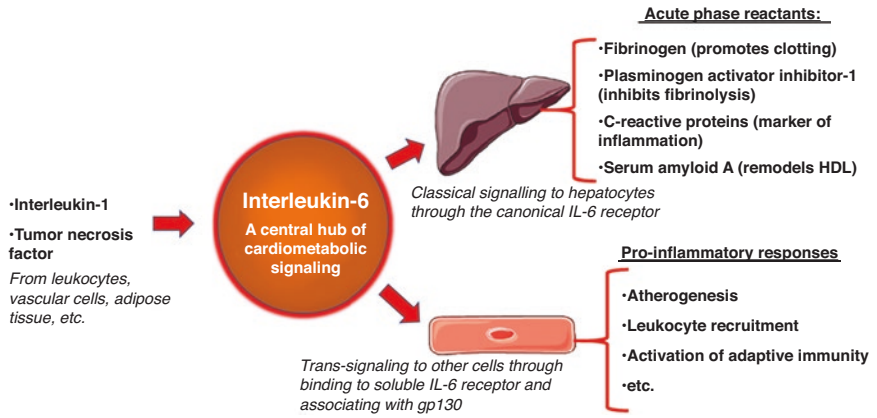

Fig. 10.2 The role of IL-6 in inflammation and atherogenesis. (Source: Libby P, Rocha VZ. All roads lead to IL-6: A central hub of cardiometabolic signaling. Int J Cardiol. 2018 May 15;259:213–215)

37]. This suggests a link between a high-fat diet and the risk of developing type 2 DM. TLR activation via signaling activates the *NLRP3* (*NLR family pyrin domain 3*) and *NLRP3 inflammasome complex*, first described by Martinon in 2002. Over the past two decades, the inflammasome has become an area of intense research interest due to its association with both atherosclerosis and insulin resistance [38, 39]. Additionally, the complex also leads to the activation of Caspase and to programmed cell death, *apoptosis.* After encountering a stress signal, the NLRP3 sensor proteins activate oligomerization domains [40]. Oligomerization leads to a wheel-shape configuration of NLRP3 [41] that, in turn, leads to the activation of inactive Caspase-1, and cleaves pro-IL-18, and IL-1B (Fig. 10.3).

These cytokines then lead to activation of *Pro-gasdermin D*, which leads to pore formation in the cell membrane, releasing active cytokines. The cytokines then attract phagocytes and cause apoptosis. Animal models suggest that IL-1b and IL-18 are upregulated in mice with impaired glucose metabolism and diabetes mellitus. In a recent study of 232 Chinese patients with impaired glucose regulation (IGR) and diabetes mellitus, IL-18 was significantly increased when compared with subjects with normal glucose metabolism [42]. A more direct relationship between *inflammasome* and diabetes mellitus remains to be explored.

Obesity is another well-understood risk factor for ongoing inflammatory response in humans and animal models. For example, adipose tissue incrementally recruits immune cells releasing chemo-attractants for inflammatory cells. This has a direct impact on insulin sensitivity as seen with downregulation of these chemo-attractants MCP-1 in animal models with obese mice [43, 44].

The adaptive immune system also skews to a pro-inflammatory state in obesity. T cells are divided into CD4+ and CD8+ cells. The CD4+ T cells can differentiate to either TH1 cells or TH2 cells based on the circulating inflammatory markers. The presence of certain pro-inflammatory interleukins that can occur in obesity such as

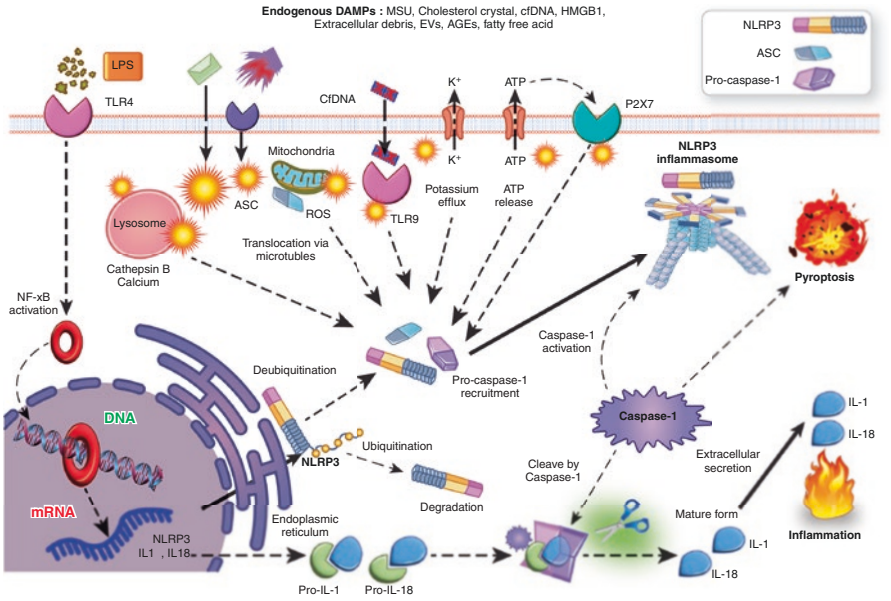

Fig. 10.3 A schematic representation of inflammasome NLRP3 assembly activation with pathogen-associated molecules (PAMPs) and DAMPs. The NLRP3 inflammasome then activates pro-IL-1b and IL-18 which are then indirectly implicated in atherosclerosis and diabetes mellitus. (Source: Shirasuna K, Karasawa T, Takahashi M. Role of the NLRP3 Inflammasome in Preeclampsia. Front Endocrinol (Lausanne). 2020 Feb 25;11:80)

IL-6 leads to the differentiation of T cells to TH1 cells which play a role in multiple inflammatory conditions [45–47].Cytokines produced in response to the activation of transcription factors such as TNF-α, IL1b, and IL-6 result in the downregulation of *Peroxisome Proliferator-Activated Receptor-γ* (*PPAR-γ*) [48] a modulator of insulin sensitivity [49].

Type II DM Causing an Inflammatory Response

Patients who already have diabetes also have a myriad of circulating signaling proteins that induce an inflammatory response. Several proteins, i.e., IL-1, IL-6, IL-10, *leptin*, *adiponectin*, and a collective group of proteins secreted from primarily white adipose tissue termed *adipokines* play an important role in diabetes-induced inflammation [50–56]. Type II DM also leads to an imbalance in T cell subtypes including *Tregs*, with a skew of T cell subtypes towards a pro-inflammatory subset that promotes a chronic inflammatory response with further release of cytokines [57].

Chronic hyperglycemia in diabetic population with poor control has been associated with many of the major complications seen in this disease. Chronic hyperglycemia may activate inflammation that in turn plays an important role in causing these complications. Specifically, the glycation of proteins produces *advanced glycated endproducts (AGE*) with subsequent interaction of AGE with cellular

receptors (*RAG*). This triggers a pro-inflammatory signaling pathway which leads to the activation of *Nuclear factor-kappa B* (*NF-kB*), *Phosphatidylinositol 3 kinase/protein kinase* (*B Pi3K/AkT*) and *Mitogen-activated protein kinase* (*MAPK*) [58]. Another method through which hyperglycemia results in inflammation is through the accumulation of nicotinamide adenine dinucleotide (NADH) in its reduced form. As glucose enters the cells, glycolysis takes place and glucose is cleaved to pyruvate and NAD+ (the oxidized form of NADH) is turned to NADH. NADH is then transported to the mitochondria. The excess NADH overwhelms the mitochondria leading to the formation of oxygen-derived free radicals (ROS) resulting in cell damage and subsequent inflammation [59].

Over the past decade, there has been an increasing amount of evidence emerging on the influence of intestinal bacteria and their role inflammation. In 2004, a hallmark study revealed an association between obesity and the gut microbiome [60]. However, the study of the gut microbiome remains a challenging process with broad differences in the bacterial composition based on age, diet, and genetic variations among many others [61]. The gut wall is part of the innate immune system. Alterations in the intestinal microbiome, also known as dysbiosis, has had a growing base of evidence describing its association with diabetes mellitus as well as low-grade inflammation [61, 62]. Dysbiosis is believed to affect gut wall integrity, and as a result, different particles can cross the barrier to the circulation.

The Role of Diabetes-Related Inflammation with Cardiovascular Disease and Atherosclerosis

Age-related diseases could be as a result of an antigenic stress that triggers the inflammatory cascade with the subsequent development of full-blown disease. Low-grade inflammation as a result of infections, autoimmune processes or toxic waste products related to dietary habits can lead to an increased risk of heart attacks, strokes, and cancer [63–65]. Atherosclerosis and the subsequent development of cardiovascular disease are the most significant complication of the diabetes and, as such, the major cause of morbidity and mortality in diabetes [66]. This topic is covered in more detail in the Chap. 10.

The ‘Response to Injury’ hypothesis for the development of atherosclerosis is predicated on an injury to the endothelial cell. The proposed causes of this injury to the endothelial cell include hypertension, diabetes mellitus, smoking, and dyslipidemia. The consensus from numerous investigators is that the inflammatory cascade occurs in response to that injury and results in the development of atherosclerosis as well as plaque formation, progression, and destabilization [67–70] (Fig. 10.1).

But what is that evidence? Histological studies on atherosclerotic plaques of diabetic patients showed a higher inflammatory cell infiltration compared to non-diabetics [71, 72]. There is also evidence of adventitial inflammation that extends into the tunica media inducing atrophy and fibrosis [71]. This inflammatory process leads to the production of vascular-stimulating factors that promote neovascularization [73]. The newly formed blood vessels are friable and at risk of

microhemorrhage and extravasation of blood cells [74, 75]. Prevention of angiogenesis could suppress the positive feedback cycle generated by macrophage stimulation and macrophage infiltration through microhemorrhagic vessels [76].

Clinical evidence shows elevated inflammatory markers such as CRP, TNF-α, and IL-6 as prognosticators for the development of atherosclerosis as well as disease progression in patients who have already been diagnosed with coronary artery disease [69, 70]. In the Women's Health Study (WHS), women with an elevated hsCRP (high sensitivity CRP) greater than 3 mg/L and metabolic syndrome had twice the relative risk of cardiovascular disease than those below this hsCRP threshold [31] (Fig. 10.4). The JUPITER trial further elaborates the use of anti-inflammatory properties in similar population with reduction in primary end-point.

Inflammation may be important even in the consequences of coronary artery disease. Several studies have highlighted the role of inflammation and the immune system in acute and chronic heart failure [77–80]. Acute myocardial injury results in an inflammatory response that leads to tissue remodeling and scar formation [81, 82]. However, there are limited data on the effect and role of the continued inflammatory process observed in chronic heart failure. Pro-inflammatory markers such as TNF-α as well as IL1β were found to be elevated in patients with abnormal heart function [83, 84]. This suggests that a continued inflammatory process in chronic heart failure can be a contributor to the progression of heart failure through remodeling and dysfunction.

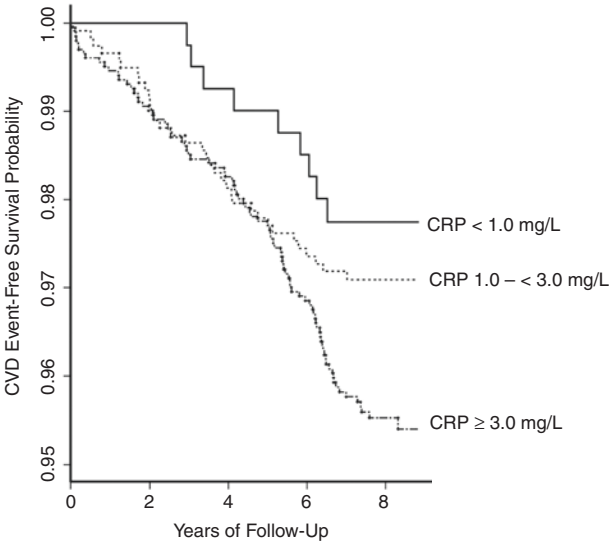

Fig. 10.4 Cardiovascular event-free survival in analyses stratified by CRP levels <1, 1 to 3, and ≥3 mg/L. Data are shown for 3597 study participants with metabolic syndrome at study entry. CVD indicates cardiovascular disease. (Source: Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. Circulation. 2003 Jan 28;107(3):391–7)

The proposed cellular and molecular mechanisms and pathways that induce the inflammatory cascade are several fold and reviewed in detail elsewhere in this volume (see Chap. 10). These include shear stress within blood vessels as a result of blood flow which can trigger an inflammatory response, resulting in the activation of pro-inflammatory transcription factors such as NF-Kβ. Inhibiting NF-Kβ in mice has been shown to prevent atherogenesis [85]. Another such pathway involves the hyperglycemic milieu of diabetes resulting in the previously described advanced glycation end products (AGE), activating RAGE receptors present on endothelial cells with subsequent activation of NF-Kβ and triggering an inflammatory response [86].

Furthermore, another mechanism involves an early response of endothelial injury (for details see Chap. 8), the decreased production of nitric oxide. Nitric oxide is hypothesized to prevent atherosclerosis by inducing vasodilation resulting in (a) reduced mechanical stress in the blood vessel wall; (b) reduced interaction between platelets and leukocytes with endothelial cells; (c) reduction and prevention of smooth muscle proliferation and migration [87–89]; and (d) reduced formation of macrophage foam cells (one of the first morphological changes associated with atherosclerosis) [90–94]. Therefore, any decrease of NO will accelerate atherogenesis. Another important contributor to the inflammatory processes of diabetic atherogenesis is excess adipose tissue. As mentioned earlier, MCP-1 secreted by adipose tissue can decrease insulin sensitivity and attract immune cells. Adipocytes can increase the expression of adhesion proteins such as ICAM-1, VCAM-1, and E-selectin which would attract monocytes to the endothelium of blood vessels [95]. These monocytes migrate to the subendothelial tissue adjacent to the adipocytes and differentiate into macrophages contributing to atherogenesis [96].

Current Treatments, Novel Therapies, and Future Potential

The Effect of Oral Hypoglycemic Medications on Inflammation (Table 10.1)

Metformin is the first line of medical treatment for Type II diabetes mellitus and has shown benefits in reducing cardiovascular disease in diabetics [102–104]. Metformin has anti-inflammatory properties which can be one of the mechanisms by which it aids in treating both diabetes and cardiovascular disease [105–107]. The exact mechanism by which metformin exerts its anti-inflammatory effect is not fully understood. Studies have shown that metformin is able to reduce cytokine production such as IL-6 through the inhibition of TNF-α, TN-signaling cascade, and subsequently NF-Kβ [105]. Metformin induces *AMPK* (5′-AMP activated protein kinase), a key regulator in cellular energy homeostasis, which has been shown to directly decrease the formation of reactive oxygen species [108, 109]. The beneficial effects of metformin on cardiovascular disease can possibly be independent of its effect on hyperglycemia with observational studies in animal models showing effects on infarct size in coronary events as well as effects on the progression of

Table 10.1 The effects of oral hypoglycemic medications on inflammation

Drug	Mechanism of action	Main finding	Limitations/ remarks	References
Metformin	Activate AMPK	Reduce cytokine production such as IL6 through the inhibition of TNF-α-signaling cascade and subsequently NF-Kβ	No effect on carotid intima thickness	[115]
Sulfonylurea	K-ATP channels of β cell plasma membranes	Modest to no reduction in inflammatory markers	Risks of hypoglycemia	[118, 125, 130–132]
GLP-1 agonists	Activates GLP-1 receptor	Increase insulin secretion; decrease in inflammatory markers such as TNF-α, IL1, and IL6; decrease in the expression of pro-inflammatory nuclear transcription factors and the production of endothelial adhesion molecules	Decrease risk of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke	[119–122]
Thiazolidinediones	Activate nuclear transcription factor PPAR-γ	Decrease serum levels of MMP-9 and CRP, inflammatory markers; increase adiponectin	Increase risk of CHF, MI, mortality	[112–119]
SGLT2 inhibitors	Inhibits SGLT2 receptors at the proximal tubule	Reduction in TNFR1, IL-6, MMP7, and FN1 as well as leptin	Increase risk of euglycemic ketoacidosis, amputation, and vulvovaginitis	[122–125]
DPP4 inhibitors	Inhibit DPP-4 activity, increase post prandial incretin levels	Decrease inflammatory cytokines, and biomarkers and CRP	Protection against endothelial dysfunction and atherosclerosis progression	[133–140]

SGLT2 sodium–glucose cotransporter 2, *PPAR-γ* peroxisome proliferator-activated receptor-γ, *AMPK* 5′ adenosine monophosphate-activated protein kinase, *TNF* tumor necrosis factor, *ATP* adenosine 5′-triphosphate, *NF-Kβ nuclear factor kappa B*, *MI myocardial infarction, IL interleukin*, *CRP* C-reactive protein, *TNFR* tumor necrosis factor receptor, *GLP* glucagon like peptide, *DPP-4* dipeptidyl peptidase 4, *MMP7/9* matrix metallopeptidase 7/9, *FN1* fibronectin 1

heart failure [110–113]. However, human studies remain inconclusive when it comes to the beneficial effect of metformin on cardiovascular disease in non-diabetic patients [102, 114, 115].

Thiazolidinediones (TZDs), a family of Peroxisome Proliferator-Activated Receptor (PPAR-γ) agonists, have been used in the treatment of diabetes mellitus. PPAR-γ plays a role in the inflammatory process in diabetes and cardiovascular disease as mentioned earlier in this article. TZDs can improve insulin sensitivity by

regulating the expression of several proteins in the insulin-signaling pathway [116]. One member of the TZD family, rosiglitazone, showed a reduction in CRP levels when administered to patient with type II DM [117] in an observational study. However, clinical results are mixed. In one study, the use of pioglitazone in type 2 DM subject showed a reduction in cardiovascular morbidity [118] while a subsequent study revealed that in the older populations, rosiglitazone was associated with an increased risk of congestive heart failure, acute myocardial infarction, and mortality when compared with other combinations of oral hypoglycemic agents [119].

Glucagon-like peptide-1 (GLP-1) agonists are another class of medications used in diabetes that has shown some anti-inflammatory effects. GLP-1 is an intestinal hormone that increases glucose stimulated insulin secretion [120, 121]. Several studies have shown a decrease in inflammatory markers such as TNF-α, IL-1, and IL-6 [122]. They also show a decrease in the expression of pro-inflammatory nuclear transcription factors and the production of endothelial adhesion molecules [123–125].

The Sodium-Glucose co-Transporter 2 (SGLT2) inhibitors, a relatively newer class of oral hypoglycemic agents, has benefit in cardiovascular disease [126] but limited data on its effect on inflammation despite some evidence on reduction in cytokines such as IL-1, IL-6 as well as leptin [127–129] suggesting the possibility that the cardiovascular benefits of this class may be related to its anti-inflammatory effect.

Sulfonylureas increases insulin secretion via the stimulation of pancreatic beta cells. There have been multiple studies to determine if sulfonylureas have any anti-inflammatory effects. In most studies, there has been a modest to no reduction in inflammation compared to other agents used in type II DM [118, 125, 130–132].

Dipeptidyl peptidase 4 (DDP-4) inhibitors (referred to as gliptins) are another class of oral hypoglycemic agents that exert their effects through inhibition of the enzyme dipeptidyl peptidase as their name suggests. This enzyme is responsible for degrading incretins, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic peptide which results in more insulin and less glucagon secretion. Studies on the effects of DDP-4 inhibitors show a significant reduction in inflammation. DDP-4 inhibitors have been shown to significantly reduce the pro-inflammatory markers such as Toll-like receptors, i.e., TLR-2, TLR-4, and CRP and IL-6 as well as providing protection against endothelial dysfunction and atherosclerosis progression [133–140].

The Effect of Anti-inflammatory Drugs on Type 2 Diabetes Mellitus and Cardiovascular Disease

Trials on Type 2 DM: (Table 10.2)

Several trials have attempted to target-specific molecular pathways involved in the inflammatory cascade. High-dose aspirin in one study not only improved glucose metabolism in type 2 DM [154]. It has been shown to inhibit I(kappa) B kinase-beta which, in turn, reduces translocation of NF-Kβ into the nucleus as part of the inflammation cascade [155]. TNF-α antagonists used to treat inflammatory conditions

Table 10.2 Effect of anti-inflammatory drugs on type 2 diabetes mellitus

Drug	Mechanism	Study name	Main finding	References
Diacerein	Decreases cytokine concentrations TNF-α and IL-1β, with an unknown mechanism of action	N/A	Increase in insulin secretion, decreases in fasting glucose and HbA1c ($P < 0.001$)	[141]
Methotrexate	DHFR inhibitor—DMARD combination with sulfasalazine and hydroxychloroquine	N/A	Decrease levels of HbA_{1c} by 0.8 mmoles/mole (−0.08%) ($P = 0.018$)	[142]
Methotrexate Hydroxychloroquine	DMARD	N/A	Decrease risk of diabetes with and HR of 0.77 (95% CI, 0.53–1.13) for methotrexate, and 0.54 (95% CI, 0.36–0.80) for hydroxychloroquine	[143]
Hydroxychloroquine	DMARD	N/A	Decrease risk of diabetes with a RR of developing diabetes of 0.23 (95% confidence interval, 0.11–0.50; $P < 0.001$)	[144]
Methotrexate Hydroxychloroquine	DMARD	N/A	Decrease in HbA_{1c} 0.54% greater in patients on HCQ vs MTX ($P = 0.041$)	[145]
Canakinumab	Monoclonal antibody targeting interleukin-1β	N/A	Reduction of HbA_{1c} between 0.19% and 0.31% with maximal effect noted in the 50 mg dose of canakinumab	[146]
Canakinumab	Monoclonal antibody targeting interleukin-1β	N/A	Increase insulin secretion	[147]
Canakinumab	Monoclonal antibody targeting interleukin-1β	N/A	Reduce major cardiovascular events but not incidence of diabetes with an HR of 1.02 (95% CI: 0.87 to 1.19; $P = 0.82$)	[148]
Anakinra	Interleukin-1 receptor antagonist (IL-1Ra)	N/A	No change in FBG or HbA1c levels ($P = 0.03$)	[149]
Anakinra	IL-1Ra	N/A	Improvement of the proinsulin/insulin ratio and markers of systemic inflammation (by −0.07 [95% CI −0.14 to −0.02], $P = 0.011$)	[150]

Table 11.2 (continued)

Drug	Mechanism	Study name	Main finding	References
Anakinra	IL-1Ra	N/A	Decrease HbA1c by 46% ($P = 0.03$), increase C-peptide secretion ($P = 0.05$), decrease ratio of proinsulin to insulin ($P = 0.005$), Il-6 ($P < 0.001$), and CRP ($P = 0.002$)	[151]
Etanercept	TNF-α antagonism	N/A	Decrease CRP and interleukin 6	[152]
TNF-a-neutralizing antibody (CDP571)	Neutralizing TNF-α	N/A	No effect on insulin sensitivity	[153]
Salicylates	Inhibit IKKβ activity	N/A	Improved fasting (24%) postprandial hyperglycemia, decrease basal rates of hepatic glucose production (22%) and insulin clearance ($P < 0.0001$); enhances peripheral insulin sensitivity	[154]

DHFR dihydrofolate reductase, *TNF* tumor necrosis factor, *DMARD* disease-modifying antirheumatic drug, *IKKβ* inhibitor of nuclear factor kappa-B kinase subunit beta, *CRP* C-reactive protein, *FBG* fasting blood glucose, *NLRP* NLR family pyrin domain containing 3

have been associated with improved glycemic control. However, most of the studies did not show effects on insulin sensitivity [135–140, 154–156]. The IL-1 receptor blocker, Anakinra was also used to target the inflammatory cascade in type 2 DM. Anakinra reduced HbA1c levels, increased insulin C peptide secretion, and reduced the production of IL-6 and CRP. There was no drug-related adverse effect [149–151]. The IL-1beta antagonist, Canakinumab, also showed a similar decrease in HbA1c in some of the studies as well as a reduction in inflammatory markers including CRP and IL-6 [146–148, 150, 157].

Immunosuppressants used in the treatment of rheumatoid arthritis such as methotrexate and hydroxychloroquine have shown some benefit in the control of diabetes. Hydroxychloroquine has been associated with a decreased incidence of diabetes as well as in reduction of HbA1C [143–145]. Similarly, Methotrexate was also associated with decreased levels of HbA1c [142].

Diacerein, a medication that is used to treat some rheumatological diseases, is believed to decrease inflammation with a reduction in IL-1beta, TNF-α, IFN-gamma, and IL-12 through unknown mechanisms [158]. When administered to diabetics, diacerein caused an increase in insulin secretion with a resultant decrease in fasting blood glucose levels as well as HbA1c [141].

Trials on Targeting the Inflammatory Process Cardiovascular Disease as the End-Point

Two major trials have recently been published that target inflammation to potentially reduce acute cardiovascular disease. The Cantos Trial evaluated the effectiveness of Canakinumab in a randomized double-blinded trial that involved 10,061 patients with a previous myocardial infarction and an elevated high sensitivity CRP(>2 mg/L). Patients were given either subcutaneous canakinumab every 3 months or placebo. The canakinumab arm was split into three groups with each group getting a different dose of the IL-1beta antagonist (50 mg, 150 mg, 300 mg). Forty percent of the subjects in each arm were diabetic. The patients were followed for 2 years. The primary end-points were nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death. The study showed a decrease in the rate of cardiovascular events in the group receiving a 150 mg dose of canakinumab vs placebo with an event rate of 4.5 events per 100 person-years in the placebo and 3.86 events per 100 person-years in the 150 mg canakinumab group. There was also a reduction of primary end-point events in the other groups receiving canakinumab, but they did not reach statistical significance when compared with the placebo group. The study also noted a reduction in CRP levels. However, there was a higher incidence of fatal infection in the canakinumab groups. Most importantly, there was no significant difference in all-cause mortality in any of the groups [159]. This trial did not publish whether diabetics had different or similar cardiovascular outcomes from the rest of each of the groups (Table 10.3).

The CIRT trial involved a low dose of methotrexate administered (15–20 mg weekly) to patients who had a previous myocardial infarction or multivessel disease with either type 2 DM or metabolic syndrome. The patients were divided into a low-dose methotrexate arm and a control arm and followed for 2.3 years. The primary end-point was a composite of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death (near the end of the trial, hospitalization for unstable angina that led to urgent revascularization was added to the primary end-point). Results revealed that low-dose methotrexate did not lower inflammatory markers IL-1b, IL-6, or CRP nor did it result in lower cardiovascular events compared to the control group. Moreover, methotrexate was also associated with elevations in liver enzyme levels, reductions in leukocyte counts and hematocrit levels, along with a higher incidence of nonbasal-cell skin cancers compared with the placebo group [101].

Colchicine is another anti-inflammatory drug that down regulates multiple inflammatory pathways and modulates the innate immune system [160–162]. Colchicine has broad anti-inflammatory action, which includes the disruption microtubules and having anti-mitotic effects as well as targeting the NLRP3 (NLR family pyrin domain containing 3) inflammasome activation of which leads to downstream IL-1beta and IL-6 upregulation. It may also inhibit the local inflammation caused by cholesterol crystals within plaques thereby reducing plaque disruption and acute coronary syndrome. The COLCOT trial [99] demonstrated the clinical efficacy of once-daily colchicine in patients with a recent admission for acute cardiovascular disease in subsequent admissions for further cardiovascular events including myocardial infarction, unstable angina, sudden death, and stroke. Both LoDoCo and LoDoCO$_2$ trials tested whether low-dose colchicine influenced

Table 10.3 Effect of anti-inflammatory drugs on cardiovascular disease

Drug	Mechanism	Study name	Main finding	References
Colchicine	Disrupts microtubules, has anti-mitotic effects as well as targeting the NLRP3 inflammasome, inhibits local inflammation caused by cholesterol crystals within plaques	LoDoCo LoDoCo-2	Lower risk of acute cardiovascular events ($P < 0.001$) (NNT = 11) Lower the incidence of cardiovascular death or spontaneous myocardial infarction (composite end-point), ischemia-driven coronary revascularization, and spontaneous (HR = 0.69; 95% confidence interval [CI], 0.57 to 0.83; $P < 0.001$)	[97, 98]
Colchicine	Disrupts microtubules; anti-mitotic effects; targeting the NLRP3 inflammasome; inhibits local inflammation caused by cholesterol crystals within plaques	COLCOT	Lowers the risk of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, and for urgent hospitalization for angina leading to coronary revascularization. (Hazard ratio, 0.71; 95% CI, 0.56 to 0.90)	[99]
Colchicine	Disrupts microtubules; anti-mitotic effects; targeting the NLRP3 inflammasome, inhibits local inflammation caused by cholesterol crystals within plaques	COPS	Higher rate of total death, non-cardiovascular death, in the colchicine group ($P = 0.024$)	[100]
Methotrexate	DHFR inhibitor—Inhibit binding of interleukin-1β to its receptor	CIRT	No reduction in Il-1β, Il-6, CRP nor in cardiovascular events. (Hazard ratio, 0.96; 95% confidence interval [CI], 0.79 to 1.16)	[101]

cardiovascular outcomes in patients with stable coronary artery disease. In patients with acute [(LoDoCo) and chronic $LoDoCO_2$] coronary disease, there was a composite decrease in further acute cardiovascular events and out-of-hospital cardiac arrests in patients receiving 0.5 mg of colchicine daily compared with the placebo arm [97, 98]. The diabetics fared no differently in terms of cardiovascular events from their non-diabetic cohorts. However, the Australian COPS study [100] (of which 20% were diabetic) demonstrated that similarly dosed colchicine used in the same dosage as in previous studies caused an increase in the rate of all-cause death and in particular, non-cardiovascular deaths in the colchicine group compared to the placebo group with a specific increase in sepsis-related deaths. In conclusion, while targeted anti-inflammatory treatment is promising, more research is necessary before it becomes a mainstay in our treatment of high-risk patients with cardiovascular disease, like those with diabetes mellitus (Table 10.4).

Table 10.4 The metabolic and inflammatory effects of inflammatory mediators

Inflammatory mediator	Site of production/ release	Metabolic effects	Inflammatory effects
Interleukin-1 β	Adipose tissue	Potentiate insulin secretion from pancreatic β cells by increasing exocytosis	Neutrophil recruitment; stimulate synthesis of acute phase reactants by the liver
Interleukin-6	Adipose tissue, immune cells, skeletal muscles	Insulin resistance contributing to the development of diabetes and potentially the metabolic syndrome	Stimulate hepatic acute phase reactants production [C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, haptoglobin, and α1-antichymotrypsin]
Interleukin-18	Macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells, and Kupffer cells	Associated with obesity, insulin resistance, and has been shown to be elevated in subjects with metabolic syndrome	Involved in several autoimmune diseases, metabolic syndrome, psoriasis, inflammatory bowel disease, macrophage activation syndrome, and acute kidney injury
Interleukin-33	Epithelial cells in barrier tissues, fibroblastic reticular cells in lymphoid organs and glial cells in nervous tissues with species specific differences	Affects glucose uptake; glycolysis, improve glucose tolerance; and attenuate cellular insulin sensitivity	Involved in inflammatory diseases such as rheumatoid arthritis, asthma, psoriasis and CNS; affects macrophage polarization and T-regulatory cells activation
Tumor necrosis factor-alpha	Adipose tissue, skeletal cells	Insulin resistance and development of type II DM	Involved in apoptosis, differentiation, and cell recruitment promoting inflammation
C-reactive protein	Hepatic tissue, smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes	Insulin resistance, positively correlated with higher resting metabolic rate	Plays a role in apoptosis, phagocytosis, nitric oxide (NO) release; production of cytokines, particularly interleukin-6 and tumor necrosis factor-α
Interleukin-1 β	Adipose tissue	Potentiate insulin secretion from pancreatic β cells by increasing exocytosis	Neutrophil recruitment; stimulate synthesis of acute phase reactants by the liver
Interferon-gamma	T-cells and NK cells	Insulin resistance, increase adipose tissue size	Potentiate pro-inflammatory signaling by priming macrophages; inducing nitric oxide (NO) production and inhibiting NLRP3 inflammasome activation

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Part II
Associated Conditions of Type 2 Diabetes Mellitus

Chapter 11
The Role of Sleep Apnea in Diabetes Mellitus and Cardiovascular Disease

Amit Anand, Jay Patel, and Melanie Pogach

Introduction

Sleep-disordered breathing (SDB), which encompasses both obstructive sleep apnea (OSA) and central sleep apnea (CSA), is highly prevalent in patients with cardiometabolic disease. A growing body of evidence supports a causal association between SDB and incidence and morbidity of hypertension (HTN), coronary heart disease (CAD), arrhythmia, heart failure (HF), stroke, and type 2 diabetes (T2DM) [1]. While the subgroups of SDB are attributed to different pathophysiologies, they often coexist in the same patient, especially in patients with co-morbid cardiometabolic disease, such as HF or atrial fibrillation (AF). Recognition and treatment of SDB may impact cardiovascular disease morbidity. This chapter will discuss current understanding of the pathophysiology and mechanisms of SDB, links between SDB and cardiovascular disease, and the impact of SDB treatment on cardiovascular outcomes.

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Definitions

OSA is a type of sleep-related breathing disorder characterized by recurrent episodes of total (apnea) or partial (hypopnea) upper airway collapse during sleep, despite ongoing respiratory effort, which results in intermittent hypoxia and hypercapnia and terminates in arousal from sleep with reopening of the airway [2].

CSA is a sleep breathing disorder characterized by repetitive cessation or decrease in both airflow and ventilatory effort during sleep. The condition can be primary (i.e., idiopathic CSA) or secondary. Secondary CSA can occur with Cheyne-Stokes breathing (CSB), a medical condition, a drug or substance (such as with narcotics), or high-altitude periodic breathing [3]. CSA is defined as ≥5 central apneas and hypopneas per hour of sleep (central apnea hypopnea index, CAHI ≥ 5) with CAHI accounting for >50% of all respiratory events [4].

The diagnostic and scoring criteria used for SDB are biased toward obstruction, in part due to difficulty reliably differentiating central verses obstructive hypopneas [5]. Central and obstructive physiologies may coexist, the central component often unrecognized clinically, impacting treatment tolerance and efficacy.

The severity of sleep apnea is determined by the number of respiratory events (apneas and hypopneas) per hour of sleep during a sleep study, referred to as the apnea–hypopnea index (AHI). The AHI 4% index requires that hypopneas are associated with at least a 4% decrease in oxygen saturation. The AHI 3% or arousal index, the alternative Academy of Sleep Medicine (AASM) definition, includes hypopneas that are associated with at least 3% decrease in oxygen saturation or an arousal from sleep. The higher the AHI, the more severe the sleep apnea (mild SDB = AHI ≥ 5 and < 15 events/h of sleep; moderate = AHI = 15–30 events and severe = AHI ≥ 30 events) [6]. The respiratory disturbance index (RDI), a marker of sleep fragmentation, includes apneas, hypopneas, and respiratory effort-related arousals (RERAs) per hour of sleep on a sleep study. These different definitions are often used interchangeable in the literature and it is important to know which definition was used (or is recognized by various insurers for treatment coverage) (Figs. 11.1, 11.2, and 11.3).

Prevalence and Risk Factors

SDB is a common disorder. Population-based studies suggest that OSA of all severities affects up to 34% of middle-aged men and 17% of middle-aged women [8]. The prevalence is considerably higher in patients with cardiometabolic disease and continues to rise as the population grows more obese [9–11]. Accurate phenotyping of the different types of sleep apnea is limited using conventional polysomnography such that the true prevalence of CSA remains uncertain.

While OSA is attributable to upper airway anatomy, it is also affected by control of breathing and arousal threshold. CSA (in states of hyperventilation) is primarily due to unstable control of breathing or chemoreflex instability, though upper airway features also contribute. In some patients with SDB, increased arousability or low

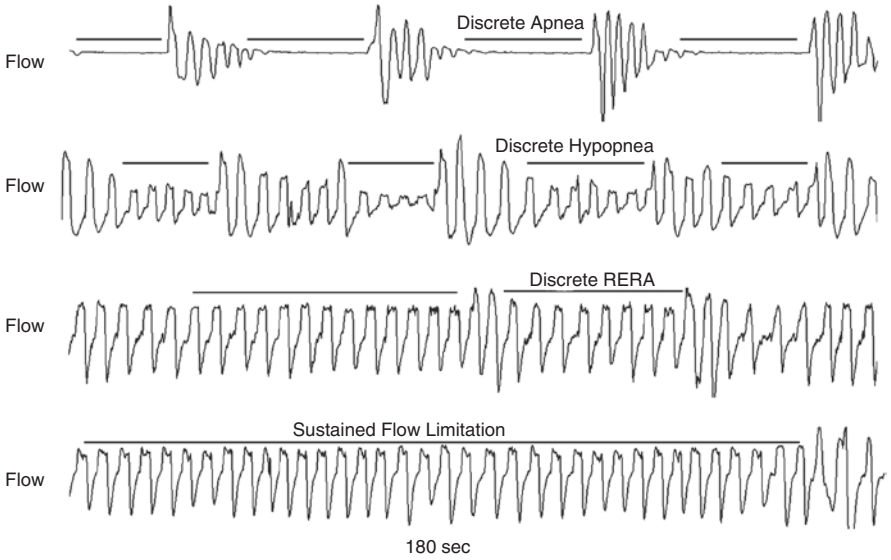


Fig. 11.1 Ventilatory events in sleep-disordered breathing detected in sleep studies. RERA: respiratory effort-related arousal [7]

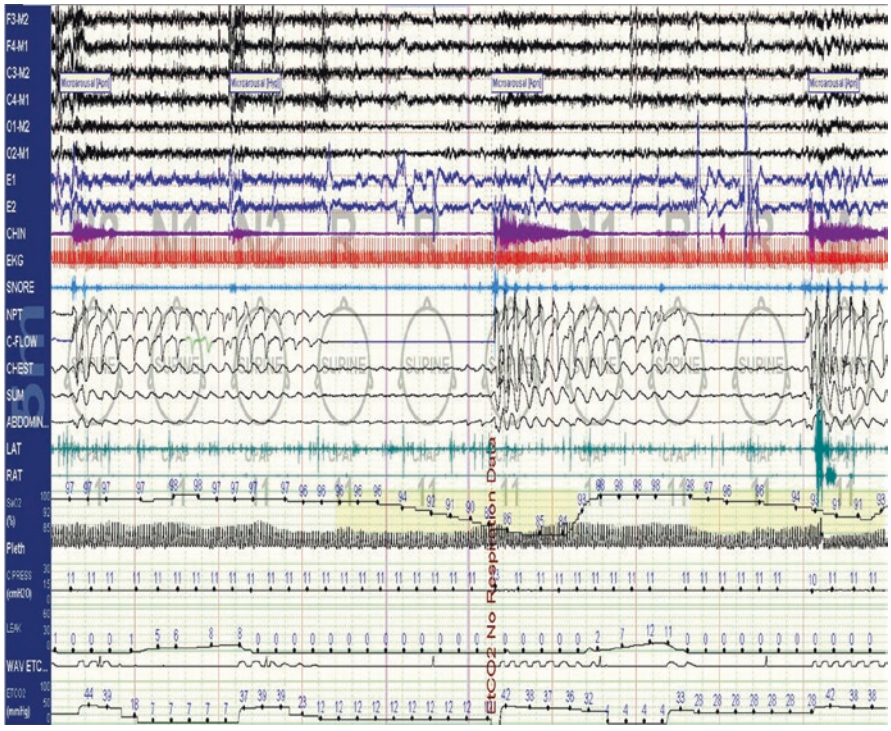


Fig. 11.2 Polysomnogram snapshot from a patient with obstructive sleep apnea with REM-dominant obstructive events. Ten-minute compression, each vertical line is 30 s

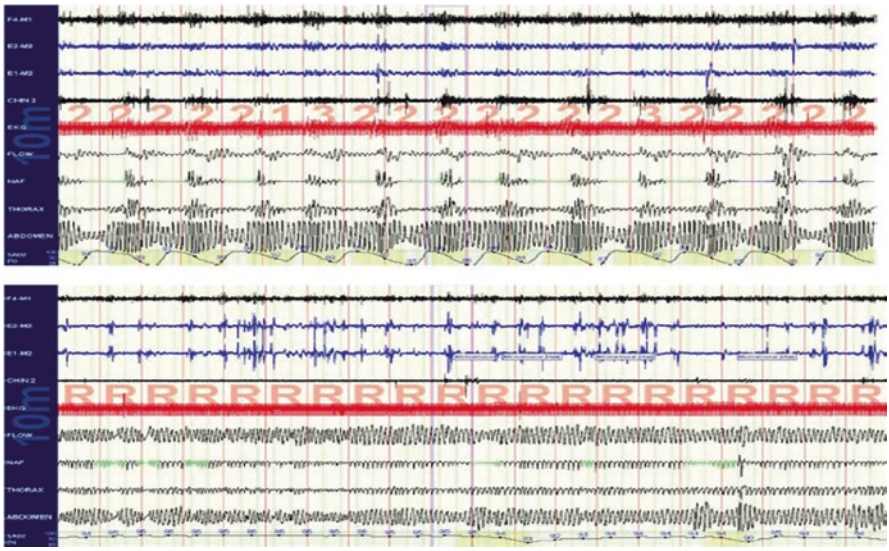


Fig. 11.3 Central sleep apnea with long cycle periodic breathing. Polysomnogram snapshot from a patient with congestive HF. Ten-minute compression, each vertical line is 30 s. In the top portion, depicting non-REM sleep, there is self-similar waxing and waning flow and effort with 45–50 s cycle duration. Breathing stabilizes during the bottom portion, depicting REM sleep

arousal threshold amplifies the abnormalities of upper airway anatomy or breathing control. Obesity is the primary risk factor for OSA. However, 20–40% of patients with OSA are not obese. Certain craniofacial features, such as retrognathia or narrowed nares also contribute. HF is the main cause of CSA.

Pathophysiology

Obstructive Sleep Apnea

There have been significant advances in the understanding of OSA over recent decades. The pathogenesis of OSA is attributed to complex interactions between upper airway anatomy, arousal threshold, and breathing control that alter the balance between forces promoting airway patency and those promoting airway collapsibility. There are four main physiological traits or OSA phenotypes that are recognized: (1) upper airway anatomy that is narrow and/or collapsible; (2) inadequate responsiveness of the upper airway dilator muscles during sleep; (3) low respiratory arousal threshold; and (4) unstable or overly sensitive respiratory control, a concept referred to as high loop gain (defined as a large ventilatory response to a change in ventilation) [12–15].

Anatomic contributions from adipose deposition, soft tissue (i.e., tonsillar hypertrophy, enlarged uvula), craniofacial features (such as small mandible or maxilla and/or narrowed or obstructed nares), large base of tongue, and elongated palate contribute to a reduced cross-sectional area of the upper airway during sleep The impact of body and neck position on the airway lumen, recumbent fluid shifts from the lower extremities and trunk, and snoring-related vibratory damage to intramucosal nerve endings may also contribute to airway collapsibility in OSA. During wakefulness, the pharyngeal dilator muscles (which include the genioglossus) have increased tone known as the "wakefulness drive." These dilator muscles relax during sleep, most pronounced in rapid eye movement (REM) sleep, contributing to upper airways resistance [16, 17]. Other factors that promote upper airways resistance during sleep include negative intrathoracic pressure during apneic events and decreased tracheal tug from reduced lung volumes (especially during supine body position) [18, 19]. Repeated arousals lead to fragmented sleep and result in chronic partial sleep deprivation and possible symptoms of insomnia, excessive daytime sleepiness, decreased quality of life (QOL), mood disturbance, impaired vigilance and attention, and increased risk of motor vehicle and workplace accidents [20]. High loop gain is an exaggerated response of the respiratory system to slight increases in the CO_2 level. An event of OSA causes hypoxia and hypercapnia, leading to an increase in neuronal activity and ventilatory drive and arousal. The increased ventilatory drive in turn results in negative luminal pressure, further increasing the likelihood of airway collapse [21].

Chronic exposure to SDB events is associated with a profile of systemic disturbances that include increased inflammation, oxidative stress, sympathetic activation, fatty acid lipolysis, alterations of the hypothalamic–pituitary axis, endothelial dysfunction, and coagulopathy. These pathophysiologic disturbances are the intermediaries linking OSA to cardiac and metabolic disease.

Central Sleep Apnea

CSA is attributed to heightened peripheral and central chemo-responsiveness. In CSA there is a tendency toward hyperventilation during both sleep and wake, resulting in relative hypocapnia. As a result, one's CO_2 level during sleep (most notably during non-REM) falls below their apnea threshold (hypocapnic apnea threshold, HAT), the level of PaCO2 below which a central apnea occurs. Pulmonary congestion which may occur in HF also contributes to a state of relative hyperventilation [22, 23]. The cycle length (the cycle duration of the periodic breathing pattern) in CSA is proportional to the lung to chemoreceptor circulation time, and inversely proportional to cardiac output. In systolic HF, periodic breathing cycle length is longer (>45 s cycles), while short cycle periodic breathing (<45 s cycles) is seen in idiopathic CSA, high altitude, and complex apnea (when LVEF is preserved) (Fig. 11.4, Table 11.1).

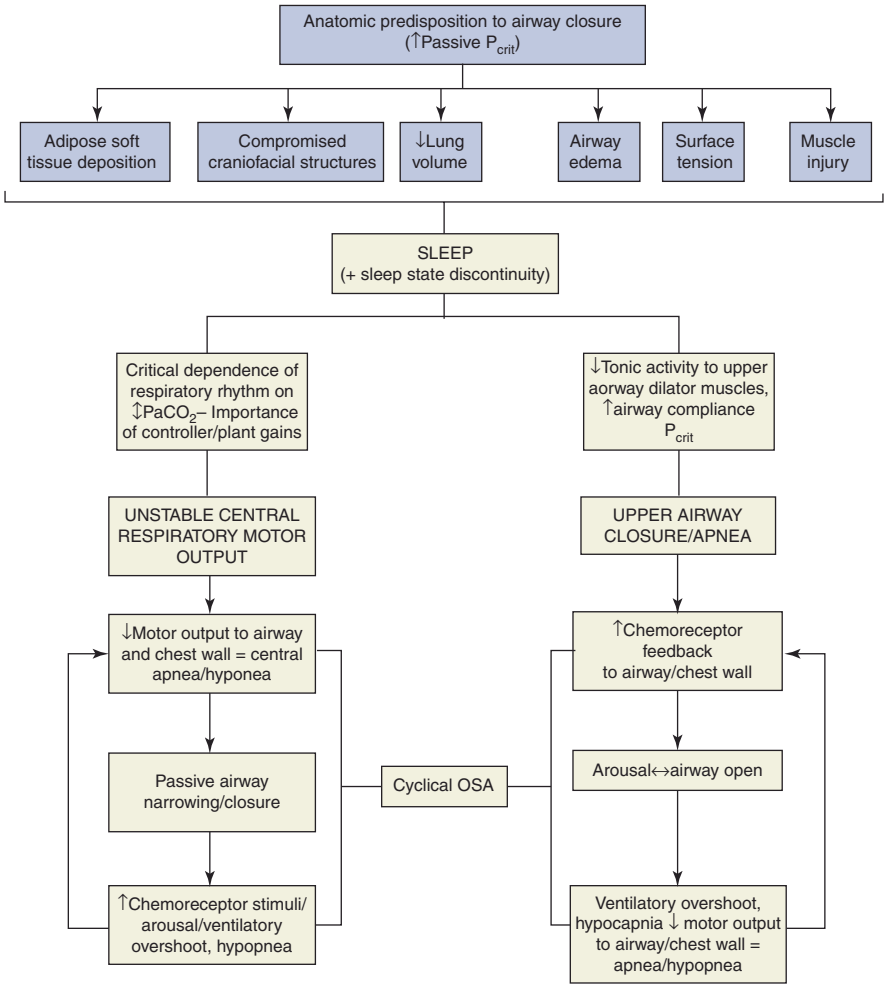


Fig. 11.4 Schematic demonstrating proposed pathogenetic mechanisms leading to obstructive apneas [24]

Table 11.1 OSA and CSA features [25]

Sleep apnea types and characteristics		
Feature	Obstructive sleep apnea	Central sleep apnea, periodic breathing
Fundamental mechanism	Upper airway collapsibility	Decreased (hypercapnic CSA) or increased sensitivity (hypocapnic CSA and PB) of the respiratory chemoreflex
Dominant presentation	Excessive sleepiness/fatigue	Insomnia/fragmented sleep
Sleep stage of expression	REM > NREM (can be nearly exclusively REM)	NREM (can be exclusively NREM)
Visual morphology	Absent or reduced flow during ongoing or increasing effort	Absent of reduced flow with absent effort, or concordant reduction in flow and effort
	Deep "V" shaped oxygen desaturations Individual respiratory event durations tend to vary	PB has metronomic waxing-waning appearance and regular cycle time from one peak or one event to the next (clone/mirror image-like)
	Can cause long periods (tens of seconds) of partially obstructed flow	"Band" oxygen desaturations (consecutive dips are identical over a several minutes)
		Opiates induced NREM-dominant mixtures of obstructive and central apnea, with an ataxic pattern
Associated conditions	Obesity, anatomical narrowing, male gender, and race effects	Heart failure (systolic or diastolic), high altitude. Race effects not known at sea level.
		Hypertrophic cardiomyopathy-typically mixed obstructive and central
		Opiates have unique features—slightly hypercapnic
		COPD, neuromuscular disorders, neurological disorders for hypercapnic CSA
		Craniovertebral junction anomalies including Arnold-Chiari malformation

(continued)

Table 12.1 (continued)

Sleep apnea types and characteristics		
Feature	Obstructive sleep apnea	Central sleep apnea, periodic breathing
Treatments	CPAP, BPAP for pressure intolerance, surgery, treatment of upper airway allergies, oral appliance, Provent (nasal EPAP), and weight loss	Adaptive ventilation, O_2 minimizing hypocapnia, acetazolamide, sedatives
	Surgical approaches include tonsillectomy, nasal turbinate reduction or septoplasty, advancing the tongue base, maxillomandibular advancement	
	Soft tissue reduction uses various techniques, each with relative advantages and disadvantages, including the "standard knife", laser, coblation, somnoplasty.	
Complications	Hypertension, congestive heart failure, stroke, depression, metabolic syndrome and hyperglycemia—these are relatively well established	Atrial fibrillation—possible. This arrhythmia is common (40%) even in young individuals with idiopathic central sleep apnea
	Triggering of cardiac arrhythmias including atrial fibrillation	Progression of heart failure remains unresolved Oxygen desaturations usually not severe enough to cause pulmonary hypertension
	Possibly cancers, via hypoxia induced increases in vascular endothelial growth factor (VGEF) increases; supportive data in humans and rodents is very preliminary but biological plausible	
	Pulmonary hypertension	

SDB evaluation, Screening, and Diagnosis

Despite growing awareness about SDB from health professionals and the public, upwards of 80% of clinically relevant sleep apnea remains undiagnosed, disproportionally impacting women, ethnic and minority groups, and the elderly [26, 27], and routine screening for SDB in the general primary care practice has not been established due to insufficient supporting evidence [28]. However, in subgroups of patients for whom there is a known high prevalence of SDB (as shown in Table 11.2), including those encountered in cardiology practices, screening and evaluation for SDB are strongly advised [31].

Questionnaires and clinical tools can inform screening for sleep apnea and provide insight regarding risk stratification, but no questionnaire or tool is able to definitively rule in or out SDB. Validated questionnaires (shown in Figs. 11.5, 11.6, and 11.7 and Table 11.3) include the following: (1) The Epworth Sleepiness Scale, an eight-question scale used to measure the degree of daytime sleepiness; (2) The Berlin Questionnaire, a ten-question test (divided into three categories) used to

Table 11.2 High-risk conditions for SDB

Severe obesity (BMI > 35 kg/m^2)
Preoperative screening for gastric bypass surgery
Congestive heart failure
Hypertrophic cardiomyopathy
Recurrent atrial fibrillation
Nocturnal dysrhythmias
Treatment-resistant hypertension
Polycystic ovarian syndrome
Congestive heart failure
Type 2 diabetes
Stroke
Pulmonary hypertension
Commercial drivers
Adults with chromosomal abnormalities, such as Down's Syndrome [29, 30]

How likely are you to doze off in the following situations?	No Chance	Slight Chance	Moderate Chance	High Chance
Sitting and reading	❍ 0	❍ 1	❍ 2	❍ 3
Watching television	❍ 0	❍ 1	❍ 2	❍ 3
Sitting inactive, in a public space	❍ 0	❍ 1	❍ 2	❍ 3
Lying down to rest in the afternoon when circumstances permit	❍ 0	❍ 1	❍ 2	❍ 3
Sitting and talking to someone	❍ 0	❍ 1	❍ 2	❍ 3
Sitting quietly after a lunch without alcohol	❍ 0	❍ 1	❍ 2	❍ 3
As a passenger in a car for an hour without a break	❍ 0	❍ 1	❍ 2	❍ 3
In a car, while stopped for a few minutes in traffic	❍ 0	❍ 1	❍ 2	❍ 3
			TOTAL SCORE:	

Fig. 11.5 The Epworth Sleepiness Scale [30, 32]

predict high or low risk for OSA; and (3) The STOP-Bang, a questionnaire focused on identifying SDB risk in the perioperative population. Overnight oximetry is not considered an adequate screening tool for SDB due to loss of data and risk of false-negative (which can be seen in mild, non-hypoxic SDB) and false-positive (movement artifacts) results.

The sleep study, including attended polysomnogram (PSG) and home sleep testing (HST) using portable monitoring (PM), remains the gold standard method to diagnose SDB. Attended or full montage PSG provides information on body position, respiratory pattern (flow and effort), oximetry, heart rate/rhythm (using single lead electrocardiogram), sleep architecture, motor activation, parasomnia activity, and when appropriate, capnography. Type III PMs, which include at least oximetry, nasal pressure and airflow, and respiratory effort (using plethysmography) and usually also body position (via an accelerometer), have been validated to

Berlin Questionnaire

BMI

Male / Female

Category 1

1. Do you snore?
   a. Yes
   b. No
   c. Don't know If you snore
2. Your snoring is:
   a. Slightly louder than breathing
   b. As loud as talking
   c. Louder than talking
   d. Very loud – can be heard in adjacent rooms
3. How often do you snore
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never
4. Has your snoring ever bothered other people?
   a. Yes
   b. No
   c. Don't Know
5. Has anyone noticed that you quit breathing during your sleep?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

Category 2:

6. How often do you feel tired or fatigued after your sleep?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never
7. During your waking time, do you feel tired, fatigued or not up to par?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

Fig. 11.6 The Berlin Questionnaire [33]

8. Have you ever nodded off or fallen asleep while driving a vehicle?
   a. Yes
   b. No

If yes: 9. How often does this occur?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

Category 3:

10. Do you have high blood pressure?
   Yes
   No
   Don't know

Scoring :

High Risk: if there are 2 or more Categories where the score is positive.
Low Risk: if there is only 1 or no Categories where the score is positive.

Category 1 is positive if the total score is 2 or more.
   Item 1: if 'Yes', assign 1 point
   Item 2: if 'c' or 'd', assign 1 point
   Item 3: if 'a' or 'b', assign 1 point
   Item 4: if 'a', assign 1 point
   Item 5: if 'a' or 'b', assign 2 points

Add points.

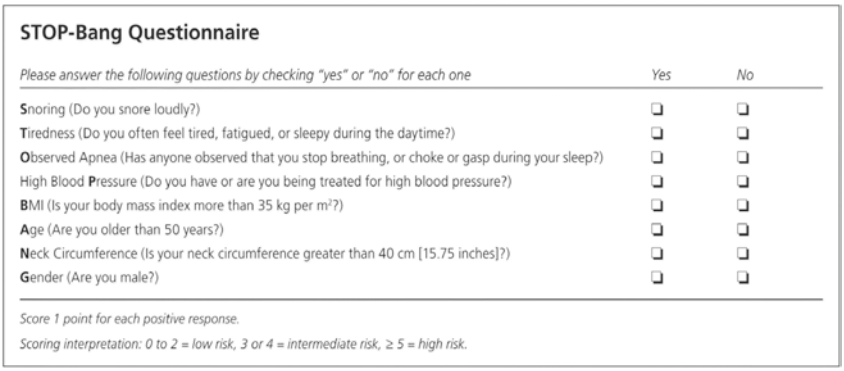
Category 2 is positive if the total score is 2 or more points
   Item 6: if 'a' or 'b', assign 1 point
   Item 7: if 'a' or 'b', assign 1 point
   Item 8: if 'a', assign 1 point
   (item 9 should be noted separately).

Add points.

Category 3 is positive if the answer to item 10 is 'Yes' OR if the BMI of the patient is greater than 30kg/m 2.

Fig. 12.6 (continued)

evaluate for OSA when there is a high pretest probability of moderate or severe disease as determined by a comprehensive sleep evaluation (clinical history, exam, comorbidities). PMs, which lack EEG data and thus provide no sleep stage information or arousal scoring, underestimate the true severity of SDB compared to attended PSG. PM is not recommended for use in conditions concerning for hypoventilation (such as neuromuscular disorders or advanced respiratory disease), cognitive impairment that may preclude ability to perform home testing, or CSA.



STOP-Bang Questionnaire

Please answer the following questions by checking "yes" or "no" for each one	*Yes*	*No*
Snoring (Do you snore loudly?)	❑	❑
Tiredness (Do you often feel tired, fatigued, or sleepy during the daytime?)	❑	❑
Observed Apnea (Has anyone observed that you stop breathing, or choke or gasp during your sleep?)	❑	❑
High Blood **P**ressure (Do you have or are you being treated for high blood pressure?)	❑	❑
BMI (Is your body mass index more than 35 kg per m^2?)	❑	❑
Age (Are you older than 50 years?)	❑	❑
Neck Circumference (Is your neck circumference greater than 40 cm [15.75 inches]?)	❑	❑
Gender (Are you male?)	❑	❑

Score 1 point for each positive response.

Scoring interpretation: 0 to 2 = low risk, 3 or 4 = intermediate risk, ≥ 5 = high risk.

Fig. 11.7 STOP-Bang Questionnaire to assess the risk of obstructive sleep apnea [34]

Table 11.3 Clinical sleep apnea questionnaire and diagnostic accuracy [35]

Questionnaire	Summary of questionnaire contents	Diagnostic accuracy compared with AHI (>15 events/h) [267]
Berlin Questionnaire	10 questions pertaining to the following 3 symptoms/signs:	• *Sensitivity*: 0.77 (0.73–0.81)
	• Snoring	• *Specificity*: 0.44 (0.38–0.51)
	• Daytime sleepiness	
	• Hypertension	
	Patients classified by score as having low risk or high risk of OSA	
STOP Questionnaire	4 questions regarding the following signs/symptoms:	• *Sensitivity*: 0.89 (0.81–0.94)
	• Snoring	• *Specificity*: 0.32 (0.19–0.48)
	• Sleepiness	
	• Observed apneas or choking	
	• Hypertension	
STOP-BANG Questionnaire	4 questions regarding signs/symptoms plus 4 clinical attributes:	• *Sensitivity*: 0.90 (0.86–0.93)
	• Snoring	• *Specificity*: 0.36 (0.29–0.44)
	• Sleepiness	
	• Observed apneas or choking	
	• Hypertension	
	• Obesity (BMI > 35 kg/m^2)	
	• Age (>50 years)	
	• Neck size	
	• Sex	
	Patients classified as low, intermediate, or high risk for OSA	
Epworth Sleepiness Scale	8 questions asking patients to rate the likelihood of falling asleep in various daytime contexts	• *Sensitivity*: 0.47 (0.35–0.59)
	Patients classified as having normal sleep, average sleepiness, or severe and possibly pathologic sleepiness	• *Specificity*: 0.62 (0.56–0.68)

AHI indicates apnea–hypopnea index, *BMI* body mass index, *OSA* obstructive sleep apnea

Treatment for SDB in General

The treatment goals for SDB include normalization of the AHI, preservation of oxygenation, resolution of symptomatology, as well as reduction in attributable cardiovascular disease morbidity and mortality.

Continuous positive airway pressure (CPAP) is considered the gold standard treatment for SDB, especially effective in OSA [28, 30, 36]. Depending upon disease severity, patient preference and comorbidities, and upper airway and physical exam characteristics, a mandibular advancement device (MAD or dental appliance) may be a PAP alternative [37–39]. Most upper airway surgeries are considered adjunctive, not curative for SDB, as they can improve upper airway obstruction to allow for lower efficacious PAP pressure. Maxillomandibular advancement (MMA), a jaw advancement surgery, can provide long-term effective control of OSA in appropriate candidates [40]. Hypoglossal nerve stimulation can be considered in ideal candidates, specifically those with moderate to severe OSA intolerant to CPAP, without severe obesity who have anterior-posterior predominant retropalatal collapse as determined by drug-induced laryngoscopy. The therapy, which involves an implantable device (similar to a pacemaker) that senses chest wall movement and stimulates the hypoglossal nerve during sleep, enlarges the upper airway via contraction of the genioglossus muscle and protrusion of the tongue [41, 42].

Treatment for CSA can be more challenging to optimize. In addition to positive airway pressure treatment, off-label therapies to stabilize breathing and prevent ventilatory overshoot may be needed. Such treatments include medications (acetazolamide, topiramate), carbon dioxide modulation, and supplemental oxygen [43, 44]. Phrenic nerve stimulation, a relatively new therapy for CSA, can also be considered [45].

Weight loss can lower the AHI and should be recommended in overweight and obese patients with SDB [46, 47]. A sleep study should be repeated after substantial weight loss (≥10% of body weight) to assess extent of residual SDB and whether treatment is still indicated or needs adjustment [29]. Body position during sleep impacts the size and patency of the upper airway, typically more narrowed and compromised in supine vs. side position [48, 49]. Positional training with maximization of side and avoidance of supine sleep may help in both OSA and CSA to minimize upper airways resistance and respiratory instability [46, 50–52]. Sedative hypnotic medications can be considered to increase arousal threshold and also to stabilize breathing [44].

Sleep Apnea and Heart Failure

Over 50% of patients with HF have comorbid SDB [53–58]. Moderate to severe SDB has been found to occur in 66% of patents with asymptomatic left ventricular systolic dysfunction [59]. More varied prevalence of SDB has been reported when the LV ejection fraction (EF) is preserved (HFpEF). Lanfranchi and colleagues found that only 25% of patients with HFpEF have SDB, while other studies report similar prevalence of SDB between HFrEF and HFpEF [59, 60]. The prevalence of diastolic dysfunction also increases with worsening severity of OSA [61]. CSA predominates in those with HFrEF, while OSA may be more common in HFpEF. Features of both types of SDB frequently coexist in the same patient, though OSA with high loop gain pathophysiology may be underrecognized clinically.

There are complex bidirectional interactions between HF and SDB pathophysiologies. Relative hypocapnia from pulmonary vascular congestion and heightened central and peripheral chemo-sensitization in HF contributes to ventilatory overshoot, respiratory instability, and central apnea. The prolonged circulation time in HFrEF leads to a long cycle length of PB [62, 63]. SDB promotes sympathoexcitation contributing to catecholamine induced myocyte injury; causes both systemic and pulmonary hypertension; and results in ischemic and inflammatory injury, cardiac remodeling, and cardiac interstitial fibrosis. Chronic exposure to SDB events impacts left ventricular filling resulting in reduction in stroke volume and cardiac output [64], contributes to diastolic dysfunction and chronic pressure overload [65], and is associated with increases in concentric cardiac remodeling (ratio of LV mass and volume) [66]. Echocardiographic indices of dysfunctional diastole (increased E/A ratio or the ratio of peak velocity blood flow from LV relaxation in early diastole to peak velocity flow in late diastole due to atrial contraction and reduced isovolumic relaxation) are more pronounced in patients with SDB compared to controls [61, 67, 68].

Asymptomatic LV dysfunction is a known predictor of incident symptomatic HF, and SDB is a likely contributing factor. In fact, prospective studies have demonstrated that SDB independently predicts new-onset HF in men [69] and in women [70]. A prospective study in which men and women without HF at the time of baseline PSG were followed over nearly 9 years showed that OSA predicted incident HF in men, but not in women, and that men with severe OSA had a 58% increased likelihood of developing HF compared to men without OSA [69]. Prospective data over 14 years from the Atherosclerosis Risk in Communities Study (ARIC) showed a 30% increased incidence of HF or death in women with vs. without SDB [70].

Cheyne-Stokes respiration (CSR) is a predictor of increased HF severity and worse prognosis [71]. The Outcomes of Sleep Disorders in Older Men Study (MrOS) demonstrated that in older men, the presence of CSA and CSR predicted a nearly twofold increased incidence of HF [72]. In patients with HF, SDB is also a predictor of HF exacerbations, impaired QOL, worsening functional status, more frequent hospitalizations, arrhythmias, and increased mortality in patient. A single-center prospective study of nearly 1000 patients with chronic stable HFrEF treated with guideline-based therapy found that patients with comorbid CSA had the lowest survival [73].

Treatment of SDB in HF

Regardless of SDB phenotype, cardiopulmonary and volume status should be medically optimized prior to pursuit of outpatient PSG, as rostral fluid shifts from the lower extremities to the neck and upper airway can worsen obstruction and pulmonary edema can exacerbate sleep hypoxemia and promote respiratory instability [53, 74, 75]. A meta-analysis evaluating the impact of cardiac resynchronization therapy (CRT), also called **biventricular pacing**, on sleep apnea in patients with HFrEF, found a significant reduction in AHI in CSA but not OSA [76]. In patients with HF, the presence of OSA has been shown to be associated not only with a decreased response to CRT but also with an increase in all-cause mortality [77]. Weight loss, when appropriate, and exercise are advised. Compression stockings can help reduce rostral fluid redistribution.

In HF as in any condition, treatment approaches for SDB and demonstrated clinical benefits vary depending on SDB phenotype.

In patients with HF and OSA, CPAP treatment has been show to promote cardiovascular benefits. CPAP use during sleep lowers awake sympathetic nervous system activity [78]. Several studies have shown that CPAP treatment improves LVEF [79–81], including a meta-analysis that found treatment of OSA with CPAP was associated with a 5.2% improvement in LVEF [82]. The reported impact of CPAP use on diastolic function has been inconsistent. While some studies have found no improvement [79], one RCT demonstrated improvement in diastolic dysfunction with CPAP treatment for OSA in patients with HFpEF [83]. In a large retrospective observational study of over 30,000 Medicare beneficiaries (from 2003 to 2005) with newly diagnosed HF and without prior diagnosis of SDB, Javaheri and colleagues found that SDB was highly underdiagnosed (only 4% were suspected to have SDB and only 2% of the cohort were tested). However, those subjects diagnosed with and treated for SDB had fewer readmissions, reduced overall health care cost, and reduced mortality [84]. In light of the available evidence supporting a pathophysiologic relationship between SDB and cardiovascular disease, the American Heart Association recommends screening for and if present treating SDB in patients with HF [85, 86].

Treatment for CSA in HF is more complicated and optimal treatment remains unclear.

While effective in the vast majority of OSA patients, CPAP is only partially effective in patients with CSA. When periodic breathing persists and CSA is not controlled, CPAP use in patients with HF may actually be harmful [87]. Respiratory stimulants, such as theophylline and acetazolamide, can stabilize breathing control in CSA, demonstrated in small studies and RCTs [88, 89]. Heart transplant cures CSA [90].

Adaptive servo-ventilation (ASV), a mode of non-invasive ventilatory treatment created for patients with central or complex sleep apnea, uses an algorithm that continuously adapts to the patient's breathing pattern by delivering an auto-adjusting pressure support to stabilize periodic breathing [91]. The Treatment of Predominant Central Sleep Apnoea by Adaptive Servo Ventilation in Patients With Heart Failure

(SERVE-HF) trial evaluated the impact of ASV (using the RESMED device) on all-cause mortality, life-saving cardiovascular interventions, or unplanned hospitalizations for worsening HF in 1325 patients with symptomatic HFrEF (<45%) and coexisting moderate to severe CSA. Results revealed a significantly higher (34%) increase in CVD mortality in the ASV arm compared to controls, with the most pronounced findings in those with lower LVEF [92]. Potential explanations for the results include notably low adherence in the treatment arm, suboptimal control of CSA with ongoing pressure cycling, excessive ventilation, and possible adverse effects on hemodynamics in vulnerable patients. Given results of the SERVE-HF trial, ASV is felt to be contraindicated to treat predominantly CSA in patients with HFrEF with EF < 35% [93].

Another ongoing Multi-Centre, Randomized Study, the Effect of ASV on Survival and Hospitalizations (ADVENT-HF), anticipated to be completed in August 2021, is testing an alternative ASV device in patients with OSA or CSA. Preliminary data shows superior adherence compared to prior studies.

Use of nocturnal oxygen supplementation (NOS) has been extensively evaluated in CSA. NOS stabilizes chemo-sensitization, reducing respiratory instability, loop gain, and periodic breathing and mitigates sleep hypoxemia and has also been shown to improve exercise capacity [94, 95]. Randomized studies have shown that NOS decreases muscle sympathetic nerve activity (MSNA), LVEF, and QOL [96, 97]. However, additional studies including RCTs are needed to clarify the role of NOS in the treatment of CSA in HF.

Transvenous unilateral phrenic nerve stimulation (**remedē System**, Respicardia, Inc., Minnetonka, MN, USA) is a potential therapy for patients with moderate to severe CSA. The system is a battery-powered device with two leads that is implanted in the upper chest. One lead provides therapy through stimulation of the phrenic nerve to cause movement of the diaphragm, while the other lead senses breathing via changes in intrathoracic pressure. Safety and efficacy data have been published [98]. The Pivotal trial, a prospective, multicenter, RCT of 151 patients with CSA (AHI > 20 events/h with at least 50% central apneas; 64% of those enrolled had HF, including both HFrEF and HFpEF), randomized to 6 months of transvenous unilateral phrenic nerve stimulation (PNS) vs. no stimulation, demonstrated improved severity of sleep apnea (reduction in AHI from 51% to 11%, $p < 0.001$; CAI, ODI), QOL, and daytime sleepiness in the treatment arm. At 12 months, 91% of subjects remained without serious therapy-related adverse events [99].

Sleep-Disordered Breathing and Arrhythmias

Data accumulated from epidemiologic and clinic-based studies shows that up to 60% of patients with SDB demonstrate some types of cardiac arrhythmia during sleep [100–102]. SDB is associated with a variety of cardiac rhythm disturbances including AF, ventricular fibrillation (VF), ventricular tachycardia (VT), premature ventricular complexes (PVCs), accelerated idioventricular rhythm (AIVR), and pronounced bradycardia and sinus arrhythmia [103]. Sudden cardiac death (SCD) has

also been linked to SDB. SDB events have been shown to directly trigger arrhythmia, including episodes of VT and AF, with a 17-fold increase in rate of arrhythmias following apneic episodes compared to normal breathing [104].

There are several proposed mechanisms, supported by experimental data from human and animal studies, linking SDB to cardiac arrhythmia, including intermittent hypoxia and hypercapnia, dysfunctional endothelium, atrial remodeling, inflammation, hypercoagulability, and autonomic dysfunction. Autonomic nervous system fluctuations from OSA may precipitate conduction changes, predisposing the atria to arrhythmia and resulting in atrial remodeling [105]. Repeated changes in intrathoracic pressure that occur during sleep apnea events impact cardiac afterload, atrial size, and also contribute to atrial remodeling [106]. Since increased respiratory effort is absent in CSA, other mechanisms causing sympathoexcitation including intermittent hypoxia, catecholamine excess, and repeated arousals are implicated [107].

Sleep Apnea and Atrial Fibrillation

AF is the most common cardiac arrhythmia. As seen with OSA, AF increases with age and BMI, though there is a stronger association between OSA and AF than between BMI and AF [108]. Both OSA and AF are often asymptomatic and thus are believed to be significantly underdiagnosed conditions.

Multiple epidemiologic, clinic-based, and experimental studies have established a direct association between SDB and AF [109]. Data from the Sleep Heart Health Study (SHHS) and the Osteoporotic Fractures in Men (MrOS) cohort of community dwelling older men show strong associations between SDB severity and arrhythmia [110, 111]. Findings from SHHC showed a two- to threefold higher odds of developing AF in CSA patients compared to controls [112], while data from MrOS showed a fivefold higher odds of AF in those with CSR compared to controls [111]. OSA severity and nocturnal hypoxia are strong predictors of new-onset AF [109]. AF patients with OSA compared to those without have a higher risk of hospitalization and more severe symptomatology [113]. Analysis of SHHS data reveals a temporal relationship between SDB events and episodes of arrhythmia, with an increased risk for paroxysms of AF observed during the 90 s after a respiratory disturbance as compared to normal breathing [104].

The 5-year AF recurrence rate following catheter ablation or cardioversion in general ranges from 25% to 60% [114]. The coexistence of OSA increases the AF recurrence rate by 40% [115].

While AF and OSA share important risk factors and comorbidities, including obesity, increasing age, HTN, and diastolic dysfunction, evidence supports independent causal effects of OSA on cardiac function and structure [116, 117]. Multiple observational studies support that CPAP decreases the risk of AF recurrence following cardioversion and ablation [9, 113, 118–120].

In a cohort ($n = 426$) of AF patients who underwent pulmonary vein isolation (PVI), in which 62 had confirmed OSA, those using CPAP ($n = 32$) had a significant AF-free interval (72% vs. 37%) compared to the untreated OSA patients ($n = 30$) and their

AF-free survival rate was similar to those without OSA [119]. A meta-analysis (which included eight studies, one of which was a RCT) of 698 CPAP users and 549 non-CPAP users after an AF intervention found a 42% decreased risk of AF in those treated with CPAP, with the most pronounced benefit seen in younger, male, and obese patients [121]. Evaluation for and treatment of OSA should be pursued in patients with AF, especially in those with recurrent arrhythmia after cardioversion or ablation procedures.

OSA, Bradycardia, and Sick Sinus Syndrome

SDB event-associated increases in vagal tone promote bradycardia and atrioventricular blocks and contribute to sick sinus syndrome (SSS). In a cohort of 98 patients with implanted pacemakers, 59% were found to have undiagnosed SDB identified by PSG [122, 123]. In patients with moderate to severe OSA who had loop recorders implanted, underwent two 24-Holter recordings, and were followed for 16 months, mostly nocturnal cardiac arrhythmias were detected in 47% of participants, with nearly half displaying severe bradycardic events or prolonged sinus pauses. Arrhythmias were significantly reduced by CPAP, within 8 weeks of use. There was significant weekly variation in the arrhythmia episodes such that the Holter recordings were insufficient at detecting the true prevalence or the beneficial impact of CPAP treatment [124]. A 2-year prospective study of 38 participants (mean age 67, 68% male and 58% with comorbid HTN) with SSS found a considerably higher prevalence of SDB (32%, AHI > 10/h) as compared to in the general population [125].

OSA and Ventricular Arrhythmias

SDB has also been associated with ventricular arrhythmias including ventricular premature complexes, VT, and VF. Patients with OSA have significantly higher frequency of premature ventricular contractions (PVCs) compared to non-OSA patients (67% vs. 0–12%) [100], with the majority occurring during sleep and in association with apneic, not hypopneic episodes [126, 127]. Conversely, in CSA, hypopneas, rather than apneas, are more frequently associated with ventricular ectopy [128]. Namtvedt et al. [129] studied 486 subjects of whom 271 (56%) had OSA and 72 (14.8%) had severe OSA (as defined by AHI ≥ 30/h). Those with increasing severity of OSA had more ventricular premature complexes at night and during the day compared to patients without OSA [129]. Night-time hypoxemia, acidosis, increased sympathetic tone, and alterations in intrathoracic pressure during sleep are plausible explanations for OSA-associated nocturnal ventricular arrhythmias [130–132]. CPAP treatment in OSA has been shown to decrease the 24-h heart rate and reduce PVC frequency during sleep [133, 134].

SDB and Stroke

SDB is not only extremely common, impacting nearly three-quarters of post-stroke patients [135–140], but is associated with worse outcomes, including higher mortality [141–143] and worse functional status [144, 145]. A majority of stroke patients have OSA, with only 7% having primarily CSA, though, as described earlier, central features tend to be underrecognized [146]. Several small studies, one of which identified a relationship only in men but not women, have found that patients with wake-up strokes have a higher prevalence of SDB than patients with non-wake-up strokes [147–149]. Multiple studies have established that SDB is an independent risk factor for incident (twofold increased risk) and recurrent stroke [150]. Given the high prevalence of SDB in patients with stroke, the American Academy of Sleep Medicine's Adult Obstructive Sleep Apnea Task Force considers patients with stroke as a high-risk group for SDB and recommends performing a sleep study in stroke or TIA patients with SDB symptoms [30].

In light of the relationship between SDB and stroke, many studies have evaluated whether CPAP, the gold standard treatment for SDB, improves outcomes after stroke or TIA. Older studies, including several RCTs, have shown inconsistent results, attributed to small numbers with insufficient sample size to detect a treatment effect [139, 140, 151, 152]. More recent studies remain conflicted, but suggest that CPAP treatment, when initiated early for ischemic stroke patients with moderate to severe OSA, improves long-term outcomes [153, 154]. Sleep Apnea Cardiovascular Endpoints (SAVE, 2016) trial, which included 2717 subjects between 45 and 75 years old with CAD or cerebrovascular disease and moderate to severe sleep apnea who were randomized to CPAP plus usual care verses usual care alone and followed for a mean of 3.7 years, found no difference in stroke incidence (a secondary endpoint and component of the primary endpoint) between the two groups [11]. However, interpretation of the study results was limited by exclusion of patients with excessive daytime sleepiness or recent stroke and suboptimal CPAP use in the intervention group (3.3 h overall mean nightly duration, with only 42% using CPAP for ≥4 h) leaving significant residual untreated disease and diminishing ability to identify a difference between the study arms.

Based on the accumulating evidence regarding a relationship between OSA and stroke and suspected benefit of OSA treatment on stroke outcomes, the American Heart Association/American Stroke Association 2014 Guidelines for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack states "1. A sleep study might be considered for patients with an ischemic stroke or TIA on the basis of the very high prevalence of sleep apnea in this population and the strength of the evidence that the treatment of sleep apnea improves outcomes in the general population 2. Treatment with CPAP might be considered for patients with ischemic stroke or TIA and sleep apnea given the emerging evidence in support of improved outcomes" [155].

OSA and Hypertension

Obstructive apneas during sleep are associated with marked oscillations in arterial pressure, heart rate, and ventricular function. In normal human subjects, arterial pressure consistently decreases from waking to stable non-REM sleep. This drop in pressure is primarily attributable to a decline in cardiac output that occurs as a consequence of decreases in heart rate. Numerous clinical studies have established that the normal decrease in arterial pressure is lacking in patients with OSA, i.e., "non-dipping" [9]. Instead, patients with sleep apnea experience repetitive surges in arterial pressure with the peak arterial pressure occurring 5–7 s following apnea termination. These hemodynamic events occur in association with changes in sleep state, chemo-stimulation, lung volume, as well as intrathoracic pressure. These oscillations in pressure may be extreme and are greater in REM than in non-REM sleep even when matching for degree of oxygen desaturation [156]. These pressure increases are further augmented after an arousal from apnea. MSNA recordings from the peroneal nerve in patients with sleep apnea demonstrate a crescendo increase from the beginning to the end of each episode and contribute to increases in systemic vascular tone from a state of chronic sympathoexcitation [130, 157–159]. These patients have elevated levels of circulating catecholamines, angiotensin II, endothelin-1, and aldosterone levels compared with control subjects (Fig. 11.8) [161].

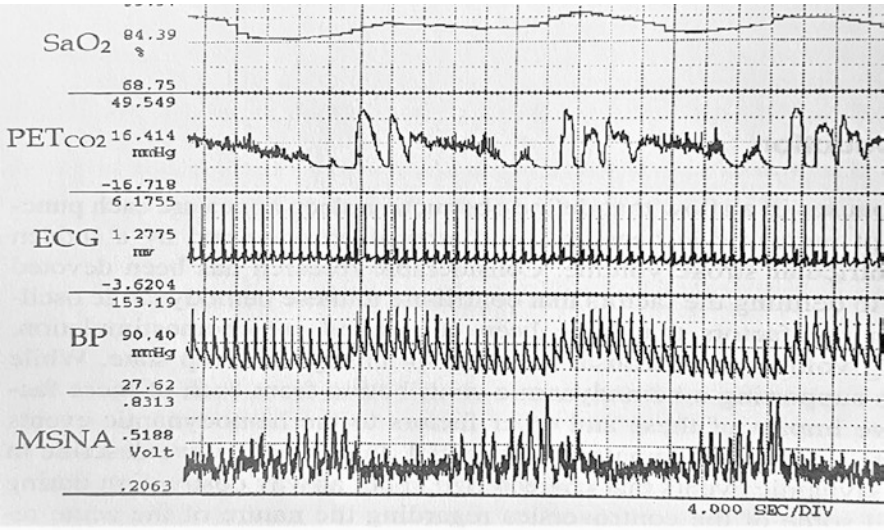


Fig. 11.8 Recording of oxygen saturation (SaO_2), end-tidal carbon dioxide ($PETCO_2$), heart rate on electrocardiogram (ECG), arterial pressure by digital photoplethysmography (PPG, BP below), and muscle sympathetic nerve activity (MSNA) in a patient experiencing repetitive obstructive apneas during sleep. The peaks of arterial pressure occur after the resumption of ventilation [160]

Epidemiologically, the relationship between HTN and SDB is well established. Approximately, 30% of patients with essential HTN have SDB and this increases to 70% in patients with resistant HTN [162]. Moreover, nearly 50% of patients with SDB have HTN [163]. There appears to be a dose response relationship between the severity of OSA and incidence of elevated diurnal blood pressures [164, 165]. The Sleep Heart Health Study (SHHS) ($n = 6132$ patients) found the prevalence of HTN was 59%, 62%, and 67% in mild, moderate, and severe sleep apnea, respectively [166, 167]. The correlation between OSA and isolated diastolic or combined systolic–diastolic HTN was stronger than that for isolated systolic HTN. A pooled meta-analysis estimated a 48% increased risk of HTN among individuals with OSA (pooled Hazard ratio 1.48; 95% CI 1.04–1.91) [168] even after controlling for confounders, such as age and obesity. The seventh national report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure (J.N.C. 7) identified OSA as a treatable cause for HTN [169].

It is widely accepted that the use of CPAP for the treatment of sleep apnea portends benefit with respect to blood pressure control, even though the findings have not been universally consistent. Marin and colleagues followed a large cohort of patients for 12 years and found an increased risk for incident HTN among those with untreated OSA compared with those who adhered to a CPAP regimen and a control group (HR of 2.0 vs HR of 0.7) [170]. In a meta-analysis of nearly 1900 patients prescribed CPAP, there was a relatively small reduction in systolic blood pressure (2.6 mmHg) [171]. However, the presence of uncontrolled or resistant HTN at baseline, as well as excessive daytime sleepiness, may be important predictors of a reduction in blood pressure with CPAP therapy, independent of the severity of OSA [172, 173]. The effectiveness of other therapeutic measures, i.e., oral appliances or upper airway surgery on blood pressure are less well studied. A meta-analysis of patients prescribed mandibular advancement therapy for sleep apnea demonstrated similar reductions in systolic and diastolic blood pressures compared to patients prescribed CPAP [174]. It is well established that even small but sustained decreases in blood pressure are associated with meaningful reductions in major cardiovascular outcomes, such as stroke and HF [175]. It is conceivable that the lack of larger reductions in blood pressure with treatment may be attributable to the presence of vascular remodeling from long-standing disease and may suggest a role for early therapy of sleep apnea as primary prevention of HTN.

OSA and CAD

OSA and CAD share many risk factors, including obesity, advancing age, diabetes mellitus (DM), and male gender, promoting their coexistence in patients. Repetitive episodes of upper airway occlusion in OSA lead to intrathoracic negative pressure around the heart, increased left ventricular afterload, and increased myocardial oxygen consumption at a time when oxygen delivery is compromised due to obstructed breathing and hypoxemia. This imbalance between myocardial oxygen demand and

supply is most heightened during REM sleep when apneas are characteristically longer and the severity of intermittent hypoxemia is greater. REM sleep itself has been associated with nocturnal angina, possibly through associated changes in autonomic tone.

In the SHHS [105], a total of 1927 men and 2495 women 40 years of age or older and free of CAD and HF at the time of baseline PSG were followed for a median of 8.7 years in a prospective longitudinal manner. After adjustment for multiple risk factors, OSA was found to be a significant predictor of incident CAD (myocardial infarction, revascularization procedure, or CAD death) in men less than 70 years of age, but not in older men or in women of any age. Among men 40–70 years old, those with severe OSA (AHI $\geq$ 30/h) were 68% more likely to develop CAD than those without OSA (AHI $\leq$ 5/h).

Conversely, SDB is also highly prevalent in patients with CAD. In a cohort of 1425 patients with confirmed CAD starting cardiac rehabilitation who were screened for SDB, the prevalence of SDB (AHI $\geq$ 5/h) was 83%, with moderate to severe SDB (AHI $\geq$ 15/h) present in 53% [104]. Up to 70% of coronary artery bypass graft (CABG) recipients had an AHI $\geq$ 15/h vs. 33% of those who had not undergone CABG.

There is reasonable evidence that the development of significant hypoxemia during sleep in patients with coexisting OSA and CAD can provoke myocardial ischemia reflected by either nocturnal angina or ST segment depression on ECG monitoring. It also appears that the treatment of OSA with nasal CPAP not only treats the OSA but also significantly reduces the prevalence of accompanying myocardial ischemia during sleep.

Multiple prospective cohort studies have been conducted to better understand the relationship between CPAP use and its protective effects on CAD patients. Treatment with CPAP, even for a duration as short as 2 weeks, can reduce sympathetic activation, inflammation, endothelial dysfunction, and oxidative stress [122]. Increased duration of adherence to CPAP demonstrated greater benefits with respect to CAD end-points, such as myocardial infarction, ACS, or stroke [8, 123, 124]. While this was most evident in patients with severe OSA (mean AHI > 42/h) [176], there were also benefits in patients with mild to moderate disease [177]. Moreover, in a multicenter randomized (CPAP vs. no active intervention) trial including 725 patients without history of cardiovascular events who had moderate to severe OSA (AHI > 20/h) and no daytime sleepiness, a post-analysis of the data found that CPAP resulted in significant reduction of blood pressure and cardiovascular events when it was used for at least 4 h each night [178].

OSA and Sudden Cardiac Death

Sudden cardiac death is defined as an unanticipated natural death from cardiac pathology within 1 h of symptom onset in a person without a known prior condition that would appear to be fatal [179]. According to the American Heart Association

(AHA), SCD is a leading cause of CVD mortality, with greater than 379,000 SCDs occurring per year [180].

SDB and nocturnal hypoxia are associated with SCD. In a single-center study of 10,701 patients who underwent PSG and were followed for up to 5 years, Gami and colleagues discovered independent associations between nocturnal oxygen saturation nadir and SCD [181]. Every 10% decrease in nadir O_2 saturation (cohort mean [SD] 93% + 3) was associated with a 14% increase in the risk of SCD [182]. A 2005 retrospective study demonstrated that the relative risk of SCD was 2.57 times higher between midnight and 6 a.m. in patients with OSA compared to the general population and the risk increased with increasing OSA severity [183]. Mutations in SCN5A that alters repolarization and predisposes individuals to ventricular arrhythmias have become increasingly recognized as a contributing factor in SCD [184, 185]. The electrophysiological changes associated with OSA may contribute to nocturnal SCD in patients with channelopathies and altered repolarization [184, 185].

From multiple studies conducted, there is little statistical evidence showing CPAP prevents ventricular arrhythmias and SCD. A study done by Craig et al. which satisfied all four Cochran criteria showed no significant change in ventricular arrhythmias in OSA patients following initiation of CPAP [133]. In another study, the prevalence of PVCs was reduced but the prevalence of VT remained unchanged [186]. Gami et al. have proposed that OSA patients have higher levels of SCD during sleeping hours compared to the rest of the population since patient with OSA lose the cardioprotective period of increased vagal tone and autonomic stability seen in normal sleep [181–183].

Sleep Apnea and Diabetes

The prevalence of DM has increased dramatically in the last three decades, with an estimated 29 million people, or 9.3% of the U.S. population, suspected of having diagnosed or undiagnosed disease and an additional 86 million adults estimated to have pre-diabetes (Centers for Disease Control and prevention, Diabetes 2014 report card). T2DM represents 90–95% of all cases and with nearly 200,000 annual deaths, it ranks as the seventh leading cause of death in the U.S. Diabetes-related microvascular complications and cardiovascular disease are major causes of morbidity, mortality, and worsening QOL for affected patients [187].

Similarly, the adverse health outcomes associated with another global epidemic—the obesity epidemic—has also reached staggering proportions. Increasing rates of weight gain have no doubt played a pivotal role in the rise of pre-diabetes and T2DM. It is estimated that 35–40% of U.S. adults have "Metabolic Syndrome," a term used to ascribe the many health risks associated with "visceral" or "central" obesity (i.e., elevated blood pressure, insulin resistance, and abnormal lipid profiles) [188].

The increased prevalence of OSA (14–55% of the adult U.S. population) has mirrored the surge in obesity over the past two decades. A 4-year follow-up study of

the Wisconsin Sleep Cohort reported that a modest 10% weight gain predicted 32% increase in the AHI as well as sixfold odds of developing moderate to severe OSA. As a corollary, they reported that a 10% weight loss predicted a 26% decrease in AHI [189].

Not surprisingly, the prevalence of OSA is markedly elevated in both community and clinic-based, diverse ethnic cohorts of patients with T2DM, despite being underdiagnosed. Among individuals with OSA, the prevalence of T2DM has been estimated to be between 15% and 30%, with a higher prevalence in those with severe OSA [190–193]. Although obesity is often comorbid with T2DM and OSA, there is growing evidence that the relationship between OSA and T2DM is independent of obesity. OSA severity was shown to be positively associated with the incidence of T2DM independent of adiposity, during 12.8 years (median) of follow-up in a subpopulation ($n = 1453$) of participants of both the SHHS and Atherosclerosis Risk in Communities (ARIC) study [194]. A dose–response association was seen between severity of OSA and incident diabetes. Even after adjusting for adiposity, obese participants with severe OSA were at 2.03-times greater risk of incident diabetes than obese participants without OSA. A meta-analysis of ten studies that included a total of 64,101 participants showed OSA is associated with incident diabetes, with an unadjusted pooled relative risk of 1.62 (95% CI, 1.45–1.80) and an adjusted pooled relative risk of 1.35 (95% CI, 1.24–1.47). The effects size of OSA on T2DM is larger than that for physical inactivity (RR of 1.20) but smaller than that for having a family history of diabetes (RR of 2.33) [195, 196].

Pathophysiology in Relation to T2DM

OSA is a syndrome of cyclic upper airway obstruction that results in bouts of intermittent hypoxemia (IH) and intrathoracic pressure–volume changes that terminate in repetitive cortical micro-arousals and blood pressure surges. Accumulation of excess fat in the neck, which is associated with visceral abdominal obesity, contributes to upper airway narrowing, increased collapsibility, decreased efficiency of dilator muscle contractility, and skeletal muscle dysfunction due to lipid accumulation. There is a convincing literature showing that intermittent hypoxia (IH) and sleep fragmentation in OSA results in sustained increases in MSNA [130] and elevation in markers of local and systemic inflammation [197]. The local and systemic inflammation of OSA may have contributory roles in the development of metabolic derangements, including insulin resistance associated with OSA.

OSA-associated inflammation is thought to arise from mechanical de-formation of the upper airway and intermittent hypoxia. The pattern of oxidative stress seen in OSA is similar to that seen with ischemia–reperfusion injury [198], resulting in acceleration of redox-activated signal transduction pathways. Hypoxia-inducible factor 1-alpha (HIF-1α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) play key roles in inflammation, especially in adipocytes, hepatocytes, and skeletal muscles [199, 200]. Gaines and colleagues [201] proposed that

central obesity, which precedes the development of OSA and metabolic dysfunction, may itself be a chronic low-grade inflammatory state as it creates conditions that perpetuate a vicious cycle of macrophage recruitment, impaired adipocyte function, and activation of genes that encode pro-inflammatory proteins.

The elevations in sympathetic activity and catecholamine secretion from the hypothalamic–pituitary–adrenal system induced by IH contribute to diurnal hypertension and reduction in insulin sensitivity and insulin-mediated glucose uptake in the peripheral tissues [202, 203]. Elevations in cortisol and norepinephrine levels also effect organs involved in glucose counterregulation (i.e., pancreatic β cell secretion, hepatic glucose production, and adipocyte regulation of energy balance) [204].

A recent analysis of the SHHS demonstrated that OSA in REM stage sleep was independently associated with insulin resistance after controlling for OSA in non-REM sleep [205]. The large declines in interstitial glucose concentration during REM stage sleep in diabetic patients without SDB, likely a result of an increase in cerebral glucose utilization, were abolished in those patients with OSA. Using continuous interstitial glucose monitoring simultaneously with polysomnography, Bialasiewicz and colleagues found that the mean glucose levels were 38% higher during REM stage sleep in those patients with OSA [206]. This finding that OSA during REM sleep is adversely associated with glucose metabolism in patients with T2DM may have important therapeutic implications regarding the duration/timing of nightly CPAP usage, as REM stage sleep tends to cluster in the second half of the sleep period [207].

The relationship between diabetes and OSA is felt to be bidirectional and insulin resistance is a suspected link. OSA is not only prevalent in patients with T2DM but also in those with Type I DM, including younger and non-obese patients [208, 209]. OSA is frequent in disorders in which insulin resistance is a primary pathophysiologic abnormality. Obese women with polycystic ovarian syndrome (PCOS) have significantly higher fasting insulin levels than non-obese women with PCOS. Vgontzas and colleagues have reported that insulin resistance is the strongest risk factor for OSA in women with PCOS, stronger than even BMI or testosterone levels [210]. A study of 30 patients with T2DM hospitalized for intensification of glycemic control found not only did nocturnal glycemic profiles improved significantly but this improvement was also accompanied by 32% reduction in the 4% AHI after just 5 days. These patients did not experience any change in body weight, neck circumference, or self-reported sleep duration [211].

Screening for OSA in Patients with T2DM

In 2008, the International Diabetes Federation Task Force on epidemiology and prevention recommended that health professionals caring for patients with either T2DM or SDB consider screening patients presenting with one condition for the other. The report acknowledged that untreated OSA is associated with worse

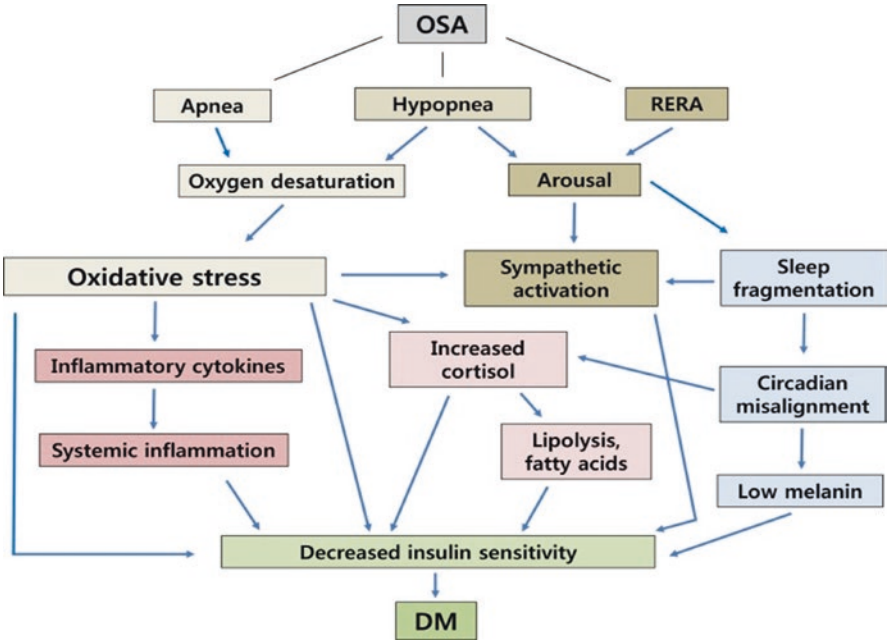


Fig. 11.9 Proposed interactions between obstructive sleep apnea (OSA) and diabetes. RERA: respiratory effort-related arousal; DM: diabetes mellitus [214]

glycemic control [212]. Westlake and colleagues compared the Berlin and Stop-Bang questionnaires with HST in 294 patients with T2DM and found that both questionnaires had low sensitivity and specificity [213]. In 2017, the American Diabetes Association recognized OSA as an important co-morbidity of T2DM and noted the benefits of OSA treatment on blood pressure control and QOL in patients with T2DM. Hence, clinicians should consider working up a diagnosis of sleep apnea using a HST monitoring devices in diabetic patients, if clinically appropriate (Fig. 11.9) [215].

Treatment

CPAP remains the gold standard treatment for patients with moderate to severe OSA with and without diabetes even though there are alternative therapies that may provide equivalent efficacy. The application of positive airway pressure establishes airway patency and has been associated with few arousals, lower AHI, improved oxygen saturation, and decreased daytime sleepiness. However, results from studies evaluating the effect of CPAP therapy on glycemic control and other markers of

inflammation have been inconsistent, despite showing evidence for improved insulin sensitivity in patients with severe disease.

In a proof-of-concept study, Mokhlesi at al. assigned 13 patients with OSA and T2DM to either nightly CPAP or sham CPAP for 1 week under nightly observation in the sleep laboratory to ensure full compliance with the allocated treatment. Using a 24-h blood sampling technique, the mean plasma glucose levels decreased significantly after 1 week of active versus sham CPAP treatment. This decrease was also associated with a trend toward lower 24-h mean insulin levels. Improvement in glucose levels was most prominent during the overnight period. Of interest, the beneficial effect with CPAP was larger in magnitude in patients with poor glycemic control at baseline [216].

There are many studies that have explored the effect of CPAP in patients with T2DM with follow-up of 1–6 months. Several randomized controlled trials have reported improvements in metabolic control, i.e., insulin sensitivity and glucose tolerance in patients with OSA treated with CPAP as compared to sham CPAP [217–219]. However, many of these studies showed no consistent effect of CPAP on glycemic control [220, 221]. In a randomized clinical trial consisting of 888 participants in the SAVE trial who were followed up for median of 4.3 years, there was no evidence that CPAP therapy affected glycemic control in those with diabetes, prediabetes, or diabetes risk over standard-of-care treatment [222]. Another recent randomized trial demonstrated improvements in inflammation, insulin resistance and serum triglycerides only in patients with OSA who combined their CPAP use with weight loss during a 24-week period [223].

These conflicting results are in large part a function of differences in baseline glycemic status, timing from disease onset/diagnosis, varying degrees of CPAP adherence and efficacy, different methodology to assess glucose metabolism, and the use of anti-hyperglycemic agents, the numbers of which have been increasing. Some of the newer diabetic medications have focused on weight loss effects along with improving glycemic control. Therapies designed to reduce visceral adiposity may address the systemic root cause of OSA and DM in many patients. Bariatric surgery is considered an effective treatment for both diabetes and sleep apnea. It is now a recommended treatment for patients with diabetes and a BMI of greater than 40 kg/m^2, with inadequate glycemic control despite lifestyle changes and optimal medical therapy.

Despite the inconclusive results, it is important to acknowledge the other benefits of CPAP in diabetic patients with OSA. Reduction in daytime sleepiness and improved QOL are favorable outcomes. CPAP does improve blood pressure control, likely a consequence of decreases in sympathetic tone which may have significant benefits on the microvascular complications of diabetes [224–226].

Additional longer-term and larger studies are needed to explore if effective treatment of sleep apnea can reduce the risk of developing T2DM. Such studies should explore the role of various lifestyle modifications, including weight reduction and physical activity, combined with CPAP therapy as a primary prevention strategy for pre-diabetes and T2DM early in the disease process.

Sleep-Disordered Breathing and High-risk Pregnancy Conditions

Preeclampsia, a potentially fatal, multisystem, progressive disorder of pregnancy impacting at least 5% of pregnancies worldwide, is a major cause of maternal and fetal morbidity and mortality. It is characterized by either new-onset HTN and proteinuria or HTN and end-organ dysfunction. The disorder typically occurs after 20-weeks gestation, but at times occurs post-partum, in previously normotensive women. The condition can also be superimposed on previously existing/chronic HTN. Gestational HTN is part of the preeclampsia spectrum. Gestational diabetes increases risk of developing both gestational HTN and preeclampsia.

The pathogenesis of preeclampsia is attributed to a combination of maternal and fetal/placental factors that promote placental oxidative stress and vascular reactivity and result in maternal systemic vascular and endothelial dysfunction, inflammation, and increased sympathoexcitation. Untreated OSA results in similar systemic pathophysiologic effects which are believed to be among the mediators linking OSA to cardiometabolic disease manifestations. Given the shared pathophysiology and mediators between OSA and preeclampsia, combined with weight gain, edema, and hormonal alterations of pregnancy that may increase the risk of developing or worsen pre-existing OSA, untreated OSA has been implicated as a potential contributor to the development of gestational diabetes, hypertensive disease of pregnancy, and preeclampsia.

The prevalence of OSA in pregnancy is not known. However, an increased report of snoring in pregnant women, compared to pre-menopausal non-pregnant women (14–45% compared to 4%) has been shown in numerous studies, though the presence of a bed partner may confound such findings [227–233]. Obesity, a major risk factor for OSA, is increasing in prevalence—between 2005 and 2014, 50% of women in the US were overweight or obese, 37% of reproductive-age women were obese, and 10% were morbidly obese (BMI 40 kg/m^2) [233]. Obese pregnant women are also more likely to have OSA [234]. Small studies using PSG to diagnose OSA in pregnant women have shown that OSA becomes more frequent later in pregnancy. In a study of 105 pregnant women with mean BMI of 33.4 kg/m^2, 26.7% in third trimester vs. 10.5% in first trimester were found to have OSA (AHI $\geq$ 5/h) [235], while another study found moderate OSA (AHI $\geq$ 15/h) in 20% of subjects studied at 48 h post-delivery [236]. In a small case–control study, the prevalence of OSA, as diagnosed by PSG (AHI 4% or arousal $\geq$5/h), was 14/17 (82%) in hypertensive, compared to 15/33 (45%) in normotensive pregnant women [237]. The primary risk factors for OSA in pregnancy include older maternal age, obesity, snoring, and history of chronic hypertension [236, 238].

OSA remains underdiagnosed both in the general population and in pregnancy. No SDB-related screening questionnaires have been specifically validated in pregnant women [239]. One prospective trial found low predictive parameters and high false-negative referral rates for pregnant woman with positive OSA screening by either the Epworth Sleepiness Scale or the Berlin questionnaire [240]. In a single,

large (over 1000 subjects analyzed) prospective trial of pregnant women, using generalized linear modeling, screening positive on the Berlin Questionnaire, but not with the Epworth Sleepiness Scale, was positively associated with hypertensive disorders of pregnancy [240]. The specificity of the STOP-Bang questionnaire increases from 37% to 85% for all OSA severities when the serum bicarbonate level is greater than 28 mEq/L in addition to a score ≥3 [241]. Although not validated in pregnancy, adding a serum bicarbonate level greater than 28 mEq/L to scores ≥3 for the STOP-Bang questionnaire may be useful in pregnancy, since serum bicarbonate levels in pregnant women are normally lower due to respiratory alkalosis [242].

Sleep apnea is more prevalent in pregnant women with high-risk pregnancy disorders than those without. While some small studies without objective testing to determine OSA have shown inconsistent results, studies that have used hypertensive disorders of pregnancy as an inclusion criteria and conducted objective testing for OSA via portable of attended PSG have found a greater prevalence of OSA in women with hypertensive disorders of pregnancy and preeclampsia [243, 244].

Pregnant women with sleep apnea also have a higher risk of adverse maternal and fetal outcomes compared to pregnant women without sleep apnea, shown in questionnaire studies, several large retrospective data-base studies, systematic reviews and meta-analyses. Associations between OSA symptoms and gestational HTN have been demonstrated [231, 232, 245–249]. Compared to pregnant women without OSA, pregnant women with OSA have a significantly higher risk of pregnancy-specific, medical and surgical complications including longer length of stay and need for Intensive Care Unit (ICU) admissions [249]. Data from the US National perinatal information center (from 2010 to 2014, including 1,577,632 pregnant women, using ICD 9 codes) showed that pregnant women with OSA have increased risk of GDM (adjusted OR 1.51, 95% CI 1.34–1.72), PEC (adjusted OR 2.22, 95% CI 1.94–2.54), and eclampsia (adjusted OR 2.95, 95% CI 1.08–8.02) and a 2.5- to 3.5-fold increase in risk of severe complications (cardiomyopathy, congestive heart failure, total abdominal hysterectomy, ICU stay and hospital length of stay) [249]. A large retrospective cross-sectional analysis using the Nationwide Inpatient Sample (NIS) database (which included almost 56 million pregnancy-related inpatient hospital discharges) found that OSA was associated with increased odds of pregnancy-related morbidities (including PEC, eclampsia, pulmonary embolism, cardiomyopathy) and that women with OSA had a fivefold increased odds of in-hospital death [250]. Pamidi and colleagues, in a systematic review and meta-analysis, found that maternal sleep apnea was significantly associated with gestational HTN and preeclampsia (pooled adjusted odds ratio (OR) 2.34; 95% confidence interval [CI], 1.60–3.09; 5 studies), and gestational diabetes (pooled aOR, 1.86; 95% CI, 1.30–2.42; 5 studies) [248]. Self-reported poor-quality sleep has been associated with longer labor, cesarean section, and preterm births [251, 252]. Obesity, which is becoming increasingly common in women at the time of conception [234], is a major risk factor for both OSA and preeclampsia, and is also associated with increased cesarean sections [253]. Diagnosed OSA in pregnancy has also been associated with poor fetal outcomes [254, 255].

Treating OSA in pregnancy may have important beneficial effects on maternal and fetal health. Two consecutive PSG studies (baseline followed by auto-titrating nasal CPAP) with simultaneous continuous blood pressure monitoring conducted in 11 women with preeclampsia found to have upper airways obstruction during sleep resulted in reduction in blood pressure on the treatment night [(128 ± 3)/(73 ± 3)] when compared with the initial non-treatment study night [(146 ± 6)/(92 ± 4)], p = (0.007)/(0.002) [256]. In women with gestational diabetes, HTN, and obesity who have OSA, CPAP treatment improves maternal and fetal outcomes, when compared to pregnant women with untreated OSA [256, 257]. Inspiratory airflow limitation and improvement in vascular reactivity and HTN have also been demonstrated with CPAP treatment in preeclampsia [258].

Rather than an isolated, though potentially morbid event of pregnancy, placental implantation disorders are actually a marker of future gestational complications and later in life cardiovascular events for both mothers and their offspring [259–261]. Since the mid-1990s evidence from retrospective and prospective epidemiological registries, clinical studies, systematic reviews, and meta-analyses has been accumulating showing that women with placental implantation disorders (such as gestation HTN and preeclampsia) are at an increased risk for long-term cardiovascular disease (including HTN, CAD, myocardial infarction, stroke, peripheral arterial disease, thromboembolism, and HF). Recent systematic reviews and meta-analyses provide strong support of the association of preeclampsia and future CVD [259, 262, 263], such that the American Heart Association now recommends that a history of preeclampsia be considered a major risk factor for cardiovascular and cerebrovascular disease [264, 265]. A large (n = 506,350 women) prospective registry-based study from Norway found that the severity of the placental implantation disorders resulted in additive risk for occurrence of major coronary events: 2.1-fold in those with history of preeclampsia, and 3.3-fold and 5.4-fold, respectively, when maternal preeclampsia was combined with intrauterine growth retardation or preterm birth [266].

OSA as a possible contributor to hypertensive disease of pregnancy and preeclampsia and may be an intervenable target to halt the progression of life-long cardiovascular disease in women with high-risk pregnancy conditions and their offspring. More studies are needed to inform optimal timing for OSA assessment and treatment and to better clarify the effect of treating OSA on maternal and fetal outcomes.

Summary and Future Directions

SDB is common in the general population, but even more prevalent in patients with comorbid cardiac and metabolic disease. Chronic exposure to SDB events is associated with a profile of systemic disturbances that are felt to contribute to and exacerbate the progression of cardiometabolic disease, while treatment of SDB has beneficial effects on these disorders and their progression. Treatment of OSA

lowers blood pressure, reduces rates of refractory HTN, increases left ventricular ejection fraction, decreases ventricular ectopy and the recurrences of and progression of AF, and may improve blood glucose control. CPAP, when used consistently in OSA and when the treatment is efficacious, leads to reduction in cardiac and cerebrovascular events and improvement in mortality. CSA is associated with worsened HF outcomes, though the impact of treatment of CSA on cardiovascular outcomes has not yet been clearly elucidated. Features of OSA and CSA often coexist in the same patient. Recognition of SDB phenotypes and their coexistence can inform treatment approaches and improve treatment tolerance, adherence, and clinical benefit. Randomized control trials, with attention to disease phenotype and optimal therapies, may provide further unbiased assessment of the impact of SDB treatment on cardiovascular disease morbidity and mortality.

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Chapter 12
The Metabolic Syndrome and Vascular Disease

Michael A. Via and Jeffrey I. Mechanick

Introduction

Since its initial proposal in 1988 [1], the clinical diagnosis of metabolic syndrome (MetS) based on a constellation of findings has been recognized to predict adverse metabolic outcomes, especially atherosclerotic cardiovascular disease (CVD), type 2 diabetes (T2D), and increased mortality [2, 3]. MetS is diagnosed using easily identifiable traits that signify insulin resistance in patients. Outside of a formal diagnosis, the concept that observable markers of dysmetabolism are predictive of CVD has been recognized since at least the 1920s [4]. Over the past century, changing lifestyle and environmental factors have contributed to the rising prevalence of MetS and the associated cardiovascular consequences.

The presence of MetS is defined by a grouping of individual elements (Table 12.1) associated with insulin resistance. Current evidence suggests that the combined risk of MetS may be greater than the sum of individual risks of each component [3, 11]. In an individual patient, the pathophysiology of insulin resistance is typically recognizable as MetS for years to decades prior to the development of T2D or sustaining a cardiovascular event. Early identification provides greater opportunity for intervention and meaningful mitigation [12]. Moreover, associated conditions such as hepatosteatosis, hypertension, abdominal and visceral adiposity, and polycystic

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Table 12.1 Diagnostic criteria of MetS

Organization	Year	MetS diagnostic requirements	Hyperglycemia/insulin resistance	Adiposity	Hypertension	Serum triglycerides	Serum HDL
American Heart Association/National Heart, Lung, and Blood Institute [5]	2005	3 of 5 criteria	Serum glucose >100 mg/dL or medication use	WC ≥ 102 cm—men; WC ≥ 88 cm—women	≥130/85 mmHg, or medication use	≥150 mg/dL	<40 mg/dL men; <50 mg/dL women; or medication use
International Diabetes Federation [6]	2005	Adiposity +2 of 4 other criteria	Serum glucose >100 mg/dL	Increased WC	≥130/85 mmHg, or medication use	≥150 mg/dL	<40 mg/dL men; <50 mg/dL women; or medication use
American Association of Clinical Endocrinologists [7]	2003	Insulin resistance + any of the other criteria	Impaired fasting glucose or impaired glucose tolerance, excluding T2D	BMI ≥ 25 kg/m^2	≥130/85 mmHg	≥150 mg/dL	<40 mg/dL men; <50 mg/dL women
National Cholesterol Treatment Program [8]	2001	3 of 5 criteria	Serum glucose >100 mg/dL	WC ≥ 102 cm—men; WC ≥ 88 cm—women	≥130/85 mmHg	≥150 mg/dL	<40 mg/dL men; <50 mg/dL women
European Group for the study of Insulin Resistance [9]	1999	Insulin resistance + any 2 of the other 4 criteria	Fasting plasma insulin >75th percentile; impaired fasting glucose, or impaired glucose tolerance, excluding T2D	WC ≥ 94 cm—men; WC ≥ 80 cm—women	≥140/90 mmHg, or medication use	≥150 mg/dL	<39 mg/dL
World Health Organization [10]	1998	Insulin resistance + any 2 of the other 4 criteria	Impaired glucose tolerance, impaired fasting glucose, or reduced insulin sensitivity	Waist to hip ratio ≥ 0.9 in men; ≥0.85 in women; or BMI ≥ 30 kg/m^2	≥140/90 mmHg	≥150 mg/dL	<35 mg/dL men; <39 mg/dL women

Abbreviations: *BMI* body mass index, *MetS* metabolic syndrome, *T2D* type 2 diabetes, *WC* waist circumference

ovary syndrome in women represent the systemic effects of insulin resistance that both drive and are driven by the presence of MetS. This chapter focuses on the cardiovascular risk that is present in patients with MetS and describes ways to address this common cause of morbidity and mortality.

Defining the Metabolic Syndrome

Published guideline and position statements authored by professional medical organizations generally agree that the presence of MetS should be defined by a set of commonly measured clinical markers, though minor differences in defining factors and ranges exist (Table 12.1). Broadly, these include abdominal girth (WC), hyperglycemia, hypertriglyceridemia, hypertension, and low levels of high-density lipoprotein (HDL) levels. Table 12.1 includes specific ranges for MetS definitions as well as relative weighting in each of these. Common to each of these sets of criteria is a constellation of metabolic disturbances centered on insulin resistance and obesity.

Epidemiology

The prevalence of MetS depends somewhat on the defining criteria. Using data from the National Health and Nutrition Examination Surveys, and with defining criteria given by the National Cholesterol Education Program Adult Treatment Panel III (NCEP), approximately 35% of adults and nearly 50% of those above the age of 60 in the U.S. have MetS [13]. Within the U.S., MetS prevalence varies regionally from 40% in the West North Central region to 30–35% in the South and to 29% in the Northeast and West Coastal regions [14].

Although the prevalence of *obesity* in Europe is significantly lower than in the U.S., the prevalence of *MetS* in Europe is similar to higher and ranges from 24 to 65% in women and 43 to 78% in men using NCEP criteria [15]. In Asia, the prevalence of MetS is 20–30% and shows a linear increase over the past three decades [16]. In the Middle East, a meta-analysis of 59 published studies demonstrates an average prevalence of MetS to be 26% [17].

Cardiovascular Risk

Patients with MetS universally exhibit increased cardiovascular risk. A Cochrane Library review that includes 87 prospective cohort studies using NCEP criteria to define MetS in 951,083 patients evaluated cardiovascular risks [18]. The relative risk for all-cause mortality was 1.54 (1.29–1.84; 95% CI). The relative risk for CVD mortality was 2.40 (1.87–3.08; 95% CI) and the relative risk for CVD itself was 2.35

(2.02–2.73; 95% CI). Similar increases in relative risk for myocardial infarction 1.99 (1.61–2.46; 95% CI) and stroke 2.27 (1.80–2.85; 95% CI) were observed [18].

An approximate five-fold increase in prevalence of T2D is observed in patients with MetS [19]. In the Cochrane Library data set, exclusion of patients with T2D continues to demonstrate a significant increase in relative risk for cardiovascular mortality at 1.75 (1.19–2.58; 95% CI) [18].

Insulin Resistance

Initially recognized as a reduction in systemic response to insulin and later described as the "ominous octet" [20], patients with insulin resistance exhibit a multiplicity of dysfunctions and dysregulations of metabolism. Many biochemical and hormonal pathways are involved [12]. These include pancreatic β-cell dysfunction resulting in impaired and delayed insulin release, excessive glucagon secretion during meals resulting in inappropriate hepatic glucose release, reduced release and activity of glucagon-like peptide-1 (GLP1), dysfunctional adipose tissue, leptin resistance, reduced adiponectin activity, reduced ghrelin levels, increased circulating free fatty acids, low-grade systemic inflammation, oxidative stress, generalized endoplasmic reticulum stress leading to protein misfolding, and reduced clearance of advanced glycated end products [19]. Impairment within each of these pathways contributes to reduced metabolic efficiency and confers increased risk of atherosclerotic vascular disease. In the case of MetS, pathophysiologic processes are quite similar to that of T2D, though often less severe.

Pancreatic β-Cell Dysfunction

Four separate cell lines within the pancreatic islets are active in hormone production and secretion. Meal consumption and associated rising serum concentrations of glucose, lipid, and certain amino acids induce insulin secretion by pancreatic β-cells [21]. Chronic exposure to elevated glucose and free fatty acid concentrations as well as inflammation impair β-cell function [22]. Some of the earliest evidence for this is the observed loss of first phase insulin secretion observed in states of insulin resistance [23].

While β-cell loss is a hallmark of T2D, in patients with MetS, an increase in β-cell mass is observed. Cadaveric studies of patients with obesity but without T2D, as well as pathology specimens of patients with MetS who undergo pancreatoduodenectomy, demonstrate an increase in pancreatic β-cell mass by approximately 50% [24, 25]. Prevailing opinion suggests these findings represent compensation to overcome systemic insulin resistance, leading to hyperinsulinemia [24]. Over time, sufficient loss of pancreatic β-cell activity may lead to T2D. The mechanisms for β-cell loss are not well understood, though several have been identified. One includes the accumulation of amyloid protein with pancreatic islets, which is associated with β-cell apoptosis [26, 27]. Islet amyloid deposits contain polymerized

amylin, a peptide hormone that is co-secreted with insulin by β-cells themselves, and accumulate at increased rates when higher insulin release is required, such as in MetS [27]. Additionally, oxidative stress activates macrophages, which can impair pancreatic β-cell function and induce cell death [28].

Endoplasmic reticulum (ER) stress represents another mechanism for β-cell apoptosis. In the setting of chronic nutrient overexposure, increased peptide synthesis, especially increased insulin production and release increases utilization of ER protein trafficking [29]. Accumulation of misfolded proteins within the ER of β-cells induces apoptosis, which may lead to T2D [29]. Studies of pancreatic β-cells obtained from patients with T2D demonstrate a doubling of ER volume [29].

Pancreatic β-cell dedifferentiation to nonfunctional cells or transdifferentiation to cells that produce other hormones has also been proposed as mechanisms for β-cell loss [30]. These processes have been observed in animal models of insulin resistance. Presently, it is unclear whether this mechanism has a significant role in the development of MetS or T2D in humans.

Prandial Glucose

Regulation of glucose homeostasis depends on dietary intake and hepatic glucose release from both glycogen breakdown and gluconeogenesis. In patients with MetS, the latter processes are inappropriately enhanced during meals, driven by elevations in circulating glucagon and impaired GLP1 levels [31, 32]. GLP1, the main incretin hormone, is secreted by L-cells of the distal ileum in response to meal intake and functions to stimulate insulin release, impairs glucagon secretion, reduces hepatic glucose release, and acts in hypothalamic centers of metabolic regulation [31]. Patients with MetS demonstrate a three- to fourfold reduction in prandial GLP1 secretion compared to healthy controls [32]. Through the many mechanisms of GLP1 function, this reduction in GLP1 activity contributes significantly to the pathophysiology of insulin resistance and MetS [19].

Adipokines

Adipose tissue has been recognized as a metabolically active organ that has profound effects on energy homeostasis and storage. Dysfunctional and re-distributed adipose tissue is a hallmark of MetS [6]. Hormones produced by adipose tissue, collectively known as adipokines, are recognized as important metabolic mediators and are affected in insulin resistance. These include leptin, which is secreted at higher levels in MetS, in proportion to the overall increase in adipose tissue [33]. Although levels are high, leptin resistance within the arcuate nucleus of the hypothalamus develops, conferring a relative reduction in leptin activity. This phenomenon is associated with reduced nutrient sensing and reduced satiation during meals [33]. The relative reduction in leptin activity also confers insulin resistance and may promote further weight gain [34].

Adiponectin, an adipokine that induces insulin sensitivity, diminishes hepatic glucose release, and reduces the inflammatory response is diminished in MetS [35]. The increase in visceral adiposity and relative reduction in subcutaneous adipose, especially in the limbs, is associated with reduced adiponectin activity [36]. Lifestyle modification as a means to address MetS has been shown to restore circulating adiponectin levels [35]. Other adipokines, such as resistin, apelin, visfatin, omentin, and chemerin, may also affect the pathophysiology of insulin resistance and MetS [37, 38].

Oxidative Stress

Markers of oxidative stress are ubiquitously affected in states of insulin resistance, including MetS and T2D. Many authors postulate that oxidative stress in the adipose tissue is responsible for inducing an inflammatory response, leading to systemic inflammation that exacerbates insulin resistance itself, among other detrimental effects [19]. Increased adiposity is associated with oxidative stress, increased production of reactive oxygen species (ROS), as well as consumption of nicotinamide adenine dinucleotide phosphate (NADPH), the main cellular antioxidant molecule. A reflexive increase in activity of NADPH synthase is also observed.

High levels of circulating ROS cause damage to DNA and proteins, which impairs cellular function. Detrimental effects of ROS on mitochondrial and endoplasmic reticulum have been demonstrated [39]. Oxidation of low-density lipoprotein (LDL) particles forms highly atherogenic oxidized low-density lipoprotein (OxLDL), which enhances platelet aggregation and macrophage activation, promotes release of tissue factor, diminishes endothelial thrombomodulin activity, and induces an inflammatory response [40].

These mechanisms are key contributors to the increased cardiovascular risk in MetS. However, attempts to address this with antioxidant supplements for either treatment or prevention of CVD have been largely disappointing [41]. It may be that the underlying insulin resistance and ROS production overwhelms ROS sequestration by antioxidant mechanisms [42]. Moreover, distribution of antioxidant agents to areas of high ROS production may be poor [42]. In contrast to treatment with supplements, adherence to a dietary pattern that is high in antioxidants is beneficial in cardiovascular risk reduction and mitigates many of the pathophysiological mechanisms, including a reduction in oxidative stress [43].

Systemic Inflammation

Systemic inflammation is another hallmark of insulin resistance and MetS. Mechanisms for its development are poorly understood; however, hypoxia among growing adipocytes resulted in increased inflammatory markers in both animal models and patients with MetS [44]. Evidence of inflammation includes increased interleukin (IL)-6, IL-1, tumor necrosis factor (TNF)-α, plasminogen

activator inhibitor (PAI)-1, serum amyloid A, and C-reactive protein (CRP) [45]. The risk for CVD is also increased in patients with higher levels of these markers of inflammation. Patients with MetS who are able to make lifestyle adjustments, such as improved dietary choices and increased physical activity, show reductions in serum levels of these markers of inflammation [45].

Endothelial Dysfunction

The vascular endothelium interacts directly with the hormonal mediators of MetS, responds and contributes to systemic inflammation, modulates coagulation, contributes to lipid metabolism, including the vascular accumulation of lipid-rich sclerotic plaques, and generates ROS through uncoupling of endothelial nitric oxide synthase (eNOS) [46]. Activity of eNOS is stimulated through the insulin signal cascade and patients with MetS have impairment of eNOS, diminishing nitric oxide (NO) production within the vascular endothelium. Consequently, the NO signal for vasodilation is reduced, leading to vasoconstriction and is associated with the development of essential hypertension [47]. Low circulating adiponectin and resistance to leptin induce increased expression of endothelin-1, further exacerbating vasoconstriction in MetS [47]. Additionally, eNOS activity becomes uncoupled in endothelial dysfunction and insulin resistance, leading to excessive production of peroxides, superoxide, and peroxynitrite [48]. ROS produced through eNOS uncoupling or by directly by glucose metabolism or further damages endothelial cells and yields greater OxLDL.

The vascular endothelium directly modulates thrombosis and atherogenesis. The pro-inflammatory state that develops with release of IL-6, IL-1, TNFα, and PAI-1 enhances platelet activity [49]. Circulating levels of coagulation factors, including factor VII, factor VIII, factor XIII B-subunit, fibrinogen, and von Willebrand factor (vWF) are elevated in MetS [50]. ROS activate monocytes and macrophages, inducing liberation of cell membrane microparticles with exposed phospholipids, glycoprotein IX, and release of tissue factor (TF) [49]. TF is also accumulated within the vascular endothelium, especially in proximity to atherosclerotic plaques, and TF pathway inhibitor, which reduces thrombosis formation, is diminished [51]. Fibrin synthesis is also enhanced in MetS though excessive PAI-1 and inflammatory cytokine activity released by the vascular endothelium [52].

One other function of vascular endothelium is to distribute hormone signaling, including insulin, to target tissues [53]. Insulin must pass through endothelial cells via endocytosis. Like other aspects of endothelial function, this process is impaired in patients with insulin resistance, exacerbating MetS [53].

Advanced Glycosylation End Products

Advanced glycosylation end products (AGEs) result from the Maillard reaction between sugars and protein that occurs spontaneously and nonenzymatically in all biological systems [54]. This glycosylation process leads to dysfunctional proteins,

oxidative stress among tissues, and tissue damage. The Maillard reaction kinetics vary by specific sugar molecules and are significantly slower for glucose compared to other monosaccharides, such as fructose or galactose [55].

AGEs are also consumed in the diet, though only 10–30% of dietary AGEs are absorbed [56]. The process of cooking food, especially at high temperatures used during grilling, yields higher amounts of AGE [56]. Higher concentration of sugars in blood and in tissues, such as occurs in T2D, increases local AGE formation [57]. Additionally, AGE clearance is reduced in states of insulin resistance. Protein receptors for AGE have been identified, including receptor of AGE (RAGE), AGE-R1, R2, and R3 that facilitate renal clearance and induce vascular endothelial response to these substances [54].

Patients with T2D and MetS have reduced AGE clearance and increased AGE formation [54, 57]. In addition, patients with MetS have an approximate 50% increase in dietary AGE consumption [58]. The increased systemic AGE levels in T2D and MetS are associated with the development of microvascular and macrovascular complications [56].

Renal Glucose Metabolism

The development and clinical use of sodium-glucose linked transporter 2 (SGLT2) inhibitors followed nearly three decades of study of renal glucose handling in states of insulin resistance. Additionally, circulating insulin is cleared primarily by the kidneys, and cells of the proximal tubules release a significant amount of glucose through gluconeogenesis that is regulated by insulin [59]. Patients with T2D and MetS exhibit increased renal glucose uptake by 15–20% due to increased SGLT2 and other glucose transporter activity [60, 61]. This process raises serum glucose levels and exacerbates insulin resistance.

Microbiota

Recognition of the importance of the microflora of the gastrointestinal (GI) tract in health and disease has grown over the past two decades [62]. Not surprisingly, a person's lifestyle, especially dietary choice and resultant eating patterns, has great influence on the speciation and function of the GI microflora [63]. Unhealthy choices and subsequent alteration of GI microflora may contribute to the pathophysiology of MetS [63]. In a study of healthy volunteers placed on a plant-based diet for 5 days, and separately placed on a diet with animal-based protein diet for 5 days with appropriate washout, significant changes in the GI microflora were noted [64]. At the end of the plant-based trial, a significant increase in bacteria that metabolize fiber was noted, compared to an increase in bile-tolerant bacterial

species that flourished after 5 days of the animal product diet. Microflora gene expression was also influenced. High expression of genes involved in vitamin biosynthesis and degradation pathways of polycyclic aromatic hydrocarbons that are formed during charring of cooked meat was seen after the animal product diet. Moreover, global microbiome gene expression was easily categorized by dietary intake and reflected the respective gene expression of herbivore or carnivore microbiomes.

Several lines of evidence are suggestive of the influence held by the GI microbiome over host metabolism. In one study, metabolites of the microbiome of rats fed a high-fiber diet induced host GLP-1 activity [65]. In another study, short-chain fatty acid metabolites produced by the microbiome of mice fed a high-fat diet affected gene expression in hepatocyte culture [66]. The specific genes affected are involved in regulation of free fatty acid production and diurnal fat metabolism [66]. Another animal model demonstrates host peroxisome proliferator activator receptor (PPAR)-γ is influenced by perturbations in GI microbiome [67]. In humans, significant changes in GI microflora are observed after weight loss [68] and bariatric surgery [69]. Transfer of microflora from lean humans to patients with MetS demonstrates a slight improvement in glycosylated hemoglobin (A1c) by 0.2% at 6 weeks; however, this affect is lost by 18 weeks [70]. These findings highlight the microbiome–host interrelationship affecting insulin sensitivity and MetS.

Addressing Patients with MetS

The myriad of causes of MetS are attributed to societal and individual lifestyle changes stemming from the industrial revolution and continual modernization. These include dietary choices, food production and availability, sedentary lifestyle, potential endocrine disruptors, diminished sleep hygiene, and dietary fructose consumption, among others. An effect of developing MetS is to impart a high risk of diseases stemming from insulin resistance, including T2D and its complications, especially CVD.

A multi-pronged approach to patients with MetS should be comprehensive: addressing each of the potential risks and components. Expertise in many specialties is required and a multidisciplinary team is best suited to achieve the goals of risk mitigation [71].

Many strategies for therapeutic intervention in patients with MetS may be implemented. These include identification and modification of lifestyle choices, potential benefits of supplements, pharmacological treatment, and consideration for bariatric surgery. Each of these may be tailored to individual patient needs and combined with any of the others. Many of the same concepts can be extrapolated to patients with MetS. Indeed, some of the most provocative data derive from prevention trials that include patients with MetS who have not yet developed T2D.

Intensive Lifestyle

For anyone with MetS, lifestyle modification should be universally employed. This broad term includes choice of a healthy dietary pattern, increased physical activity, improvement in sleep hygiene, and methods of stress reduction [72]. While controversy continues over which dietary pattern is best, robust evidence exists for choice of healthy dietary pattern in combination with increased physical activity in patients with MetS in order to prevent T2D, cardiovascular outcomes, and other complications.

Low-Fat/Low-Calorie Dietary Patterns

Early trials of lifestyle intervention focus on reduction in dietary fat and calorie restriction, widely held as gold standard dietary advice in the 1980s through early 2000s. One such trial is the Finnish Diabetes Prevention Study (DPS) [73]. In this trial, 522 overweight adults with impaired glucose tolerance, age 40–64 were randomized to an intensive lifestyle or standard dietary recommendations, which were essentially pamphlets with general advice and a single session with a dietician. The intensive lifestyle included seven sessions with a dietician during the first year who endorsed a low-fat dietary pattern and advised increased physical activity, followed by one session every 3 months. Members of the intensive group were also given opportunities to attend expert lectures, guided trips to local supermarkets, and cooking lessons.

Results of the DPS showed weight loss of 5.6 kg in the first year, compared to the control group that lost 1 kg in the first year. Both groups sustained weight regain over 10 years; however, average weight remained below baseline in the intensive group [74]. The intensive lifestyle group also demonstrated improved markers of MetS, including reduction of fasting glucose by 4 mg/dL, reduction of CRP by 1.24 mg/L, and reduction of waist circumference by 4.4 cm after 1 year. Compared to the control group, development of T2D in the intensive group was reduced by 58% at 3 years, 43% at 7 years, and 32% reduction at 13-year follow-up [74]. Marked improvement was also noted with respect to cholesterol including an increase in high-density lipoprotein (HDL) by 2 mg/dL and reduction in triglycerides by 18 mg/dL in the intensive group after 1 year [73]. However, no significant decrease was observed in LDL cholesterol.

A larger randomized trial, the Diabetes Prevention Program (DPP), enrolled 3234 patients with impaired glucose tolerance to standard therapy, metformin treatment, or an intensive lifestyle intervention [75]. This intensive intervention involved individually assigned lifestyle coaches that met with each participant 16 times in the first 6 months and subsequently once every 2 months for dietary advice mainly based on calorie restriction and to monitor physical activity of 150 min/week. The success of this intervention is demonstrated by the 58% reduction in development

of T2D, compared with the control group, and compared to only a 31% reduction in T2D with metformin alone [76].

Together, these pivotal trials demonstrate the efficacy of a lifestyle intervention that combines physical activity and strong adherence to a healthy dietary pattern. In contrast to the Look-AHEAD trial, in which the same intensive protocol as the DPP was studied in patients already with T2D, this type of lifestyle intervention in patients with earlier stages of insulin resistance, such as MetS, yields superior results. Although these trials mainly used calorie restriction as a guide, one of several dietary patterns may be employed to reduce cardiovascular risk in MetS.

The Mediterranean Diet

The Mediterranean diet has been long recognized to reduce cardiovascular risk [77]. Although differences exist within regional cultures, this diet generally is high in fruits, vegetables, whole grains, fish, poultry, nuts, with varying degrees of pork, meat, and wine consumption. The Mediterranean diet is characterized by a high polyphenol content, a high fiber content, a diversity of fruits and vegetables that includes at least three to four fruits and three to four servings of vegetables consumed daily, as well as healthy protein sources and a relatively high amount of n-3 fatty acids [78]. These contents provide a nutritional basis that addresses many of the molecular mechanisms of MetS.

Efficacy of the Mediterranean diet has been formally evaluated in several large randomized controlled trials. In one randomized trial comparing the Mediterranean diet to a low-fat diet or a low carbohydrate diet in patients with obesity, those assigned to the Mediterranean diet had the greatest weight loss of 4.4 kg over 2 years, as well as significant reduction in LDL by 3 mg/dL, triglycerides by 22 mg/dL, fasting serum glucose, and fasting plasma insulin levels [79]. HDL levels increased by 6 mg/dL. In another randomized trial, patients with overweight or obesity assigned to the Mediterranean diet demonstrated a 30% reduction in cardiovascular events and cardiovascular mortality over 6 years, compared to subjects assigned a low-fat diet [80]. Additionally, there was a 30% reduction in the incidence of T2D among subjects on the Mediterranean diet in this trial [81].

The DASH Diet

The Dietary Approaches to Stop Hypertension (DASH) diet is high in fruits, vegetables, fish, nuts, and low-fat dairy products. With this diet, an average of 1.5 kg weight loss is observed, along with a 1.05 cm reduction in waist circumference [82]. Additionally, systolic blood pressure reduces by an average of 6.7 mmHg, while diastolic blood pressure reduces by 3.5 mmHg [83]. A slight reduction in fasting insulin is observed after 16 weeks of the DASH diet; however, no change in fasting

glucose was noted [84]. After an average of 15 years of follow-up, patients following the DASH diet demonstrate a 20% reduction in cardiovascular events, and an approximate 30% reduction in development of congestive heart failure [85].

The Ornish Diet

The Ornish diet is somewhat similar to the DASH and Mediterranean diets in that it contains high amounts of fruits, vegetables, legumes, and nuts, with minor differences that are mainly reduction in animal and fish protein sources. Only 15% of calories are derived from fat, and 10% of calories derived from protein. The majority, approximately 75% of calories, are in the form of complex carbohydrates.

Studies of the Ornish diet are limited in that they are observational in nature. Still, cohort studies of subjects on the Ornish diet demonstrate reduced fasting glucose and A1C, as well as reduced total LDL content, and shifts to larger LDL particle size [86, 87].

Dietary Supplements

Numerous dietary supplements are marketed as inducing weight loss or demonstrating diabetes-controlling properties. Clinical evidence does not support general use in patients with MetS [88]. In the case of micronutrients such as chromium or thiamin, supplementation to improve glucose metabolism is beneficial in patients with frank deficiencies. In well-nourished patients, however, benefits of excessive supplementation do not consistently improve MetS [89]. Similarly, a large randomized trial of vitamin D supplementation in patients with T2D failed to demonstrate benefit [90].

A number of plant polyphenols, such as resveratrol, quercetin, epigallocatechin gallate, and curcumin, demonstrate improvement in insulin resistance and systemic inflammation in pre-clinical, epidemiological, observational, or small randomized trials [91–93]. However, widespread use should be cautioned due to a lack of large-scale trials to corroborate these suggestive findings [94].

Pharmacologic Therapies

As an adjunct to healthy lifestyle choices, appropriate use of medications that address individual components of MetS reduce CV risk. Hypertension, cholesterol abnormalities, and dysglycemia may be treated with pharmacological agents. A combination of agents to address the multiple pathways may provide the greatest benefit.

Hypertension

Based on the high cardiovascular risk, antihypertensive therapy should be initiated in patients with MetS to target blood pressures of 140/90 mmHg or less [95, 96]. Angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) should be considered as first-line agents due to their high efficacy in blood pressure lowering, prevention of albuminuria, and reduced risk for development of T2D [96]. A 25% reduction in cardiovascular events is observed with ACEi or ARB [97]. Calcium channel blocking agents also demonstrate reduction in albuminuria may be considered as second line [98]. Thiazide diuretics and β-blocking agents are effective and low cost; these may be considered as additional therapies for blood pressure reduction in MetS.

Cholesterol

Cholesterol-lowering therapies yield significant cardiovascular risk reduction and should be considered in MetS. The presence of MetS predicts similar cardiovascular risk as other scoring systems, such as Framingham Risk Score, especially over the longer term (>10 year risk) [99, 100]. Treatment of patients with MetS should target LDL cholesterol levels less than 100 mg/dL [101, 102]. In most patients, statin therapy should be first choice due to significant LDL and cardiovascular risk reduction with a relatively low cost. Proprotein convertase subtilisin/kexin type 9 inhibitors can be considered in individuals with atherosclerotic disease who are unable to achieve LDL targets with statins or in patients with familial hypercholesterolemia. Ezetimibe has only modest effects in lowering LDL levels, but may be given in addition to statins to achieve LDL targets, or to patients with only mild elevations in LDL cholesterol.

The bile acid sequestrant colesevelam has several activities that may be beneficial for patients with MetS. Treatment with colesevelam lowers LDL levels by approximately 18% through reduced intestinal absorption, and it lowers serum glucose levels and insulin requirements through diminished intestinal carbohydrate absorption [103]. In a trial of men with MetS already taking statins, colesevelam treatment increased insulin sensitivity and reduced fasting serum glucose by 6 mg/dL, postprandial serum glucose by 17 mg/dL, and LDL cholesterol by 22 mg/dL [103].

When considering a lipid-lowering regimen, hypertriglyceridemia, a hallmark of MetS, should also be addressed. In many cases, initiation of statin therapy combined with lifestyle interventions either with or without insulin sensitizing agents can substantially reduce triglyceride levels. In patients with persistent hypertriglyceridemia, treatment with fibrate medications or long-chain n-3 fatty acids is beneficial. Several large trials suggest pharmacologic intervention in individuals with triglyceride levels greater than approximately 200 mg/dL and HDL levels less than approximately 35 mg/dL reduces the risk of cardiovascular events by 27–31%

[104–106]. Treatment with icosapent ethyl, a concentrated form of eicosapentaenoic acid, reduces triglyceride levels by 22% and CRP levels by 40–80% in patients with MetS [107]. In a randomized trial of 8179 patients at high cardiac risk with moderately elevated triglycerides (150–499 mg/dL), treatment with icosapent ethyl reduced cardiovascular outcomes by 25% over nearly 5 years [108]. Treatment of hypertriglyceridemia in high risk patients, such as MetS, represents a commonly overlooked opportunity for cardiovascular risk reduction [109].

Insulin Resistance

Therapies to improve insulin sensitivity have potential to drastically alter the course of MetS. In many instances, though, large randomized trials have not been completed. The United States Food and Drug Administration (FDA) has not approved use of insulin sensitizing medications in MetS, except in the case of medicines approved in the treatment of obesity.

Metformin

While not approved by the FDA for use in MetS, metformin has been commonly administered to patients with MetS due to its low cost, safety profile, and efficacy. Results from the DPP trial demonstrate a 27% reduction in development of T2D with metformin therapy [110]. Additionally, metformin can improve hepatosteatosis that is commonly present in patients with MetS [111]. Mechanistically, metformin use is associated with reduced TNFα and increased adiponectin levels, and raises endothelial NO production, which mitigates MetS development [112, 113].

Acarbose

Acarbose is an α-glucosidase inhibitor that acts to slow the hydrolysis and absorption of dietary carbohydrates. This effectively reduces serum glucose levels, though only to a relatively low effect in patients with T2D. A single large trial investigating use of acarbose demonstrates a reduction in incidence of T2D by approximately 38% among patients with MetS [114]. A post hoc analysis of patients with MetS included in this study demonstrates a reduction in the incidence of T2D by 38%, a reduction in incidence of hypertension by 34%, and a reduction in cardiovascular events by 49%a after 3.3 years of follow-up [114, 115].

PPARγ Agonists

Agonists of the PPARγ nuclear receptor improve insulin resistance. The initial design of the DPP trial included a group of patients treated with troglitazone, a PPARγ agonist that was withdrawn due to hepatotoxicity. For the average of 10 months of troglitazone use in this trial, incidence of T2D was reduced by 75%, compared to a reduction of 58% for the intensive lifestyle group [116]. More recently, use of the PPARγ agonist pioglitazone was shown to reduce the incidence of subsequent ischemic stroke by 28% in a randomized trial of patients without T2D but with insulin resistance who had sustained either transient ischemic attack or ischemic stroke [117]. Additionally, pioglitazone reduced the incidence of T2D by 48% over 4.8 years in this trial. However, potential adverse effects that are commonly observed with PPARγ agonist therapy, including weight gain, edema, bone fractures, and possible exacerbation of heart failure that may limit their use in patients MetS.

GLP1 Receptor Agonists

Through potential weight loss, increased insulin sensitivity, impaired glucagon release, and enhanced pancreatic β-cell function, GLP1 receptor agonists (GLP1ra) are effective therapeutic options in MetS. The use of liraglutide is approved by the FDA for weight loss in patients with obesity. Several GLP1ra including liraglutide, dulaglutide, and semaglutide demonstrate reduced cardiovascular events in patients with T2D [118]. GLP1ra therapy may be considered in patients with MetS.

SGLT2 Inhibitors

SGLT2 inhibitors prevent reuptake of glucose in the renal proximal collecting tubules, promoting glucose excretion within urine and can mitigate the higher glucose reuptake threshold that is seen in MetS and T2D [119]. In addition to minimizing hyperglycemia, these agents induce weight loss, reduce waist circumference, insulin resistance, and triglyceride levels, increase GLP1 levels, and may preserve normal pancreatic β-cell function [120]. In one randomized trial that included patients with MetS, 58% of those treated with dapagliflozin no longer met criteria for MetS after 90 days [121]. In another trial that included patients with heart failure, empagliflozin therapy reduced rate of hospitalization and rate of renal function decline [122]. These results held true in patients with T2D, and in those without T2D. SGLT2 inhibitor therapy may be considered in high cardiovascular risk conditions other than T2D, such as MetS.

Medications for Weight Loss

Drugs that are FDA approved for weight loss therapy may be beneficial for patients with MetS. Although weight loss with these therapies is generally modest at approximately 3–5% that is maintained over 1–2 years of follow-up [123, 124], this approaches the target weight loss (5–7%) of the DPP and Finish DPS trials.

The use of orlistat, a pancreatic lipase inhibitor, has been shown to improve insulin resistance and induce weight loss in patients with MetS. In clinical trials, use of orlistat reduced fasting glucose levels, triglycerides, and blood pressure [125]. Potential adverse effects of orlistat include loose bowel movements and malabsorption of fat-soluble vitamins, which may limit its use [126]. Additionally, in a head-to-head trial, liraglutide induced more than twofold greater weight loss and a 2 cm greater reduction in waist circumference compared to orlistat treatment in patients with MetS [127]. Liraglutide is also FDA approved for weight loss in patients with obesity.

Other medications approved for weight loss exist as combination therapies. These include phentermine and topiramate, which reduces appetite and successfully induces weight 5–10% weight loss in approximately 60% of patients given this medication. Care should be taken to avoid use of phentermine/topiramate in patients with underlying CVD due to tachycardia induced by phentermine [124]. The combination of bupropion and naltrexone affects both appetite regulation and reward pathways and demonstrates modest weight reduction.

Surgical Treatment

A number of procedural interventions that alter the GI tract either through surgery or by endoscopic intervention are in clinical use. Presently, bariatric surgery is approved for patients with BMI $\geq$ 40 kg/m^2, or $\geq$35 kg/m^2 in the presence of T2D, CVD, or other weight-related complications [128]. In patients with MetS and obesity, bariatric surgical procedures can drastically alter the risk for atherosclerotic disease and T2D [129]. Surgically induced changes in the GI tract affect energy physiology, insulin resistance, hypertension, hyperlipidemia, and other weight-related complications [130].

Approved bariatric surgical procedures include the Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy, biliopancreatic diversion with or without duodenal switch, and laparoscopic gastric banding. Endoscopic insertion of either a gastric balloon or a gastric aspiration tube has also been approved, though clinical use has been limited [131].

Analysis of multiple trials show bariatric surgeries to be highly effective in weight loss and comorbidity reduction. Pooled analyses of patients who undergo bariatric procedures demonstrate a 61% reduction in incidence of T2D, a 53%

reduction in cardiovascular events, resolution of hypertension in 44–68% and resolution of MetS in 80% [132]. In a study with over 24 years of follow-up, a 23% reduction in mortality was observed in patients who undergo bariatric surgery [133].

Conclusion

As an easily identified and highly prevalent clinical syndrome, identification of MetS represents an important opportunity for early intervention to prevent the development of T2D and cardiovascular outcomes. Pathophysiological mechanisms of insulin resistance in MetS are similar to those of T2D, albeit at earlier stages and therefore all the more likely to respond to treatment. An intensive lifestyle intervention that includes significant dietary modification and increased physical activity should be universally recommended. Consideration for medical therapies that target individual components of hyperlipidemia, hypertension, insulin resistance, and weight loss should be tailored to the individual patient. Bariatric procedures may also be considered and yield substantial CVD risk reduction. Most importantly, clinical attention to patients with MetS allows a dialogue for the consideration of these impactful treatments.

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Chapter 13
Diabetes and Hypertension

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Introduction

Diabetes is a major public health problem that is rapidly approaching epidemic proportions in the United States and worldwide [1–3]. In the United States, more than 34 million people of all ages had diabetes in 2018 [4]. Furthermore, the economic burden of diabetes to the US economy is monumental. In the year 2017, diagnosed diabetes cost the United States an estimated $327 billion in medical costs and reduced productivity [5]. Cardiovascular disease (CVD) is by far the leading cause of death in people with diabetes accounting for up to 80% of mortality in this patient population [6–9].

Risk factors for CVD that cluster in diabetes include hypertension, central obesity, dyslipidemia, microalbuminuria and coagulation abnormalities, and left ventricular hypertrophy (LVH) [9] (Table 13.1). Among those risk factors, hypertension is approximately as twice as frequent in patients with diabetes compared to those without the disease and accounts for up to 85% of the excess CVD risk. Conversely, patients with hypertension are more prone to have diabetes than are normotensive persons [10]. In a large prospective study of 12,550 adults, the development of type 2 diabetes was almost 2.5 times as likely in patients with hypertension as in their

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Table 13.1 Risk factors for CVD that cluster in diabetes mellitus

1. Hypertension
2. Central obesity
3. Microalbuminuria
4. Low high-density lipoprotein cholesterol levels
5. High triglycerides levels
6. Small, dense low-density lipoprotein cholesterol particles
7. Hyperinsulinemia
8. Endothelial dysfunction
9. Increased fibrinogen levels
10. Increased plasma activator inhibitor-1 levels
11. Increased C-reactive protein and other inflammatory markers
12. Absent nocturnal dipping of blood pressure and pulse
13. Left ventricular hypertrophy
14. Increased uric acid levels
15. Decreased renal function

normotensive counterparts after adjustment for age, sex, race, education, adiposity, family history with respect to diabetes, physical activity level, and other health-related behaviors [11].

Epidemiological Aspects of Hypertension in Diabetes and the Metabolic Syndrome

As a component of the metabolic syndrome, hypertension is much more common than diabetes. Data from the Third National Health and Nutrition Examination Survey (NHANES III) [12], involving a representative sample of 8814 adult Americans and using the National Cholesterol Education Panel (NCEP) Adult Treatment Panel III (ATPIII) definition [13], the prevalence of hypertension (as defined by blood pressure [BP] >130/85 mmHg, or the use of antihypertensive medications) was 34% compared to only 12.6% of those with hyperglycemia [12]. In this analysis, hypertension was the second most prevalent component of the metabolic syndrome, compared to central obesity, which was the most prevalent component (38.6%). Hypertension was followed in prevalence by low high-density lipoprotein (HDL) cholesterol (37.6%), hypertriglyceridemia (30%), and diabetes (12.6%) [12]. However, it is important to emphasize that although most patients with the metabolic syndrome do not have diabetes, the prevalence of this syndrome in the diabetic population is very high (86%) [14, 15]. Furthermore, the prevalence of the metabolic syndrome in patients with impaired glucose tolerance (IGT) was 31% and 71% in those with impaired fasting glucose (IFG; >110 mmHg) [14, 15].

An analysis of the NHANES data from 1998 to 2012 showed that prevalence of metabolic syndrome increased for every sociodemographic group and by 2012,

more than 30 percent of all adults in the US suffered from metabolic syndrome [16]. Another analysis of NHANES data aiming to evaluate the risk of coronary heart disease (CHD), among people over the age of 50 years with the metabolic syndrome with and without diabetes showed that the overall prevalence of this syndrome is 44% [17]. In this analysis, which also used the NCEP definition [13], the prevalence of diabetes was 17% for the entire population and 86% for those with the metabolic syndrome [17]. Hypertension was almost as twice as common in diabetic patients with the metabolic syndrome (82.7%), compared to diabetic patients without the syndrome (43%) [17]. These data underscore the common occurrence of diabetes and hypertension as components of the metabolic syndrome, particularly in the older population. Hypertension was the strongest predictor for the presence of CHD in patients over the age of 50, followed by low HDL cholesterol and diabetes. The odds ratio, 95% confidence interval was 1.87 (1.37–2.56), 1.74 (1.18–2.58), and 1.55 (1.07–2.25) for hypertension, low HDL cholesterol, and diabetes, respectively [17].

A third analysis, from Europe [18], using the World Health Organization (WHO) definition [19], was conducted to evaluate the prevalence and CVD risk associated with the metabolic syndrome. In this analysis, 4483 patients, aged 35 to 70 years participating in a large study of type 2 diabetics in Finland and Sweden (the Botnia study) [20] were examined. The metabolic syndrome, as defined by the WHO, was present in 10% of those without diabetes compared to 50% of those with IFG/IGT, and 80% of those with diabetes [18]. The prevalence of the metabolic syndrome in diabetic patients in this European population, using the WHO definition, was close to that of the US population using the NCEP criteria (80% vs 86%, respectively) [17, 18]. Furthermore, hypertension occurred frequently in those with diabetes (59%); this prevalence increased with age and was 67% in those 60 to 69 years of age [18]. In this study hypertension was only second to microalbuminuria as the most potent predictor for CVD mortality [18]. These data from the United States and Europe, across different ethnic populations using the NCEP or WHO, consistently demonstrate the high prevalence of the metabolic syndrome that is approaching epidemic proportions in the United States and worldwide. These data also demonstrate the frequent occurrence of hypertension and diabetes mellitus (DM) as components of the metabolic syndrome conferring high risk for CVD in this patient population [12, 17, 18]. Presence of undiagnosed hypertension especially in female individuals or certain populations which might be affected disproportionately such as younger adults or non-Hispanic Blacks needs to be considered [21].

Hemodynamic and Metabolic Characteristics of Hypertension in Diabetics

Hypertension in patients with diabetes, compared to those without diabetes, has unique features, such as increased salt sensitivity, volume expansion, loss of nocturnal dipping of BP and pulse, increased propensity to proteinuria, orthostatic hypotension, and isolated systolic hypertension [10]. Most of these features are

considered risk factors for CVD [6] and are particularly important for selecting the appropriate antihypertensive medication. For example, low-dose diuretics should be considered for the treatment of volume expansion, while angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) used for those with proteinuria.

Loss of Nocturnal Decline of BP (Non-dipping)

In normotensive individuals and most patients with hypertension, there is a reproducible circadian pattern to BP and heart rate during 24-h ambulatory monitoring [22]. Typically, the BP is highest while the patient is awake and lowest during sleep, a pattern called "dipping," in which BP decreases by 10% to 15%. Patients with loss of nocturnal decline in BP "non-dippers" have less than 10% decline of BP during the night compared to daytime BP values [23]. In patients with diabetes, and many of those with the cardiometabolic syndrome, there is a loss of nocturnal dipping as demonstrated by 24-h ambulatory monitoring of BP. This is particularly important because the loss of nocturnal dipping conveys excessive risk for stroke and myocardial infarction (MI) [23–25]. Indeed, ambulatory BP has been reported to be superior to office BP in predicting target organ involvement, such as LVH and proteinuria [24, 25]. About 30% of MIs and 50% of strokes occur between 6 AM and noon [26]. This is especially important in deciding the optimal dosing strategies of antihypertensive medications where drugs that provide consistent and sustained 24-h BP control will be advantageous [26]. Indeed, nighttime BP control may be especially important in those diabetic patients with elevated nocturnal BP [10]. Diabetic patient and advanced aged have found having uncontrolled nocturnal hypertension and subsequently high risk of cardiovascular complications, such as heart failure. Considering the fact that most of the patients with diabetes are of advanced age demonstrates the significance of this issue [27].

Volume Expansion and Salt Sensitivity

Alterations in sodium balance and extracellular fluid volume have heterogeneous effects on BP in both normotensive and hypertensive subjects [28]. Increased salt intake does not raise BP in all hypertensive subjects and sensitivity to dietary salt intake is greatest in the elderly, those with diabetes, obesity, renal insufficiency, low renin status, and African Americans [29, 30].

Indeed salt sensitivity in normotensive subjects is associated with a greater age-related increase in BP [29]. This is particularly important to consider in management of hypertension in patients with diabetes, especially elderly persons, because the prevalence of both diabetes and salt sensitivity also increases with age. Thus, a decreased salt intake along with other aspects of diet, such as reduced fat and increased potassium, is important to institute in these patients [10].

Microalbuminuria

There is considerable evidence that hypertension in type 1diabetes is a consequence, rather than a cause of renal disease and that nephropathy precedes the rise in BP [10]. Persistent hypertension in patients with type 1 diabetes is often a manifestation of diabetic nephropathy as indicated by an elevation of the urinary albumin at diagnosis of diabetes [10, 31].

Both hypertension and nephropathy appear to exacerbate each other. In type 2 Diabetes, microalbuminuria is associated with insulin resistance [6, 32], salt sensitivity, loss of nocturnal dipping, and other components of the metabolic syndrome [32, 33]. Elevated systolic BP (SBP) is a significant determining factor in the progression of microalbuminuria [33, 34]. Indeed, there is an increasing evidence that microalbuminuria is an integral component of the metabolic syndrome associated with hypertension [6, 10, 33]. This concept is important to consider in selecting the pharmacological therapy for hypertension in patients with diabetes, as medications that decrease both proteinuria and BP, such as ACE inhibitors and ARBs, have evolved as increasingly important tools in reducing the progression of nephropathy in such patients. These agents also appear to improve insulin sensitivity [10]. Furthermore, aggressive BP lowering, often requiring several drugs, is very important in controlling the progressive course of diabetic renal disease. Other factors that are important include cholesterol and glycemic control and smoking cessation [10].

Isolated Systolic Hypertension

With the progression of atherosclerosis in patients with diabetes, the larger arteries lose elasticity and become rigid and the SBP increases disproportionately because the arterial system is incapable of expansion for any given volume of blood ejected from the left ventricle leading to isolated systolic hypertension, which is more common and occurs at a relatively younger age in patients with diabetes [10, 35]. Indeed, the relationship between systolic elevations in BP and micro/macrovascular disease is especially pronounced in patients with DM [10].

Orthostatic Hypotension

Pooling of blood in dependent veins during rising from a recumbent position normally leads to decrease in stroke volume and SBP with a concomitant sympathomimetic reflex induced increase in systemic vascular resistance, diastolic BP (DBP), and heart rate. In patients with diabetes and autonomic dysfunction, excessive venous pooling and impaired baroreflex sensitivity can cause immediate or delayed orthostatic hypotension, and thus may result in a reduction in cerebral blood flow leading to intermittent lightheadedness, fatigue, unsteady gait, and syncope

[36–38]. This is important to recognize in patients with diabetes and hypertension because it has several diagnostic and therapeutic implications.

For example, discontinuation of diuretic therapy and peripheral vasodilators and volume repletion might be necessary for the treatment of chronic orthostasis. Also, in the subset of patients with "hyperadrenergic" orthostatic hypertension as manifested by excessive sweating and palpitation, the use of low-dose clonidine might be necessary to blunt an excess sympathetic response [39]. Furthermore, increased propensity for orthostatic hypertension in patients with diabetes renders peripheral α-adrenergic receptor blockers less desirable and second-line agents for these patients. Additionally, doses of all antihypertensive agents must be titrated more carefully in patients with diabetes who have greater propensity for orthostatic hypertension while having high supine BPs.

Pathophysiology of Hypertension in Diabetics

The relation between hypertension, obesity, insulin resistance, and diabetes is complex. For example, hypertension is considerably more prevalent in diabetic patients than non-diabetics [6, 10]. When matched to age, gender, ethnicity, adiposity, level of physical activity, and family history, hypertension is 2.5 times more likely to develop in type 2 diabetics than non-diabetics [6, 10]. Possible reasons for the increased propensity to develop diabetes in persons with essential hypertension have been reviewed extensively [6, 10, 40]. These include an altered skeletal muscle tissue composition (i.e., more fat and less insulin-sensitive slow-twitch fibers), decreased blood flow to skeletal muscle tissue as a result of vascular hypertrophy, rarefaction and vasoconstriction, and impaired post-receptor regulatory responses to insulin [10] (Table 13.2).

In type 1 diabetics, hypertension is uncommon in the absence of diabetic renal disease [10]. BP readings start to rise about 3 years after the onset of microalbuminuria [6, 10]. In contrast, in the Hypertension in Diabetes Study, 3648 patients

Table 13.2 Pathophysiological mechanisms of insulin resistance associated with hypertension

Decreased delivery of insulin and glucose to skeletal muscles
1. Increased vasoconstriction
2. Vascular hypertrophy
3. Vascular refraction
Alteration of skeletal muscle fibers
1. Decreased insulin-sensitive slow-muscle twitch fibers
2. Increased fat interspersed with skeletal muscle fibers
Post-receptor insulin-signaling defect
1. Decreased PI3 kinase/AKT signaling responses to insulin
2. Decreased insulin-mediated glucose transport
3. Decreased glycogen synthase activity

recruited for the United Kingdom Prospective Diabetes Study (UKPDS) were examined; hypertension already existed in 39% of newly diagnosed type 2 diabetes cases [41]. In these patients, hypertension was often associated with other components of the metabolic syndrome, such as obesity, elevated triglycerides, and elevated hyperinsulinemia. The prevalence of microalbuminuria in this hypertensive group was 24% [41]. These findings highlight differences in hypertension pathophysiology of types 1 and 2 diabetes, with hypertension in the latter being more closely linked to other components of the cardiometabolic syndrome [10].

Microalbuminuria is often the first clinical sign of diabetic nephropathy. It is not only a risk factor for diabetic nephropathy but also a risk factor of CVD morbidity and mortality in both diabetic and non-diabetic patients [42]. Microalbuminuria reflects generalized endothelial cell dysfunction, including that occurring in renal glomeruli [6, 10]. Hypertension and diabetic nephropathy exacerbate each other and contribute to a cycle of progressive hypertension, nephropathy, and CVD. Several other renal-related factors contribute to the increased propensity to develop hypertension and subsequent complications in diabetic patients. Diabetic patients have an increased propensity to sodium retention and volume expansion [43]. Increased salt sensitivity in these patients involves multiple mechanisms, including hyperglycemia-induced renal sodium reabsorption in the proximal renal tubule [44], hyperinsulinemia, and renal abnormalities in renin–angiotensin–aldosterone system (RAAS) [45]. Thus, restriction of salt in the diet of these patients is important in the management of their hypertension [10].

Insulin resistance and hyperinsulinemia increase sympathetic activity, which is associated with renal sodium retention, and predispose to increased vascular resistance [6, 10]. Insulin normally enhances vasodilatation and increases muscle blood flow, which facilitates glucose utilization [45–48]. This effect is mediated, in part, by increased production of nitric oxide (NO) production [48], as insulin increases endothelial NO synthase (NOS) activity. Insulin fails to enhance muscle blood flow in both obese and diabetic patients as a result of decreased ability to stimulate NO [49]. Hyperinsulinemia and insulin resistance do not consistently lead to hypertension. Pima Indians have an increased incidence of obesity, insulin resistance, and hyperinsulinemia, but have a relatively low incidence of hypertension [50]. These observations indicate that the relationship between insulin resistance and hypertension is complex, and dependent also on ethnic and environmental factors.

Obesity, especially central obesity, is a risk factor for both hypertension and diabetes [51, 52]. Central obesity, insulin resistance, hypertension, and diabetic dyslipidemia are parts of the cardiometabolic syndrome [6, 10, 53–66] There are other abnormalities found in the cardiometabolic syndrome, such as microalbuminuria, increased coagulability, impaired fibrinolysis, and increased inflammatory status [6]. Several definitions of the metabolic syndrome have been recently published: one by the WHO [17, 18] and another by the NCEP-ATPIII in the United States [13]. The cardiometabolic syndrome is a common disorder; the prevalence in the United States, using NCEP criteria, is 22% [17, 18]. The prevalence of this syndrome, and that of type 2 diabetes, increases progressively with advancing age, obesity, and sedentary lifestyle [9, 10].

The etiology of the cardiometabolic syndrome is complex, involving genetic and acquired abnormalities [6, 10]. Central obesity is a key element in the pathogenesis of this syndrome. It is characterized by a greater deposition of fat in the upper or central part of the body (visceral fat). Visceral adipocytes are more metabolically active and insulin resistant than peripheral adipocytes [51]. They release several cytokines, like tumor necrosis factor α and interleukin-(IL)-6 that promote inflammation, dyslipidemia, hypertension, microalbuminuria, abnormal coagulability, and impaired fibrinolysis [51, 52]. Lipolysis of the abdominal fat releases free fatty acids, which are substrates for triglycerides production in the liver [49, 53, 63]. The renin–angiotensin system (RAS) is also very active in the central adipocytes [52, 54]. Furthermore, adipocyte-derived peptides have a role in promoting the cardiometabolic syndrome. For example, leptin levels are high in obese patients, and elevated leptin levels may stimulate the sympathetic nervous system and may contribute to the pathogenesis of hypertension associated with obesity [51, 52, 65]. Adiponectin has anti-inflammatory effects and its levels are low in insulin resistance conditions [52, 53, 63]. Decreased adiponectin levels may be particularly important, given the role of adiponectin in enhancement of insulin-mediated vasodilatation and glucose transport activities [52, 54]. Finally, high concentrations of resist in (an adipocyte-derived peptide) in visceral fat are associated with both insulin resistance and obesity [55]. This peptide, in contradistinction to adiponectin, inhibits insulin metabolic actions [51, 52, 55, 58].

Cardiovascular Effects of Insulin and Insulin-like Growth Factor 1 in the Normal and in the Insulin-Resistant State

Insulin and its highly homologous peptide, insulin-like growth factor 1 (IGF-1), both have important effects on vascular tone. Insulin is produced only in the pancreas [57]. On the other hand, IGF-1 is an autocrine/paracrine peptide [56–58, 67–74] produced by endothelial cells and vascular smooth muscle cells (VSMCs) following stimulation by insulin [57, 68], angiotensin 2 [57, 69], and mechanical stress [71, 72]. Furthermore, IGF-1 receptors are expressed to a greater extent than insulin receptors in VSMCs [57, 58]. IGF-1 has many important biological effects on the vasculature, including maintenance of the normal differentiated VSMC phenotype [73], glucose transport [67, 74], and modulation of vascular tone [57, 67, 70, 74–86]. IGF-1 and insulin normally attenuate vasoconstriction/enhance relaxation through a phosphatidylinositol 3-kinase (PI3K)-dependent stimulation of vascular NOS enzyme [57, 67, 74, 75, 77, 79, 80, 82] and Na+, K+-ATPase pump activity [57, 70, 78, 85, 87, 88]. In animal models of obesity, insulin resistance, and hypertension there is accumulating evidence that resistance to PI3K signaling by IGF-1 and insulin plays an important role in the pathogenesis of hypertension [57, 77, 86, 89], impaired myocardial function [57, 79, 90–98], and attenuated glucose transport [73, 88, 92].

Thus, alterations of cardiovascular and skeletal muscle IGF-2 and insulin signaling responses may explain the common co-existence of hypertension, insulin resistance, and type 2 diabetes [57, 71, 81]. Insulin and IGF-1 normally induce

vasorelaxation, in part, by lowering VSMC intracellular calcium ([Ca2+]i) levels [73, 83, 85, 88] and myosin light chain (MLC) phosphorylation/Ca2+ sensitization [83, 88]. These actions involve activation of both vascular NOS and Na+, K+-ATPase pump activity [67, 70, 74, 77–85, 87, 88]. Upon stimulation, the β-subunit of the insulin and IGF-1 receptor not only become phosphorylated on various tyrosine sites but also induce the phosphorylation of a number of accessory molecules [58], such as insulin receptor substrate (IRS)-1, which serve as important docking sites for many kinases and phosphatases. Many insulin and IGF-1 metabolic effects are mediated by PI3K upon binding to IRS-1 through its regulatory subunit (p85) SH2 domain [58]. An important downstream target of IGF-1/insulin-stimulated PI3K is the serine–threonine kinase, Akt (protein kinase B) [58, 82, 99, 100]. Akt interacts through its pleckstrin homology domain with the phospholipids produced by PI3K. Phosphorylation of Thr308 and Ser473 of Akt is important for its activation [99, 100]. Akt is involved in insulin and IGF-1-regulated glucose transport and other cell functions [99–105]. A number of studies have demonstrated a critical role for Akt signaling in mediating the vascular actions of IGF-1/insulin [78, 82, 103–108]. Furthermore, it has been observed that angiotensin II (Ang II) inhibits IGF-1 signaling through the PI3K/Akt pathway resulting in less NOS/Na+, K+-ATPase activation in VSMCs [79]. Vascular relaxation in response to insulin and IGF-1 signaling is dependent, in part, on endothelial cells and VSMC production of NO and reductions in VSMC [Ca2+]i. The NO/cyclic guanosine monophosphate (cGMP) increase in response to insulin and IGF-1 stimulation results in inhibition of MLC phosphorylation/activation [104] by increasing the activity of the myosin-bound serine/threonine specific phosphatase (MBP) [109–112]. This effect of insulin and IGF-1 thus counterbalances the increase in [Ca2+]i and the Ca2+-MLC sensitization effects mediated by vasoconstrictor agonists, such as Ang II [113–116].

Accumulating evidence suggests that Ang II may antagonize the vasodilatory actions of insulin/IGF-1 through small-molecular-weight G-protein signaling mechanism [113–117], increasing phosphorylation and activation of MLC [114, 115]. Thus, there appears to be counterbalancing actions between insulin/IGF-1 and Ang II and other vasoconstrictors in the modulation of MLC-Ca2+ sensitization/vascular tone. Generation of vascular tissue reactive oxygen species appears to be an important mechanism by which Ang II inhibits the metabolic signaling pathways by insulin and IGF-1 [118].

Insulin and IGF-1 also regulate vascular tone by increasing the VSMC Na+, K+-ATPase pump activity in VSMCs [70, 76, 83, 85], consequently elevating the transmembrane Na+gradient that drives Ca2+ efflux via Na+/Ca2+ exchange [70, 83, 85, 87–89].

Furthermore, insulin/IGF-1 may indirectly activate the Na+, K+-ATPase pump, and MBP, in VSMC by stimulating VSMC NO/cGMP [118]. Thus, in insulin-resistant states, including that associated with type 2 diabetes, there appears to exhibit vascular resistance to the vasodilatory actions of insulin and IGF-1 [118–121]. Increasingly, it appears that increased secretion of Ang II and consequent generation of reactive oxygen species [82] in vasculature contribute to this resistance [82] (Fig. 13.1).

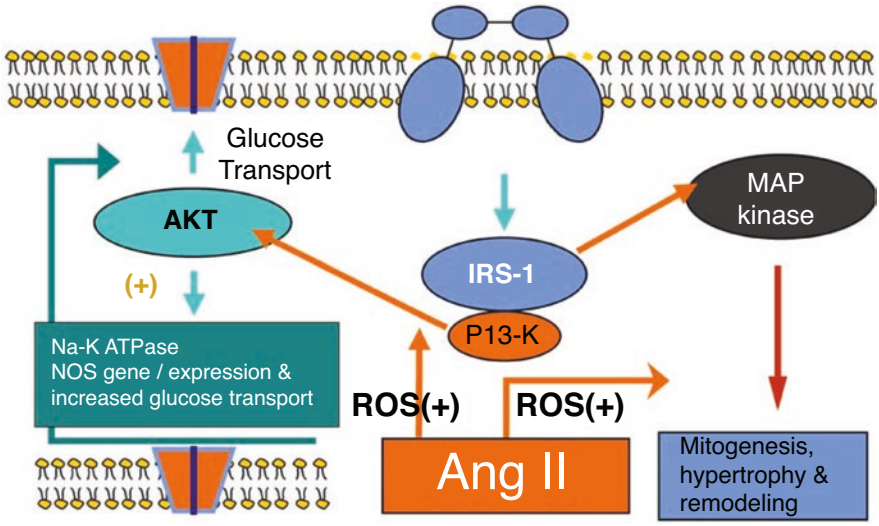

Fig. 13.1 Mechanisms by which angiotensin II antagonizes the vasorelaxing effects of insulin/insulin-like growth factor 1

Treatment Goals and Pharmacological Therapy

The goal of lowering BP in persons with diabetes and hypertension is to prevent the inordinate hypertension-associated death and disability in this population [10, 86, 122–128]. Because of increased BP variability in these patients, more BP measurements over a longer period are needed to establish the "representative BP." Because of the greater propensity to orthostatic hypotension, standing BPs should be obtained on each office visit [10, 36–38]. Therapy should begin with lifestyle modifications (Table 13.3) involving weight reduction, increased physical activity, and moderation of dietary salt and alcohol intake [129].

Drug therapy should be initiated along with lifestyle modifications to lower BP to less than 130/80 mmHg in diabetic persons, a goal that is recommended by the American Diabetes Association and the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, eighth report (JNC 8) [130]. JNC 8 recommended four classes of drugs as effective first-line therapy in these patients [130]. Each drug class has potential advantages and disadvantages. Furthermore, most diabetic patients will need several different agents to lower BP adequately.

In diabetic patients, the benefit of tight BP control is well established [10]. The UKPDS trial [122] included 1148 hypertensive patients who were followed up for about 8.4 years. Tight BP control (<140/82 mmHg) compared to less tight control (<180/105 mmHg) was associated with a 24% reduction in diabetic-related endpoints, 32% in death related to diabetes, 44% in stroke, and a 37% reduction in microvascular complications. Interestingly, the relative benefits of strict BP control outweighed the benefits of tight blood glucose control.

Another major study, the Hypertension Optimal Trial, demonstrated a 51% reduction in major CVD events in the diabetic subgroup that was randomized to a

Table 13.3 Lifestyle and dietary modification in hypertension management

1. Weight loss (maintain normal body weight [BMI, 18.5–24.9])
2. Exercise (aerobic physical activity) 30–45 min at least three times a week
3. Reduced sodium intake to 100 mmol (2.4 g) per day
4. Smoking cessation
5. Adequate intake of dietary potassium, calcium, and magnesium
6. Reduced alcohol intake to <1 oz of ethanol (24 oz of beer) per day
7. Diet rich in fruits and vegetables but low in fat

BMI body mass index

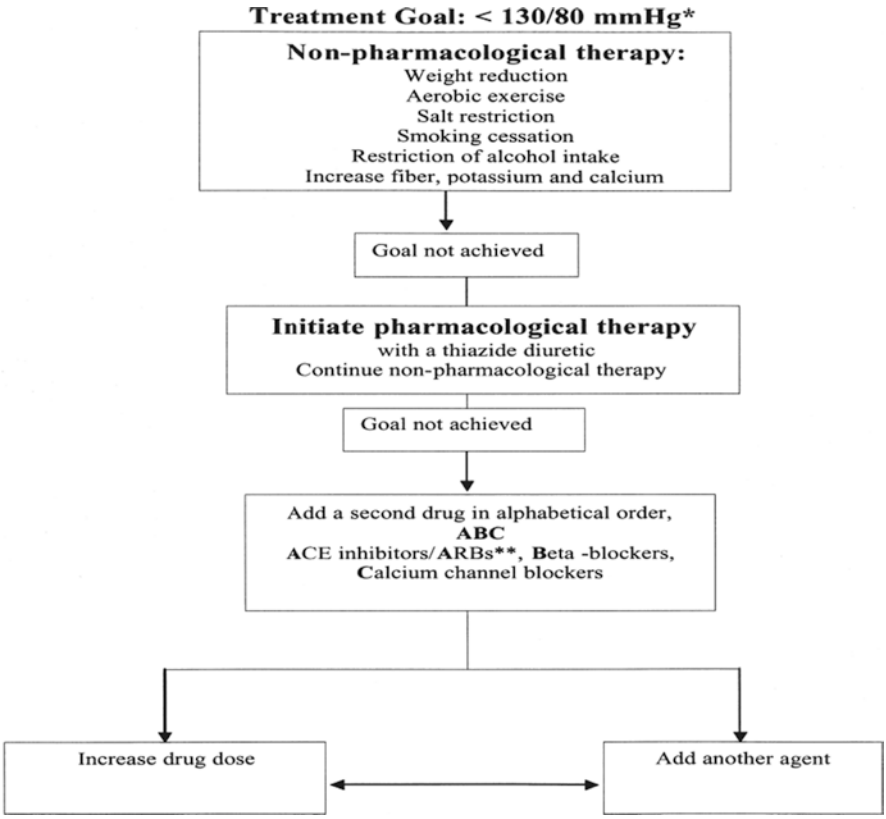

Fig. 13.2 Flow chart showing appropriate measures for lowering the blood pressure in patients with diabetes and hypertension

DBP goal of less than 80 mmHg compared to a goal of less than 90 mmHg [123]. Other studies reported significant advantages of hypertension treatment in special categories of diabetic patient like the elderly and those with isolated systolic hypertension [124, 125]. Based on the results of these clinical trials and on the data from epidemiological studies which suggested an increase in CVD events and mortality with BP more than 115/75 mmHg [126], the currently recommended BP goal in diabetic patients is now less than 130/80 mmHg (Fig. 13.2) [127–129].

In Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE trial), patients benefitted from more intensive BP (SBP < 120 mmHg) treatment regardless of baseline BP and of 10-year estimated ASCVD risk [131].

Lifestyle Modifications in Management of Hypertension (Table 13.3)

Adaptation of a healthy lifestyle is an essential component of managing hypertension in patients with diabetes or the cardiometabolic syndrome [127–129]. These interventions include weight loss, dietary sodium reduction, increased aerobic physical activity, cigarette-smoking cessation, and moderation of alcohol intake (Table 13.1) [129, 132]. The Dietary Approach to Stop Hypertension (DASH) diet, when combined with sodium reduction (2300 mg per day) is effective in lowering BP [132]. The DASH diet is rich in fiber, potassium, and calcium, low in cholesterol (150 mg per day), and low in total and saturated fat (20% and 6% of daily calories, respectively), with 55% of daily calories coming from carbohydrates. In addition to lowering BP, weight reduction and increased physical activity improve insulin resistance, serum glucose levels, and lipid profiles [133]. Exercise and weight reduction have also been shown to reduce the development of type 2 diabetes in patients with IGT [134]. The protective effects of physical activity have been demonstrated in prospective cohort studies [134–138], where the development of type 2 diabetes was significantly lower in patients who exercise regularly even after adjustment for obesity, hypertension, and family history of diabetes. In these studies, the reduction in the development of type 2 diabetes was strongest among patients with hypertension and those with the highest risk for the development of diabetes [134–136]. More recently, the Finnish study and the US Diabetes Prevention Program [136–138] have shown that diet and exercise reduce the risk of development of type 2 diabetes, by more than 50% in high risk patients with IGT. Therefore, these interventions are highly recommended in patients with hypertension who are at risk for the development of type 2 diabetes [10].

Pharmacological Therapy for Hypertension in Patients with Diabetes

Diet and lifestyle modifications are usually the first step in the management of hypertension, but most diabetic patients will also require pharmacological treatment. In fact, most diabetic patients need more than one medication to maintain their BP within the target range of less than 130/80 [127, 129]. Initiation of therapy with two drugs should be considered if BP is more than 20/10 mmHg above the goal, i.e., less than or equal to 150/90 [129]. The optimal goal BP in patients with

the cardiometabolic syndrome (without overt diabetes) is not known. However, because these patients are at high risk for CVD, it currently appears prudent to treat BP more aggressively than in the general population (i.e., same goal as in type 2 diabetic patients <130/80) [6, 10, 129].

Thiazide Diuretics

Thiazides are an important component of hypertension treatment in almost all hypertension cases. They are inexpensive and effective, especially in treating systolic hypertension [125]. The Antihypertensive and Lipid Lowering treatment to prevent Heart Attack Trial (ALLHAT) included more than 33,000 patients aged 55 or older with at least one other CHD risk factor who were followed up for a mean period of 4.9 years [139]. The study showed that there was no difference between chlorthalidone (a thiazide), lisinopril (an ACE inhibitor), and amlodipine (a calcium channel blocker [CCB]) in preventing major coronary events or in their effects on overall survival. Chlorthalidone was associated with less combined CVD, stroke, and better BP control than lisinopril, especially in African Americans. Chlorthalidone, however, was associated with less heart failure incidence rate than both lisinopril and amlodipine [139]. It should be noted that the inability to use a diuretic in those patients randomized to lisinopril may have unmasked early heart failure. In clinical practice, the combination of ACE inhibitors and thiazide diuretics is a well-accepted treatment strategy in these patients [10]. The ALLHAT study, the largest hypertension trial to date, was not designed to prospectively assess the treatment effect in the diabetic patients. However, the diabetic cohort was predesigned for subgroup analysis. About 36% of ALLHAT participants had diabetes [140]; the benefits of chlorthalidone were noticed in both diabetic and non-diabetic populations; however, for the diabetic patients, several points need to be made in order for the results of the ALLHAT trial to be viewed in the proper context:

1. Optimal control of BP in people with diabetes is difficult to achieve and requires multiple medications. Data from our investigative group showed that in a large diabetic cohort, with a mean age of 64.5 years (close to ALLHAT 66.6 mean age in the diabetic subgroup), a BP goal of 130/80 was achieved in only 25% of the patients. Furthermore, an average of 3.1 medications was required to achieve such a goal [141]. The fact that diabetic patients require multiple antihypertensive medications for BP control is documented in all the major hypertension trials. This fact makes the issue of the initial antihypertensive therapy in people with diabetes less relevant.
2. The study clearly illustrates the importance of lowering BP to improve the CVD outcome. Therefore, efforts should be directed toward improving BP control that is currently suboptimal [141].
3. Although BP reduction was in favor of the diuretic group, there was a lack of difference in the primary outcome (fatal CHD or non-fatal MI) among treatment

groups. Although ACE inhibitors have been shown to be beneficial in reducing mortality in heart failure patients [142], the observation that diuretic treatment is associated with less incidence of heart failure than ACE inhibitor treatment was likely the result, in part, of unmasking of early heart failure as a result of the inability to add a diuretic to the ACE inhibitor regimen. This also may be explained by the higher BP in the ACE inhibitor group, and in particular African American subjects who may have less BP response to ACE inhibitors than whites. The efficacy of ACE inhibitors in diabetic nephropathy is well documented. Therefore, it is of major importance to know the results of the drug comparisons in the diabetic subjects involved in the study.

4. The ALLHAT, with its simple office-based design, did not offer information that is particularly relevant for the diabetic population, such as the use of combination antihypertensive medications, or the treatment of diabetic patients with albuminuria or compromised renal function. Furthermore, it is important to note that thiazide diuretics have been shown to increase insulin resistance [10] and have some adverse metabolic side effects, such as a small increase in serum blood sugar [129], increased serum triglycerides, increased total cholesterol, increased serum uric acid, hyponatremia, hypokalemia, and hypomagnesemia. However, it is likely that some of these adverse effects can be minimized by using low doses of thiazides, such as 12.5 mg of chlorthalidone or 25 mg of hydrochlorothiazide in combination with an ACE inhibitors or ARBs.

In using diuretics, it is important to avoid volume depletion and orthostatic hypotension. Diabetic patients with autonomic neuropathy, especially elderly people, are more prone to orthostatic hypotension with subsequent risk of falls [10, 36–38]. This is particularly important in elderly diabetic patients who are often on multiple hypertensive medications [10].

ACE Inhibitors, ARBS, ARNI

The RAAS is linked to the pathophysiology of various conditions, such as hypertension, dyslipidemia, insulin resistance, and inflammation. Ang II has two major types of receptors: ATI and ATII. ATI receptors are responsible for most the deleterious effects of Ang II, including vasoconstriction and aldosterone release, and growth and remodeling [141, 142] ACE inhibitors block the conversion of Ang I to Ang II. ARBs selectively block the binding of Ang II to (AT1) receptors. ACE is also a kininase, degrading bradykinin to non-active products; thus, ACE inhibitor treatment increases kinins levels [141].

Bradykinin is a vasodilator, which might be beneficial for hypertension, as it promotes endothelial production of NO [10]. However, it might be also responsible for the cough that some patients develop while taking ACE inhibitors.

Multiple clinical trials have provided cumulative evidence that using an antihypertensive agent that interrupts the RAS results in beneficial CVD and renal

outcomes in hypertensive diabetic patients [141–143]. For example, the Heart Outcomes Prevention Evaluation (HOPE) study included 3577 diabetic patients who had also at least one other CVD risk factor. The participants were randomized to receive either ramipril (an ACE inhibitor) or placebo and were followed up for about 4.5 years. Compared to placebo, ramipril lowered the rates of MI, stroke, and all-cause mortality in diabetic patients by 22%, 33%, and 24%, respectively [144]. The CVD benefits of an ARB (losartan) were compared to a β-blocker (atenolol), in the Losartan Intervention For Endpoint reduction (LIFE) study. Losartan in diabetic hypertensive patients with LVH lowered the CVD mortality and total mortality by 37% and 39%, respectively [145]. In the HOPE trial, new-onset diabetes was decreased by 35%, and in the LIFE study, new-onset diabetes was decreased by 25% of those that began the study without evidence of clinical diabetes [144, 145]. In the recent valsartan antihypertensive long-term use evaluation (VALUE) trial, new-onset diabetes was decreased in the valsartan group by 23% compared to the amlodipine-treated patients [146].

RAAS blockade has also been shown to reduce the risk for renal disease and renal disease progression in diabetes [141]. The benefits of ACE inhibitors in renal disease in type 2 appear promising, but there is a need for more investigation [147]. On the other hand, several major clinical trials, the Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) trial [148], the IRbesartan MicroAlbuminuria type II diabetes in hypertension patients (IRMA II) trial [149], the Irbesartan in Diabetic Nephropathy Trial [150], and the Microalbuminuria Reduction with VALsartan [151], demonstrated the renal protection effects of ARBs. Indeed, the mean BP was similar in the placebo and valsartan-treated groups, indicating that ARBs renal protection effects are independent of BP reduction. In the RENAAL trial, which was done on diabetic patients with impaired renal function (creatinine 1.3–3 mg/dL) and proteinuria, losartan reduced the risk of the primary endpoint (a composite of doubling of serum creatinine, end-stage renal disease [ESRD], and death from any cause) by 16%. The risk of doubling of serum creatinine and ESRD was reduced by 25% and 28%, respectively [148]. Furthermore, the risk of the first hospitalization for congestive heart failure was reduced by 32%. Treatment with 300 mg of irbesartan in the IRMA II trial increased regression of microalbuminuria back to values within the normal range by 37% [149]. The combination of an ACE inhibitor and an ARB in the Candesartan And Lisinopril Microalbuminuria trial was associated with significantly more reduction in urinary albumin to creatinine ratio (50%) than with either agent alone (24% for candesartan and 39% for lisinopril) [152].

ACE inhibitors are generally well tolerated by patients; they have no adverse effects on lipids or cation metabolism. Clinically important side effects are cough (up to 15%), hyperkalemia, and rarely angioedema. In patients with underlying renal disease or longstanding hypertension, initiation of ACE inhibitor therapy might cause a small increase in serum creatinine levels, which does not necessitate discontinuation of the agents. If creatinine levels rise to more than 30% or show progressive increase on repeated measurements, treatment should be stopped and volume status examined carefully [153].

Many of these patients will be hypovolemic as a result of over treatment with diuretics, and with the resumption of more normal volume status, the ACE inhibitors can be safely reinitiated. ACE inhibitors are relatively contraindicated in patients with bilateral renal artery stenosis or unilateral renal stenosis if they have one kidney as a result of a greater risk of these patients to develop acute renal failure. ARBs are very well tolerated, and the incidence rates of cough and angioedema are much lower than those of ACE inhibitor treatment [154, 155]. Both hyperkalemia and azotemia may be associated with ARB and ACE inhibitory therapy. The JNC 7 recommended the use of an ARB as one of several alternative first-line therapies for patients with hypertension who cannot tolerate or who do not respond to the recommended first-line medications [133]. Additionally, ARBs were also recommended as an initial therapy for those who could not tolerate ACE inhibitors (usually because of cough) and in whom ACE inhibitors are recommended [133], such as patients with diabetes and proteinuria, heart failure, systolic dysfunction, post-MI, and those with mild renal insufficiency.

The combination of ACE inhibitors/ARBs with thiazides helps to minimize the adverse effect on serum potassium levels. The interesting finding from HOPE and LIFE trials suggests that both ACE inhibitors and ARBs decrease the incidence of new-onset diabetes [144, 145]. Ongoing prospective studies are more definitely evaluating the potential of these agents to lessen the development of clinical diabetes in patients with essential hypertension and others with high risk for developing diabetes [134]. Angiotensin receptor neprilysin inhibitor (ARNI) might improve glycemic control. In PARADIGM-HF trial, patients with heart failure and diabetes who received sacubitril/valsartan had a greater long-term reduction in HbA1c than those receiving enalapril [156].

β-Blockers

β-Blockers are useful in the treatment of hypertensive diabetic patients with ischemic heart disease. Also, β-blockers have been established as a mainstay of treatment for heart failure [157]. There are some controversial concerns regarding their metabolic adverse effects [158]. β-Blocker might increase risk of hypoglycemia among diabetic patients [159]. In the Atherosclerosis Risk In Communities study, β-blocker treatment was associated with28% increased risk of developing diabetes compared to no-medication group [160]. β-Blockers are a heterogeneous class of medications, having disparate metabolic and hemodynamic properties [160]. Both selective β-1 blockers and non-selective β -blockers increase insulin resistance. In contrast, vasodilating β-blockers may improve insulin action [161]. In a study of 45 hypertensive diabetic patients who were treated with either carvedilol or atenolol, carvedilol was associated with 20% increase in glucose disposal (vs 10% decrease with atenolol), a 20% reduction in serum triglyceride levels (vs 12% elevation), and an 8% increase in serum HDL cholesterol (vs 12% decrease with atenolol) [162]. In the UKPDS study, atenolol was as effective as captopril in reducing microvascular

and macrovascular events [122]. There was no significant difference in all-cause mortality between selective β1-adrenergic receptor blocker (metoprolol succinate) versus non-selective β1-β2-α1-blocker (carvedilol), in patients with diabetes and heart failure [163].

Calcium Channel Blockers

CCBs are generally classified into two classes, dihydropyridines (DHP-CCBs; e.g., nifedipine and newer agents, like amlodipine) and non-dihydropyridines (NDHP-CCBs; e.g., verapamil and diltiazem). Long-acting DHP-CCBs and NDHP-CCBs are safe, effective, and have no adverse effects on serum lipids. They can be added to ACE inhibitors and diuretics in diabetic patients to achieve the BP target of 130/80 mmHg [35]. In the ALLHAT study, amlodipine had comparable effects to chlorthalidone on CHD, stroke, and all-cause mortality rate. Interestingly, non-cardiovascular mortality rate was significantly lower and renal function better preserved in amlodipine group [139]. The ALLHAT study underscores the value of DHP-CCBs, as one of the antihypertensive drugs that are useful in treating the patients with diabetes and hypertension [35, 139]. In the VALUE study [146], amlodipine was associated with a more pronounced BP control, particularly early in the trial, compared to valsartan. However, despite BP differences, the primary composite cardiac endpoint was not different between the two treatment groups [146].

Other Pharmacological Interventions to Treat Coronary Heart Disease

Risk Factors

In addition to lifestyle modifications and antihypertensive medications, it is very important to address the other CVD risk factors, which are commonly found in hypertensive diabetic population. For example, the degree of hyperglycemia is associated epidemiologically with the incidence of microvascular and macrovascular disease. Controlling blood sugar significantly improves microvascular complications, but effects on macrovascular complications have not been proved [164]. On the other hand, statin therapy is highly beneficial for diabetic patients [165–167]. The beneficial effects of statins are independent of their classical actions on lipoproteins [167]. These effects include reductions in inflammation in the vasculature, kidney, and bone. Potential beneficial effects of these agents also include enhancement of NO production in vasculature and the kidney. These agents may improve insulin sensitivity and reduce the likelihood of persons progressing from IGT to type 2 diabetes [167].

The low-density lipoprotein (LDL) cholesterol goal in diabetic patient, as generally recognized, is less than 100 mg/dL [13], or lower. Furthermore, a non-HDL cholesterol of less than 130 mg/dL, in those with serum triglyceride levels greater than 200 mg/dL is increasingly recognized as an important target goal, as well [13]. Diabetes and hypertension associated with high risk for stroke. Using aspirin along with hypertension and lipid treatment significantly reduces the risk [168].

Finally, it is important to emphasize that adequate lowering of BP in this high-risk group of patients with hypertension and diabetes often requires a minimum of three drugs, at least one of which should be an ACE inhibitor, if tolerated. Indeed, at least six clinical trials unequivocally demonstrate the substantial benefits of aggressive BP lowering in diabetic patients.

Conclusions

Hypertension is a common co-morbidity in people with diabetes. It substantially increases the risk of CVD in this patient population. The goal of treatment of hypertension in patients with diabetes is to prevent the hypertension-associated increase risk of CVD death and disability. Persons with DM often have more labile BPs are more susceptible to postural hypotension, and often do not have a normal nocturnal "dip" of BPs.

Thus, the level of BP and the diagnosis of hypertension should be based on multiple BP measurements obtained in a standardized fashion on at least three occasions. Because of the tendency to orthostatic hypotension, standing BPs should be measured at each office visit. Furthermore, because of the increased BP variability in these patients, ambulatory BP measurements or home BP monitoring may be very useful. The consensus BP goal in diabetic persons with hypertension is less than 130/80 mmHg. Pharmacological therapy should be initiated when lifestyle modifications do not lower BP to less than 130/80 mmHg in these patients. Combination therapy is usually necessary for adequate BP control. Recent data from several clinical studies, including the UKPDS emphasizes the importance of rigorous BP control, requiring several antihypertensive medications.

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Chapter 14
Diabetes and Dyslipidemia

Kenneth R. Feingold and Carl Grunfeld

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is a major cause of morbidity and mortality in both men and women with type 1 and type 2 diabetes [1–5]. In addition to coronary disease, ASCVD includes stroke and peripheral vascular disease (PVD). PVD is common in diabetes, may be the first presentation of ASCVD, and should be recognized as needing aggressive treatment of dyslipidemia. In patients with type 2 diabetes the risk of cardiovascular disease is increased approximately three- to fourfold in women and twofold in men [1, 2, 5–7]. The annual rate of cardiovascular disease in the Framingham study was similar in men and women with diabetes, emphasizing that woman with diabetes need as aggressive treatment as men with diabetes to prevent cardiovascular disease [1, 6]. Several but not all studies have observed that patients with diabetes who have no clinical history of cardiovascular disease have approximately the same risk of having a myocardial infarction as non-diabetic patients who have a history of cardiovascular disease, i.e., diabetes is an equivalent risk factor as a history of a previous cardiovascular event [8, 9]. The duration of diabetes and the presence of other risk factors for cardiovascular disease likely determine whether a patient with diabetes has a risk equivalent to patients

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with a history of previous cardiovascular events [10, 11]. Importantly, many studies have found that patients with both diabetes and cardiovascular disease are at an extremely high risk of having another event, indicating that these individuals need especially aggressive preventive measures [4, 9]. This increased risk in patients with diabetes compared to patients without diabetes for the development of ASCVD is seen both in populations where the prevalence of cardiovascular disease is high (Western societies) and low (for example, Japan) [1]. Myocardial infections may be lethal arguing for early treatment. However, in countries where the prevalence of ASCVD is low, the contribution of cardiovascular disease as a cause of morbidity and mortality in patients with diabetes is reduced [1].

While the number of studies is not as great, the evidence indicates that patients with type 1 diabetes are also at higher risk for the development of cardiovascular disease [4, 12–14]. Notably, women with type 1 diabetes have twice the excess risk of both fatal and non-fatal vascular events compared to men with type 1 diabetes [15, 16]. It is important to recognize that developing type 1 diabetes at a young age increases the risk of cardiovascular disease to a greater extent than late onset type 1 diabetes [16]. Furthermore, the risk of developing cardiovascular disease in patients with type 1 diabetes is increased by obesity and the metabolic syndrome and approximately 50% of patients with type 1 diabetes are obese or overweight and between 8% and 40% meet the criteria for the metabolic syndrome [17].

The development of diabetes at a young age increases the risk of cardiovascular disease in patients with both type 1 diabetes and type 2 diabetes, but the deleterious impact is greater in patients with type 2 diabetes [18]. The increased risk in patients with type 2 diabetes is likely due to the increased prevalence of other cardiovascular risk factors (hypertension, dyslipidemia, obesity). Finally, concomitant renal disease in patients with both type 1 and type 2 diabetes increases the risk of cardiovascular disease [2, 12].

Of particular note is that the risk of developing cardiovascular disease in patients with diabetes has recently decreased, most likely due to better treatment of blood pressure and dyslipidemia [3, 7, 19]. This finding supports the need to aggressively treat these risk factors in patients with diabetes.

Role of Risk Factors in ASCVD

Numerous studies have demonstrated that the traditional risk factors for ASCVD play a very important role in increasing the risk of ASCVD in patients with diabetes [1–3, 20]. Patients with diabetes without other risk factors have a relatively low risk of cardiovascular disease (albeit higher than similar non-diabetic patients), whereas the increasing prevalence of other risk factors markedly increases the risk of developing cardiovascular disease [1]. The major reversible traditional risk factors are hypertension, cigarette smoking, and dyslipidemia [1–3, 12, 21]. Other risk factors include obesity (particularly visceral obesity), insulin resistance, procoagulant state (increased PAI-1, fibrinogen), family history of early cardiovascular disease,

homocysteine, renal disease, albuminuria, and inflammation (C-reactive protein, SAA, cytokines) [1–3, 20, 21]. It has become clear that to reduce the risk of cardiovascular disease in patients with diabetes, one will not only need to improve glycemic control but also address these other cardiovascular risk factors. The remainder of this chapter will focus on the dyslipidemia that occurs in patients with diabetes and how to reduce the impact of this risk factor on cardiovascular disease.

Role of Lipids in ASCVD

Similar to studies in the non-diabetic population, studies have shown that increased LDL-C and non-HDL-C levels and decreased HDL-C levels are associated with an increased risk of cardiovascular disease in patients with diabetes [1, 2, 20, 21]. In the UKPDS type 2 diabetes cohort, LDL-C levels were the strongest predictor of coronary artery disease [22]. While it is widely recognized that elevated levels of LDL-C and non-HDL-C cause atherosclerosis and cardiovascular disease, the role of decreased HDL-C is less certain. Genetic studies and studies of niacin and cholesterol ester transport proteins inhibitors (CETP inhibitors), drugs that raise HDL-C, have not supported low HDL-C levels per se as a causative factor for atherosclerosis [23]. Rather it is currently hypothesized that HDL function is associated with atherosclerosis risk and that HDL function does not precisely correlate with HDL-C levels [23].

Elevations in serum triglyceride levels also are associated with an increased risk of cardiovascular disease in patients with diabetes [2, 21, 24]. Elevated triglycerides are usually accompanied by increased non-HDL cholesterol. It is not clear whether increased triglyceride levels directly cause atherosclerosis and cardiovascular disease or whether the elevation in triglycerides is a marker for other abnormalities [2, 21, 24, 25]. Recent Mendelian randomization studies of genes affecting triglyceride metabolism have provided support for the hypothesis that elevated triglyceride levels play a causal role in atherosclerosis [25, 26], but studies have not yet demonstrated that specifically lowering triglyceride levels reduces cardiovascular events.

Lipid Abnormalities in Patients with Diabetes

In patients with type 1 diabetes in good glycemic control, the lipid profile is very similar to lipid profiles in the non-diabetic population [20]. In some studies HDL-C levels are modestly increased in patients with type 1 diabetes [27]. In contrast, in patients with type 2 diabetes, even when in good glycemic control, there are abnormalities in the lipid profile [28–31]. It is estimated that 30–60% of patients with type 2 diabetes have lipid abnormalities [3, 32]. Specifically, patients with type 2 diabetes frequently have an increase in serum triglyceride, VLDL, and IDL levels and a decrease in HDL-C levels. Non-HDL-C levels are increased due to the increase in VLDL and IDL.

LDL-C levels are usually similar to LDL-C levels in non-diabetic subjects, but there is an increase in small dense LDL, a lipoprotein particle that is particularly pro-atherogenic [33]. While triglyceride levels are the strongest predictor of the presence of small dense LDL, an increase in small particles may be present without frank hypotriglyceridemia. As a consequence of an increase in small dense LDL particles, there are more LDL particles, which together with the increases in VLDL and IDL particles, leads to an elevation in apolipoprotein B levels [28–31]. Additionally, the postprandial increase in serum triglyceride levels is accentuated and elevations in postprandial lipids are associated with an increased risk of cardiovascular disease [28–31].

It should be recognized that the lipid changes observed in patients with type 2 diabetes are characteristic of the alterations in lipid profile seen in obesity and the metabolic syndrome (insulin resistance syndrome) [34]. Since many patients with type 2 diabetes are obese, insulin resistant, and have the metabolic syndrome, it is not surprising that the prevalence of increased triglycerides and small dense LDL and decreased HDL-C is common in patients with type 2 diabetes even when these patients are in good glycemic control. Obesity is also accompanied by increased systemic inflammation.

As noted above the function of HDL may be of greater importance in determining the risk of developing atherosclerosis than HDL-C levels. Studies have demonstrated that the anti-oxidant and anti-inflammatory functions of HDL, which are hypothesized to be anti-atherogenic, are reduced in HDL isolated from patients with type 1 and type 2 diabetes [27, 35]. Additionally, the ability of HDL to facilitate cholesterol efflux, the first step in reverse cholesterol transport, is also reduced in patients with type 1 and type 2 diabetes [36, 37]. Together these findings indicate that HDL function is perturbed in patients with diabetes, which could increase the risk of developing atherosclerosis. Additionally, these observations indicate that HDL-C levels per se may not fully reflect risk of cardiovascular disease in patients with diabetes.

In patients with both type 1 and type 2 diabetes, poor glycemic control increases serum triglyceride, VLDL, IDL, and non-HDL cholesterol levels and decreases HDL-C levels [29]. Poor glycemic control can also result in a modest increase in LDL-C, which because of the elevation in triglycerides is typically in the small dense LDL subfraction. It is therefore important to optimize glycemic control in patients with diabetes because this will have beneficial effects on lipid levels.

Lipoprotein(a) (Lp (a)) levels are usually within the normal range in patients with type 1 and type 2 diabetes [38]. Many studies have observed no impact of diabetes mellitus on Lp(a) levels, while some studies have reported an elevation and some studies a decrease in Lp(a) concentrations [38]. The development of microalbuminuria and the onset of renal disease are associated with an increase in Lp (a) levels in patients with diabetes [39]. Of note low Lp(a) levels are associated with an increased risk of developing type 2 diabetes [38]. A recent very large case–control study found that Lp(a) concentrations in the bottom 10% increases type 2 diabetes risk [40]. The mechanism by which low Lp(a) levels is associated with an increased risk of developing type 2 diabetes is unknown (Table 14.1).

Table 14.1 Lipid abnormalities in patients with diabetes

Type 1 diabetes	Lipid profile is similar to controls if glycemic control is good
Type 2 diabetes	Increased triglycerides, VLDL, IDL, non-HDL-C, and apolipoprotein B. Decreased HDL-C. Normal LDL-C but an increase in small dense LDL and LDL particle number
Poor glycemic control	Increased triglycerides, VLDL, IDL, and apolipoprotein B and decreased HDL-C. Modest increase in LDL-C with increase in small dense LDL and particle number

Table from reference [41] with permission

Effect of Glucose-Lowering Drugs on Lipids

Therapies employed to lower glucose levels may have an impact on lipid levels above and beyond their effects on glucose metabolism. In reviewing the literature, it is often very difficult to separate the effects of improving glycemic control vs. the direct effect of the drugs. Moreover, many of the changes induced by drug therapy result in only small changes in LDL-C, HDL-C, and triglyceride levels, are variable from study to study, and are therefore of questionable clinical significance.

Insulin, sulfonylureas, meglitinides, DPP4 inhibitors, and alpha-glucosidase inhibitors do not appear to markedly alter fasting lipid profiles other than by improving glucose control (there are data indicating that DPP4 inhibitors and acarbose decrease postprandial triglyceride excursions, but they do not markedly alter fasting lipid levels) [42]. In contrast, metformin, colesevelam, thiazolidinediones, GLP1 receptor agonists, bromocriptine-QR, and SGLT2 inhibitors have been reported to have effects independent of glycemic control on serum lipid levels.

Metformin modestly decreases serum triglyceride and LDL-C levels without altering HDL-C levels [42]. In a meta-analysis of 37 trials with 2891 patients, metformin decreased triglycerides by 11 mg/dL when compared with control treatment ($p = 0.003$) [43]. In an analysis of 24 trials with 1867 patients, metformin decreased LDL-C by 8 mg/dL compared to control treatment ($p < 0.001$) [43]. Metformin may increase LDL particle size [44]. In contrast, metformin did not significantly alter HDL-C levels [43]. Thus, metformin has a small effect on lipid levels.

Colesevelam, a bile acid sequestrant that is approved for glucose lowering, decreases LDL-C levels by 15–20% [45, 46]. Bile acid sequestrants by binding bile acids in the intestine result in a decrease in hepatic bile acid levels leading to the increased utilization of cholesterol to synthesize bile acids in the liver. A reduction in hepatic cholesterol levels leads to the upregulation of LDL receptor expression and the enhanced uptake of circulating LDL reducing LDL-C levels [45]. The effect of bile acid sequestrants on triglyceride levels varies [45]. In patients with normal triglyceride levels, bile acid sequestrants modestly increase triglyceride levels. However, as baseline triglyceride levels increase, the effect of bile acid sequestrants on plasma triglyceride levels becomes greater, and can result in substantial increases in triglyceride levels [45]. Bile acid sequestrants are contraindicated in patients with

triglycerides >500 mg/dL [45]. Despite the increase in triglycerides, colesevelam has little effect on HDL-C levels. Surprisingly, despite the increase in triglycerides colesevelam administration is accompanied by an increase in both VLDL and LDL particle size [47].

The effect of thiazolidinediones on the lipid profile differs slightly between rosiglitazone and pioglitazone. Both drugs increase LDL-C and HDL-C levels [42]. This is accompanied by reductions in the small dense LDL subfraction and an increase in the large buoyant LDL subfraction with both thiazolidinediones [42]. However, rosiglitazone only decreases serum triglycerides if the baseline triglyceride levels are high [42]. In contrast, pioglitazone has less impact on LDL-C levels but decreases serum triglyceride levels [42]. In the PROactive study, a large randomized cardiovascular outcome study, pioglitazone decreased triglyceride levels by approximately 10%, increased HDL-C levels by approximately 10%, and increased LDL-C by 1–4% [48]. In a randomized head to head trial comparing rosiglitazone and pioglitazone, it was shown that pioglitazone decreased serum triglyceride levels and increased serum HDL-C levels to a greater degree than rosiglitazone [49, 50]. Additionally, pioglitazone increased LDL-C levels less than rosiglitazone. In contrast to the differences in lipid parameters, both rosiglitazone and pioglitazone decreased A1c and C-reactive protein to a similar extent. The mechanism by which pioglitazone induces more favorable changes in lipid levels than rosiglitazone despite similar changes in glucose levels is unclear, but differential actions of ligands for nuclear hormone receptors are well described.

Treatment with SGLT2 inhibitors results in a small increase in LDL-C and HDL-C levels [42]. In a meta-analysis of 48 randomized controlled trials SGLT2 inhibitors significantly increased LDL-C (3.8 mg/dL, $p < 0.00001$), HDL-C (2.3 mg/dL, $p < 0.00001$), and decreased triglyceride levels (8.8 mg/dL, $p < 0.00001$) [51]. The mechanism for these increases in LDL and HDL cholesterol is unknown but could be due to a decrease in plasma volume. The decrease in triglyceride levels could be secondary to weight loss and/or inflammation.

GLP-1 receptor agonists can favorably affect the lipid profile by inducing weight loss (decreasing triglycerides and very modestly decreasing LDL-C levels) [42]. In a review by Nauck et al. it was noted that GLP-1 receptor agonists lowered triglyceride levels by 18–62 mg/dL depending upon the specific GLP-1 receptor agonist while decreasing LDL-C by 3–8 mg/dL and increasing HDL-C by less than 1 mg/dL [52]. Additionally, GLP-1 receptor agonists decrease postprandial triglycerides by reducing circulating chylomicrons secondary to decreasing intestinal lipoprotein production [42, 52]. GLP-1 receptor agonists may increase the size of LDL particles [53, 54]. DPP4 inhibitors have a similar effect on postprandial triglyceride levels as GLP-1 receptor agonists while having minimal effects on fasting lipid levels [52]. GLP-1 receptor agonists decrease weight and inflammation more that DPP4 inhibitors.

Finally, bromocriptine-QR (Cycloset) decreases triglyceride levels but has no significant effect on LDL-C or HDL-C levels [55, 56]. The decrease in triglyceride levels is thought to be due to a decrease in hepatic triglyceride synthesis, due to the reduced delivery of fatty acids to the liver for triglyceride synthesis that results from a decrease in adipose tissue lipolysis (Table 14.2) [57].

Table 14.2 Effect of glucose-lowering drugs on lipid levels

Metformin	Modestly decrease triglycerides and LDL-C; increase LDL particle size.
DPP4 Inhibitors	Decrease postprandial triglycerides
GLP-1 Receptor Agonists	Decrease fasting and postprandial triglycerides and small dense LDL
Rosiglitazone and Pioglitazone	Decrease triglycerides and increase HDL-C. Small increase LDL-C but a decrease in small dense LDL
Acarbose	Decrease postprandial triglycerides
SGLT2 Inhibitors	Small increase in LDL-C and HDL-C
Colesevelam	Decrease LDL-C. May increase triglycerides
Bromocriptine-QR	Decrease triglycerides
Sulfonylureas and Insulin	No effect

Table from reference [41] with permission

Pathophysiology of the Dyslipidemia of Diabetes

There are a number of different abnormalities that contribute to the dyslipidemia seen in patients with type 2 diabetes [29–32, 58–60].

Increase in Triglycerides

Multiple mechanisms account for the increase in triglyceride levels seen in patients with type 2 diabetes, which are affected both by the level of control of glucose and by factors, such as obesity that also contribute to diabetes.

Overproduction of VLDL by the Liver

A major abnormality is the hepatic overproduction of VLDL (Fig. 14.1). The rate of secretion of VLDL is highly dependent on triglyceride availability, which is determined by the levels of fatty acids available for the synthesis of triglycerides in the liver. An abundance of triglycerides inhibits the intra-hepatic degradation of Apo B-100 allowing for increased VLDL formation and secretion.

There are three major sources of fatty acids in the liver all of which may be increased in patients with type 2 diabetes. First, the movement of fatty acids from adipose tissue to the liver is enhanced. An increased mass of adipose tissue, particularly visceral adipose tissue, results in the enhanced transport of fatty acids to the liver. Additionally, insulin inhibits the lipolysis of triglycerides to free fatty acids in adipose tissue; thus, in patients with poorly controlled diabetes due to a decrease in insulin levels or a decrease in insulin activity due to insulin resistance, the inhibition

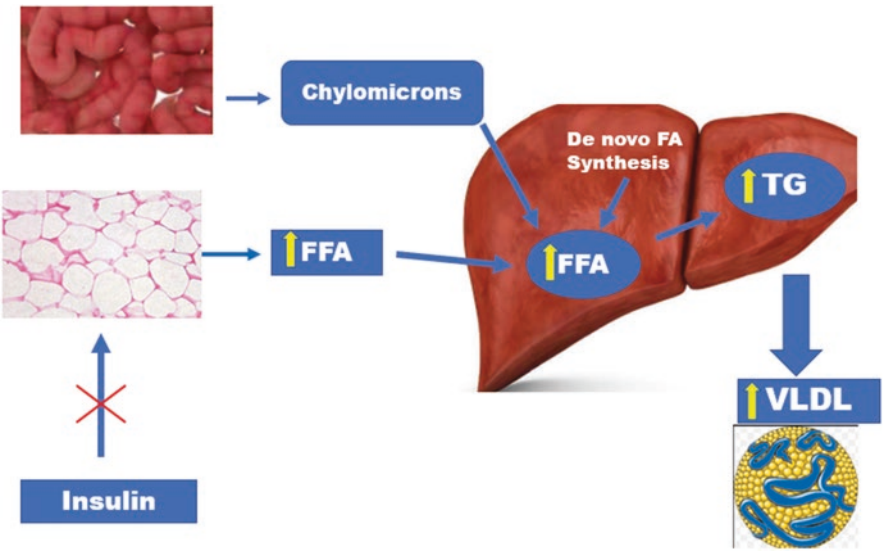

Fig. 14.1 Mechanism for overproduction of VLDL by the liver

of triglyceride lipolysis is blunted and there is increased triglyceride lipolysis leading to increased fatty acid transport to the liver. A second source of fatty acids in the liver is de novo fatty acid synthesis. Numerous studies have shown that hepatic fatty acid synthesis is increased in patients with type 2 diabetes. This increase may be due to the hyperinsulinemia seen in patients with insulin resistance. While the liver is resistant to the effects of insulin on carbohydrate metabolism when there is insulin resistance, the liver remains sensitive to the effects of insulin stimulating lipid synthesis [61]. Specifically, insulin increases the activity of SREBP-1c, a transcription factor that stimulates the expression of acetyl CoA carboxylase and fatty acid synthase, the key enzymes required for the synthesis of fatty acids. Thus, while the liver is resistant to the effects of insulin on carbohydrate metabolism, the liver remains sensitive to the ability of insulin to stimulate lipid synthesis [61]. Additionally, hyperglycemia per se can induce another transcription factor, carbohydrate responsive element binding protein (ChREBP), which also stimulates the transcription of the enzymes required for fatty acid synthesis [62]. The third source of fatty acids is the hepatic uptake of triglyceride-rich lipoproteins. Studies have shown that an increase in fatty acid synthesis in the intestines leads to the enhanced secretion of chylomicrons in animal models of type 2 diabetes. This increase in chylomicrons results in the increased delivery of fatty acids to the liver, which may in turn be secreted as VLDL.

The increase in fatty acids in the liver produced by these three pathways results in an increase in the hepatic synthesis of triglycerides and the protection of Apo B-100 from degradation resulting in the enhanced formation and secretion of VLDL. Finally, insulin stimulates the post-translational degradation of Apo B-100 in

the liver, so the decrease in insulin activity in patients with type 2 diabetes thereby allows for the enhanced survival of Apo B-100 promoting increased VLDL formation.

Decreased Degradation of Triglyceride-Rich Lipoproteins

The decreased metabolism of triglyceride-rich lipoproteins also plays a role in the elevation of triglyceride levels. There is a modest decrease in lipoprotein lipase activity, the key enzyme that metabolizes triglyceride-rich lipoproteins. The expression of lipoprotein lipase is stimulated by insulin and decreased insulin activity in patients with type 2 diabetes results in a decrease in lipoprotein lipase activity, which plays a key role in the breakdown of the triglycerides carried in chylomicrons and VLDL particles. Additionally, levels of Apo C-III, a key regulator of triglyceride metabolism, are increased in patients with type 2 diabetes. Glucose increases and insulin decreases Apo C-III expression; thus, diabetes with hyperglycemia and either insulin deficiency or insulin resistance contribute together to increases in Apo C-III levels. Apo C-III is an inhibitor of lipoprotein lipase activity and thereby reduces the clearance of triglyceride-rich lipoproteins. Additionally, Apo C-III also inhibits the cellular uptake of lipoproteins. Recent studies have shown that loss of function mutations in Apo C-III leads to decreased serum triglyceride levels and a reduced risk of cardiovascular disease [63, 64]. Notably, inhibition of Apo C-III expression leads to a decrease in serum triglyceride levels even in patients deficient in lipoprotein lipase, indicating that the ability of Apo C-III to regulate serum triglyceride levels is not solely dependent on affecting lipoprotein lipase activity [65]. Thus, in patients with diabetes, a decrease in clearance of triglyceride-rich lipoproteins also contributes to the elevation in serum triglyceride levels.

Effects of Hypertriglyceridemia on HDL and LDL

The elevation in triglyceride-rich lipoproteins has effects on the size and composition of LDL and HDL particles (Fig. 14.2). Specifically, cholesterol ester transfer protein (CETP) mediates the exchange of triglycerides from triglyceride-rich VLDL and chylomicrons to LDL and HDL. The increase in triglyceride-rich lipoproteins per se leads to an increase in CETP-mediated exchange, increasing the triglyceride content of both LDL and HDL. The triglyceride on LDL and HDL is then hydrolyzed by hepatic lipase and lipoprotein lipase leading to the production of small dense LDL and small HDL particles. Furthermore, hepatic lipase activity is increased in patients with type 2 diabetes, which will facilitate the removal of triglyceride from LDL and HDL resulting in small lipoprotein particles. Thus, the elevation in triglyceride-rich lipoprotein particles plays a key role in the production of small dense LDL and small HDL particles in patients with type 2 diabetes.

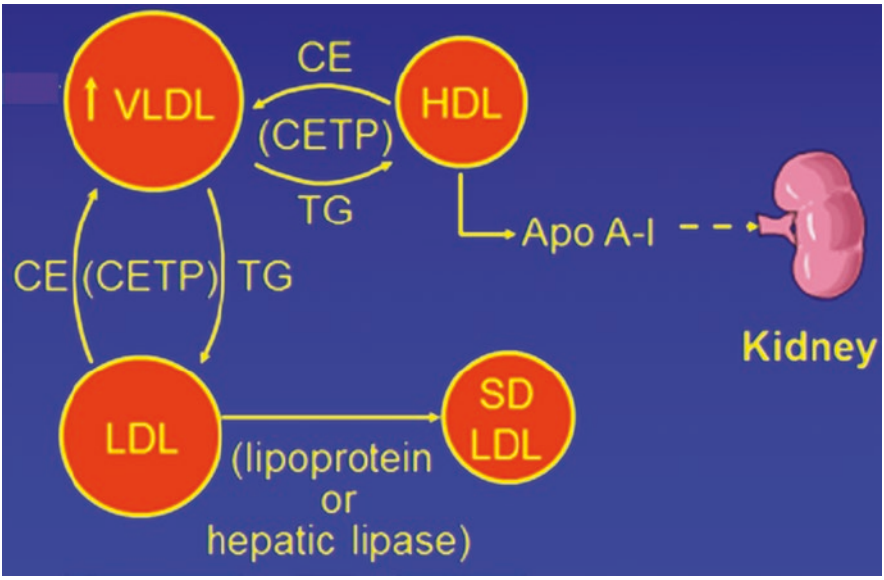

Fig. 14.2 The effect of hypertriglyceridemia on LDL and HDL (modified from reference [41] with permission)

The decrease in HDL-C levels in diabetes is due to several factors. First, the affinity of Apo A-I for small HDL particles is reduced, leading to the disassociation of Apo A-I, which in turn leads to the accelerated clearance and breakdown of Apo A-I by the kidneys. Second, the production of Apo A-I may be reduced in patients with diabetes. As noted above, high glucose levels activate ChREBP and this transcription factor inhibits Apo A-I expression. Lastly, insulin stimulates Apo A-I expression and a reduction in insulin activity may also lead to a decrease in Apo A-I expression. The increased clearance and decreased production of Apo A-I result in lower levels of Apo A-I and HDL-C levels in patients with type 2 diabetes.

Role of Poor Glycemic Control

The above described changes lead to the typical dyslipidemia observed in patients with type 2 diabetes (increased triglycerides, decreased HDL-C, and an abundance of small dense LDL and small HDL). In patients with both type 1 and type 2 diabetes, poor glycemic control can further adversely affect lipid and lipoprotein metabolism. As noted above the expression of lipoprotein lipase is stimulated by insulin. If insulin activity is very low the expression of lipoprotein lipase is severely compromised, resulting in a marked impairment in the clearance of triglyceride-rich lipoproteins. This delayed clearance of both chylomicrons and VLDL results in elevations of triglyceride-rich lipoproteins. Additionally, decreased insulin activity results in a marked increase in lipolysis in adipose tissue, leading to the release of

free fatty acids into the circulation. This increase in serum fatty acids results in the increased delivery of fatty acids to the liver, stimulating triglyceride synthesis in the liver, and the enhanced production and secretion of VLDL. Whereas patients with type 1 diabetes who are well controlled typically have normal serum lipid profiles, if their control deteriorates, they can develop hypertriglyceridemia. In patients with type 2 diabetes deterioration of glycemic control can further worsen their underlying dyslipidemia resulting in greater elevations in serum triglyceride levels. If the production of new VLDL is increased sufficiently this can result in an increase in LDL-C levels and small dense LDL. HDL-C levels may decrease due to the formation of small HDL that are more susceptible to accelerated clearance and decreased Apo A-I synthesis. Improvements in glycemic control can markedly lower serum triglyceride levels and may increase serum HDL-C levels. In patients with very poorly controlled diabetes improvements in glycemic control may also decrease LDL-C levels.

Role of Obesity and Inflammation

Many if not most patients with type 2 are obese and increasing number of patients with type 1 are also obese. Obesity is a pro-inflammatory state due to the macrophages that infiltrate adipose tissue. The cytokines produced by these macrophages and the adipokines that are produced by fat cells can also alter lipid metabolism [66, 67]. The pro-inflammatory cytokines, TNF and IL-1, inhibit the expression of lipoprotein lipase and stimulate the expression of angiopoietin, like protein 4, an inhibitor of lipoprotein lipase [68]. Together these changes decrease lipoprotein lipase activity, thereby delaying the clearance of triglyceride-rich lipoproteins. Additionally, pro-inflammatory cytokines stimulate lipolysis in adipocytes increasing circulating free fatty acid levels, which will provide substrate for triglyceride synthesis in the liver. Moreover, pro-inflammatory cytokines stimulate hepatic de novo fatty acid and triglyceride synthesis. These alterations will increase the production and secretion of VLDL. Thus, increases in the levels of pro-inflammatory cytokines will stimulate the production of triglyceride-rich lipoproteins and delay the clearance of triglyceride-rich lipoproteins, which together will contribute to the increase in serum triglycerides that occurs in obese patients with type 1 and type 2 diabetes.

Obesity and the increase in pro-inflammatory cytokines may also affect HDL-C levels [68–70]. First, pro-inflammatory cytokines inhibit the production of Apo A-I, the main protein constituent of HDL. Second, in many tissues pro-inflammatory cytokines decrease the expression of ABCA1 and ABCG1, which will lead to a decrease in the efflux of phospholipids and cholesterol from the cell to HDL decreasing the formation of mature HDL. Third, pro-inflammatory cytokines inhibit the production and activity of LCAT, which will limit the conversion of cholesterol to cholesterol esters in HDL. This conversion step is required for the formation of a normal spherical HDL particle and is crucial for the ability of HDL to increase the efflux of cholesterol from cells (including macrophages). Together these effects may lead to a decrease in HDL-C levels and a decrease in reverse cholesterol

transport. Reverse cholesterol transport plays an important role in preventing cholesterol accumulation in macrophages and thereby reduces atherosclerosis. In obesity, there may also be an increase in uptake of HDL_2 by adipose tissue [71].

Inflammation also decreases other important functions of HDL, such as its ability to prevent LDL oxidation [72]. This reduction in the ability of HDL to protect from oxidation may be mediated in part by lower levels of the enzyme paraoxonase, which is commonly seen in patients with diabetes and secondary to Inflammation [66, 73]. Likewise, the inflammation seen with insulin resistance in type 2 diabetes increases the concentration of ceramides in LDL, which promotes LDL aggregation, enhancing LDL uptake by macrophages [74, 75].

Role of Adipokines

Adipokines, such as leptin, adiponectin, and resistin, regulate lipid metabolism and the levels of these adipokines are altered in patients with obesity. Obesity increases serum leptin levels and leptin stimulates lipolysis in adipocytes leading to increases in serum free fatty acid levels [76]. The serum levels of adiponectin are decreased in patients who are obese [77]. Decreased adiponectin levels are associated with elevations in serum triglyceride levels and decreases in HDL-C levels [77]. These associations are thought to be causal as studies in mice have demonstrated that adiponectin knockout mice have increased triglyceride and decreased HDL-C levels while conversely, overexpressing adiponectin (transgenic mice) decreases triglyceride and increases HDL-C levels [77]. The adiponectin-induced decrease in triglyceride levels is mediated by an enhanced catabolism of triglyceride-rich lipoproteins due to an increase in lipoprotein lipase activity and a decrease Apo C-III, an inhibitor of lipoprotein lipase [77]. The increase in HDL-C levels induced by adiponectin is mediated by an increase in hepatic Apo A-I and ABCA1, which results in the increased production of HDL particles [77].

Resistin is elevated in patients who are obese and the levels of resistin directly correlate with plasma triglyceride levels [78]. Moreover, resistin has been shown to stimulate hepatic VLDL production and secretion due to an increase in the synthesis of Apo B, triglycerides, and cholesterol [78, 79]. Finally, resistin is associated with a decrease in HDL-C and Apo A-I levels [78].

Effect of Lipid-Lowering on Atherosclerotic Cardiovascular Events in Diabetes

In order to understand the current recommendations for the treatment of lipids in patients with diabetes it is essential to be familiar with the results of lipid-lowering trials on cardiovascular outcomes in patients with diabetes.

Life Style Studies

There are very few randomized trials examining the effect of lifestyle changes on cardiovascular outcomes in patients with diabetes. Additionally, while it is well recognized that lifestyle changes can alter lipid levels, these studies were not primarily designed to determine the role of changes in lipid levels on the effect of lifestyle alterations on cardiovascular outcomes.

The Look Ahead trial failed to demonstrate that lifestyle changes result in a reduction in cardiovascular events [80]. In this trial, approximately 5000 overweight or obese patients with type 2 diabetes were randomized to either an intensive lifestyle intervention group that promoted weight loss through decreased caloric intake and increased physical activity or to a group that received diabetes support and education (control group). After a median follow-up of 9.6 years there was no difference in cardiovascular events (hazard ratio in the intervention group, 0.95; $p = 0.51$). A major limitation of this study was that while the weight difference between groups was impressive during the first year of the trial, over time the differences greatly narrowed such that by the end of the trial the intensive group had a 6.0% weight loss, while the control group had a 3.5% weight loss. This very modest difference at the end of the trial demonstrates the difficulty in sustaining long-term lifestyle changes. In a post hoc analysis, individuals who lost at least 10% of their bodyweight in the first year of the study had a 21% lower risk of cardiovascular events (HR 0.79; $p = 0.034$) [81]. Thus, while lifestyle changes are likely to be beneficial in reducing cardiovascular events, in clinical practice they are rarely sufficient because long-term life style changes are very difficult for most patients to maintain.

In contrast to the failure of the Look Ahead trial to reduce cardiovascular events, the PREDIMED trial employing a Mediterranean diet (increased monounsaturated fats) did decrease the incidence of major cardiovascular disease [82, 83]. In this multicenter trial center trial over 7000 patients at high risk for developing cardiovascular disease were randomized to a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet. Approximately 50% of the patients in this trial had type 2 diabetes. In the patients assigned to the Mediterranean diets there was 29% decrease in myocardial infarctions, strokes, and deaths from cardiovascular disease. Subgroup analysis demonstrated that the Mediterranean diets were equally beneficial in patients with and without diabetes. The Mediterranean diet resulted in a small but significant increase in HDL-C levels, a small decrease in both LDL-C and triglyceride levels and an improvement o in lipoprotein sub-particles [84]. The Mediterranean diets also improved the functions of HDL in reverse cholesterol transport and anti-oxidant activity [85]. The results of this trial indicate that we should be encouraging our patients with diabetes to follow a Mediterranean type diet. It is likely that the beneficial effects of the Mediterranean diet on cardiovascular disease are mediated by multiple mechanisms with alterations in lipid levels making only a minor contribution.

Monotherapy Drug Studies

Statins

The Cholesterol Treatment Trialists analyzed data from 18,686 subjects with diabetes (mostly type 2 diabetes) from 14 randomized trials [86]. In the statin-treated group there was a 9% decrease in all-cause mortality, a 13% decrease in vascular mortality, and a 21% decrease in major vascular events per 39 mg/dL reduction in LDL-C levels. The beneficial effect of statin therapy was seen in both primary and secondary prevention patients. The effect of statin treatment on the reduction in cardiovascular events in patients with diabetes was similar to that observed in non-diabetic subjects. Thus, these studies indicate that statins are beneficial in reducing cardiovascular disease in patients with diabetes. Because of the large number of patients with diabetes included in the Heart Protection Study (HPS) and CARDS these two studies will be discussed in greater depth.

The HPS was a double-blind randomized trial that studied patients at high risk for the development of cardiovascular events, including patients with a history of myocardial infarctions, other atherosclerotic lesions, diabetes, and/or hypertension [87, 88]. The study therefore examined both primary and secondary prevention. Patients were between 40 and 80 years of age and had to have total serum cholesterol levels greater than 135 mg/dL. The major strength of this trial was the large number of patients studied (>20,000). The diabetes subgroup included 5963 subjects and thus was as large as many other prevention trials. The study was a 2 × 2 study design comparing simvastatin 40 mg/day vs. placebo and anti-oxidant vitamins (vitamin E 600 mg, vitamin C 250 mg, and beta-carotene 20 mg) vs. placebo and lasted approximately 5 years. Analysis of the group randomized to the anti-oxidant vitamins revealed no beneficial or harmful effects. In contrast, simvastatin therapy (40 mg/day) reduced cardiovascular events, including myocardial infarctions and strokes, by approximately 25% in all participants and to a similar degree in the diabetic subjects (total cardiovascular disease reduced 27%, coronary mortality 20%, myocardial infarction 37%, stroke 24%). Further analysis of the subjects with diabetes revealed that the reduction in cardiovascular events with statin therapy was similar in individuals with diabetes diagnosed for a short duration (<6 years) and for a long duration (>13 years). Similarly, patients with diabetes in good glycemic control (HbA1c < 7%) and those not in ideal control (HbA1c > 7%) also benefited to a similar degree with statin therapy. Moreover, both patients with type 1 and type 2 diabetes had a comparable reduction in cardiovascular disease with simvastatin therapy. The decrease in cardiovascular events in patients with type 1 diabetes was not statistically significant because of the small number of subjects. Nevertheless, this is the only trial that included type 1 diabetics and suggests that patients with type 1 will benefit from statin therapy similar to type 2 diabetics. In general, statin therapy reduced cardiovascular disease in all subgroups of subjects with diabetes (females, males, older age, renal disease, hypertension, high triglycerides, low HDL, ASA therapy, etc.), i.e., statin therapy benefits all patients with diabetes (note this study did not include patients with end-stage renal disease but

other studies have failed to show benefits of statin therapy in patients with diabetes and end-stage renal disease) [89]. It should be noted that since patients with diabetes have an increased risk of cardiovascular disease, a similar percent reduction means a greater reduction in absolute number of events.

The CARDS trial specifically studied patients with diabetes [90]. The patients in this trial were males and females with type 2 diabetes between 40 and 75 years of age who were at high risk of developing cardiovascular disease based on the presence of hypertension, retinopathy, renal disease, or current smoking. Of particular note, the subjects did not have any evidence of clinical atherosclerosis (myocardial disease, stroke, peripheral vascular disease), and hence, this study is a primary prevention trial. Inclusion criteria included LDL-C levels less than 160 mg/dL and triglyceride levels less than 600 mg/dL. It is important to recognize that the average LDL-C in this trial was relatively low (118 mg/dL). A total of 2838 patients with type 2 diabetes were randomized to either placebo or atorvastatin 10 mg/day. Atorvastatin therapy resulted in a 40% decrease in LDL-C levels with over 80% of patients achieving LDL-C levels less than 100 mg/dL. Most importantly, atorvastatin therapy resulted in a 37% reduction in cardiovascular events. In addition, strokes were reduced by 48% and coronary revascularization by 31%. As seen in the HPS, subjects with relatively low LDL-C levels (LDL < 120 mg/dL) benefited to a similar extent as subjects with higher LDL-C levels (>120 mg/dL).

CARDS and HPS, in combination with the other statin trials, provide conclusive evidence that statin therapy will reduce cardiovascular events in patients with diabetes. Importantly, the benefits of statin therapy are seen in patients with diabetes in both primary and secondary prevention trials.

Given the benefits of statin therapy, studies were designed to determine whether more aggressive lowering of LDL-C with statins would provide greater benefits. The Prove-It trial determined in patients recently hospitalized for an acute coronary syndrome whether aggressively lowering of LDL-C with atorvastatin 80 mg/day vs. moderate LDL-C lowering with pravastatin 40 mg/day would have a similar effect on cardiovascular end points [91, 92]. In this trial, approximately 18% of the patients were diabetic. As expected, the on-treatment LDL-C levels were significantly lower in patients aggressively treated with atorvastatin compared to the moderate treated pravastatin group (atorvastatin 62 mg/dL vs. pravastatin 95 mg/dL). Of great significance, death or major cardiovascular events were reduced by 16% over the 2 years of the study in the group aggressively treated with atorvastatin. Moreover, the reduction of risk in the patients with diabetes in the aggressive treatment group was similar to that observed in non-diabetics.

In the treating to new targets trial (TNT) patients with stable coronary heart disease and LDL-C levels less than 130 mg/dL were randomized to either 10 or 80 mg atorvastatin and followed for an average of 4.9 years [93, 94]. Approximately 15% of the patients had diabetes. As expected, LDL-C levels were lowered to a greater extent in the patients treated with 80 mg atorvastatin than with 10 mg atorvastatin (77 mg/dL vs. 101 mg/dL). Moreover, the occurrence of major cardiovascular events was reduced by 22% in the group treated with atorvastatin 80 mg ($p < 0.001$). In the patients with diabetes events were reduced by 25% in the high-dose statin group.

Once again, the relative risk reduction in the patients with diabetes randomized to the aggressive treatment group was similar to that observed in non-diabetics.

Finally, the IDEAL trial was a randomized study that compared atorvastatin 80 mg vs. simvastatin 20–40 mg in 8888 patients with a history of cardiovascular disease [95]. Approximately 12% of the patients had diabetes. As expected, LDL-C levels were reduced to a greater extent in the atorvastatin-treated group than the simvastatin-treated group (approximately 81 mg/dL vs. 104 mg/dL). Once again, the greater reduction in LDL-C levels was associated with a greater reduction in cardiovascular events. Specifically, major coronary events defined as coronary death, non-fatal myocardial infarction, or cardiac arrest was reduced by 11% ($p = 0.07$), while non-fatal acute myocardial infarctions were reduced by 17% ($p = 0.02$).

Combining the results of these statin trials leads one to the conclusion that aggressive lowering of LDL-C with statin therapy will be beneficial and suggests that in high-risk patients lowering the LDL-C to levels well below 100 mg/dL is desirable. Moreover, the Cholesterol Treatment Trialists reviewed five trials with 39,612 subjects designed to determine the effect of usual vs. aggressive reductions in LDL-C with statin therapy [96]. They reported that intensive control (approximately a 19 mg/dL difference in LDL-C) resulted in a 15% decrease in major vascular events, a 13% reduction in coronary death or non-fatal MI, a 19% decrease in coronary revascularization, and a 16% decrease in strokes. As will be discussed below, current treatment guidelines reflect the results of these studies. Additionally, as described in detail below, recent studies of the addition of either ezetimibe or PCSK9 inhibitors to statins provides additional support that aggressive lowering of LDL-C levels further reduces cardiovascular events.

Fibrates

The data demonstrating the beneficial effect of monotherapy with fibrates (e.g., gemfibrozil, fenofibrate) on cardiovascular disease in patients with diabetes are not as strong as with statins but suggests that this class of drug might also reduce cardiovascular events in patients with diabetes, especially in those with type 2 diabetes and hypertriglyceridemia (Table 14.3). The largest trial was the Field Trial [102]. In this trial, 9795 patients with type 2 diabetes between the ages of 50 and 75 not taking

Table 14.3 Effect of fibrate monotherapy on cardiovascular outcomes

Study	Drug	# Diabetic subjects	% Decrease controls	% Decrease diabetics
Helsinki Heart Study [97]	Gemfibrozil	135	34	60[a]
VA-HIT [98, 99]	Gemfibrozil	620	24	24
DIAS [100]	Fenofibrate	418	–	23[a]
SENDCAP [101]	Bezafibrate	164	–	70
Field [102]	Fenofibrate	9795	–	11[a]

From reference [41] with permission
[a]Not statistically significant

statin therapy were randomized to fenofibrate or placebo and followed for approximately 5 years. Fenofibrate therapy resulted in a 12% decrease in LDL-C, a 29% decrease in triglycerides, and a 5% increase in HDL-C levels. The primary outcome was coronary events (coronary heart disease death and non-fatal MI), which were reduced by 11% in the fenofibrate group but did not reach statistical significance ($p = 0.16$). However, there was a 24% decrease in non-fatal MI in the fenofibrate-treated group ($p = 0.01$) and a non-significant increase in coronary heart disease mortality. Total cardiovascular disease events (coronary events plus stroke and coronary or carotid revascularization) were reduced 11% ($p = 0.035$). These beneficial effects of fenofibrate therapy on cardiovascular disease were observed in patients without a previous history of cardiovascular disease. In patients with a previous history of cardiovascular disease no benefits were observed. Additionally, the beneficial effect of fenofibrate therapy was seen only in those subjects less than 65 years of age. The beneficial effects of fenofibrate in this study may have been muted by the increased use of statins in the placebo group, which reduced the differences in lipid levels between the placebo and fenofibrate groups. If one adjusted for the addition of lipid-lowering therapy, fenofibrate reduced the risk of coronary heart disease events by 19% ($p = 0.01$) and of total cardiovascular disease events by 15% ($p = 0.004$).

While the results of fibrate trials for prevention of ASCVD have been very heterogeneous, it should be noted that fibrate trials in patients with elevated triglyceride levels have reported a greater reduction of cardiovascular events [103]. Additionally, subgroup analysis of several fibrate trials has also suggested that the benefit of fibrates was greatest in patients with elevated triglyceride levels [103, 104].

The mechanism by which fibrates reduce cardiovascular events is unclear. These drugs lower serum triglyceride levels and increase HDL-C, but it should be recognized that the beneficial effects of fibrates could be due to other actions of these drugs. Specifically, these drugs activate the nuclear hormone receptor PPAR alpha, which is present in the cells that comprise the atherosclerotic lesions, and it is possible that these compounds directly affect lesion formation and development. In addition, fibrates are anti-inflammatory. In fact, analysis of the VA-HIT study suggested that much of the benefit of fibrate therapy was not due to changes in serum lipoprotein levels [98, 99].

To summarize, while the studies to date suggest that monotherapy with fibrates may reduce cardiovascular disease in patients with diabetes, the results are not as robust or consistent as seen in the statin trials. Of note fibrate therapy was most effective in patients with increased triglyceride levels and decreased HDL-C levels, a lipid profile typically seen in patients with type 2 diabetes.

Niacin

A single randomized trial, the Coronary Drug Project, has examined the effect of niacin monotherapy on cardiovascular outcomes [105]. This trial was carried out from 1966 to 1974 (before the introduction of statin therapy) in men with a history of a prior myocardial infarction and demonstrated that niacin therapy reduced cardiovascular events. Patients on insulin were excluded from this study. The results of this

study were re-analyzed to determine the effect of niacin therapy in subjects with varying baseline fasting and 1-h post-meal glucose levels [106]. It was noted that 6 years of niacin therapy reduced the risk of coronary heart disease death or non-fatal MI by approximately 15–25% regardless of baseline fasting or 1-h post-glucose challenge glucose levels. Reductions in events were seen in the subjects who had a fasting glucose levels >126 mg/dL or 1-h glucose levels >220 mg/dL (i.e., patients with diabetes). Thus, based on this single study from the pre-statin era, niacin monotherapy reduces cardiovascular events both in normal subjects and patients with diabetes.

Ezetimibe

A multicenter, randomized trial in Japan studied the effect of ezetimibe in patients aged ≥75 years with elevated LDL-C (≥140 mg/dL) without a history of coronary artery disease who were not taking lipid-lowering drugs [107]. Patients were randomized to ezetimibe ($n = 1716$) or usual care ($n = 1695$) and followed for 4.1 years. The primary outcome was a composite of sudden cardiac death, myocardial infarction, coronary revascularization, or stroke. In the ezetimibe group LDL-C was decreased by 25.9%, while in the usual care group LDL-C was decreased by 18.5% ($p < 0.001$). By the end of the trial 9.6% of the patients in the usual care group and 2.1% of the ezetimibe group were taking statins. Ezetimibe reduced the incidence of the primary outcome by 34% (HR 0.66; $p = 0.002$). Additionally, composite cardiac events were reduced by 60% (HR 0.60; $p = 0.039$) and coronary revascularization by 62% (HR 0.38; $p = 0.007$) in the ezetimibe group vs. the control group. Approximately 25% of the patients in this trial had diabetes and the beneficial effects were similar in the diabetic and non-diabetic patients. It should be noted that the decrease in cardiovascular events was much greater than one would expect based on the absolute difference in LDL-C levels (121 mg/dL in ezetimibe group vs. 132 mg/dL). This study was prematurely terminated by the Data Monitoring Committee due to loss to follow-up and competing risks in this elderly population. As stated by the authors "Given the open-label nature of the trial, its premature termination, and issues with follow-up, the magnitude of benefit observed should be interpreted with caution." Nevertheless, this study provides evidence that ezetimibe monotherapy can reduce cardiovascular events in patients with diabetes.

Other Drugs

With regard to PCSK9 inhibitors, bempedoic acid, and bile acid sequestrants there have been no randomized monotherapy studies that have examined the effect of these drugs on cardiovascular events in patients with diabetes. In non-diabetic patients, monotherapy with bile acid sequestrants have reduced cardiovascular events [108, 109]. Since bile acid sequestrants have a similar effect on lipid levels in diabetic and non-diabetic subjects one would anticipate that these drugs would also result in a reduction in events in the diabetic population. Additionally, bile acid sequestrants improve glycemic control [46]. However, bile acid sequestrants can

raise triglyceride levels and therefore must be used with caution in hypertriglyceridemic patients. There are no outcome studies with PCSK9 inhibitor monotherapy in patients with diabetes but given that these drugs reduce LDL-C levels and in combination with statins reduce cardiovascular events one would anticipate that PCSK9 inhibitor monotherapy will also reduce cardiovascular events. Finally, a trial of bempedoic acid in patients with cardiovascular disease or at high risk of cardiovascular disease who are intolerant of statins is currently in progress (NCT02993406).

Combination Drug Therapy

The studies with statins have been so impressive in reducing of cardiovascular events that most patients with diabetes are routinely treated with statin therapy. Therefore, a key issue is whether the addition of other lipid-lowering drugs to statins will result in a further reduction in cardiovascular events. A difficulty with such studies is that given the robust reduction in cardiovascular events induced by statin therapy, very large trials are required to see an additional benefit.

Statins + Fibrates

The ACCORD-LIPID trial was designed to determine if the addition of fenofibrate to aggressive statin therapy would result in a further reduction in cardiovascular disease in patients with type 2 diabetes [110]. In this trial, 5518 patients on statin therapy were randomized to placebo or fenofibrate therapy. The patients had diabetes for approximately 10 years and either had pre-existing cardiovascular disease or were at high risk for developing cardiovascular disease. During the trial, LDL-C levels were approximately 80 mg/dL. There was only a small difference in HDL-C with the fenofibrate groups having a mean HDL-C of 41.2 mg/dL, while the control group had an HDL-C of 40.5 mg/dL. Differences in triglyceride levels were somewhat more impressive with the fenofibrate group having a triglyceride level of 122 mg/dL, while the control group had a triglyceride level of 144 mg/dL. The primary outcome was first occurrence of non-fatal myocardial infarction, non-fatal stroke, or death from cardiovascular causes and there was no statistical difference between the fenofibrate-treated group and the placebo group. Additionally, there were also no statistically significant differences between the groups with regard to any of the secondary outcome measures of cardiovascular disease. Of note, the addition of fenofibrate to statin therapy did not result in an increase in either muscle or liver side effects. While this was a negative study, it must be recognized that most of the patients included in this study did not have a lipid profile that would usually lead to treatment with fibrates, as most subjects did not have elevated triglycerides. On further analysis, there was a possible benefit of fenofibrate therapy in the subgroup of patients in whom the baseline triglyceride levels were increased (>204 mg/dL) and HDL-C levels decreased (<34 mg/dL). This same group of patients also derived the greatest benefit of fibrate therapy in the fibrate monotherapy trials. Thus,

future fibrate–statin combination therapy trials should focus on patients with high triglycerides and low HDL-C levels.

The PROMINENT trial is exploring the effect of pemafibrate, a new selective PPAR-alpha modulator, in reducing cardiovascular outcomes in a large number (approx. 10,000) patients with diabetes with atherogenic dyslipidemia on a statin [111]. This trial was recently stopped due to futility but the details of the results have not yet been published. The negative results of this trial have reduced the appeal of using statin + fibrate combination therapy.

Statin + Niacin

The AIM-HIGH trial was designed to determine if the addition of Niaspan to aggressive statin therapy would result in a further reduction in cardiovascular events in patients with pre-existing cardiovascular disease [112]. In this trial 3314 patients were randomized to Niaspan vs. placebo. Approximately 33% of the patients had diabetes. On trial, LDL-C levels were in the 60–70 mg/dL range in both groups. As expected, HDL-C levels were increased modestly in the Niaspan-treated group (approximately 44 mg/dL vs. 38 mg/dL), while triglycerides were decreased (approximately 121 mg/dL vs. 155 mg/dL). However, there were no differences in the primary end point between the control and Niaspan-treated groups (first event of death from coronary heart disease, non-fatal myocardial infarction, ischemic stroke, hospitalization for an acute coronary syndrome, or symptom-driven coronary or cerebral revascularization). There were also no differences in secondary end points except for a possible increase in strokes in the Niaspan-treated group. The addition of Niaspan to statin therapy did not result in a significant increase in either muscle or liver toxicity. Thus, this study does not provide support for the addition of niacin to statins. However, it should be recognized that this was a relatively small study and a considerable number of patients stopped taking the Niaspan during the course of the study (25.4% of patients discontinued Niaspan therapy). In addition, most of the patients included in this study did not have a lipid profile that one would typically consider treating with niacin therapy. In the subset of patients with TG > 198 mg/dL and HDL-C < 33 mg/dL, niacin showed a trend toward benefit (hazard ratio 0.74; $p = 0.073$), suggesting that if the appropriate patient population was studied the results may have been positive [113].

HPS 2 Thrive also studied the effect of niacin added to statin therapy [114]. This trial utilized extended release niacin combined with laropiprant, a prostaglandin D2 receptor antagonist that reduces the flushing side effect of niacin treatment. HPS 2 Thrive was a very large trial with over 25,000 patients randomized to either niacin therapy or placebo. Approximately 32% of the patients in this trial had diabetes. The LDL-C level was 63 mg/dL, the HDL-C 44 mg/dL, and the triglycerides 125 mg/dL at baseline. As expected, niacin therapy resulted in a modest reduction in LDL-C (10 mg/dL), a modest increase in HDL-C (6 mg/dL), and a marked reduction in triglycerides (33 mg/dL). However, despite these lipid changes there were no significant differences in major cardiovascular events between the niacin and control

group (risk ratio 0.96). It is unknown whether laropiprant, the prostaglandin D2 receptor antagonist, might have effects that worsen atherosclerosis and increase event rates. Similar to the ACCORD-LIPID and AIM-HIGH studies, the group of patients included in the HPS 2 Thrive trial were not the ideal patient population to test for the beneficial effects of niacin treatment added to statin therapy. Ideally, patients with high triglycerides and high non-HDL-C levels coupled with low HDL-C levels should be studied.

Statin + Ezetimibe

The IMPROVE-IT trial evaluated whether the addition of ezetimibe to statin therapy would provide an additional beneficial effect in patients with the acute coronary syndrome [115]. This was a large trial with over 18,000 patients randomized to statin therapy vs. statin therapy + ezetimibe. Approximately 27% of the patients in this trial had diabetes. On-treatment LDL-C levels were 70 mg/dL in the statin-alone group vs. 53 mg/dL in the statin + ezetimibe group. There was a small but significant 6.4% decrease in major cardiovascular events (cardiovascular death, MI, documented unstable angina requiring re-hospitalization, coronary revascularization, or stroke) in the statin + ezetimibe group (HR 0.936; $p = 0.016$). Cardiovascular death, non-fatal MI, or non-fatal stroke were reduced by 10% (HR 0.90; $p = 0.003$). These beneficial effects were particularly pronounced in the patients with diabetes (Primary end point hazard ratio, 0.85; 95% confidence interval, 0.78–0.94) [116, 117].

Statin + PCSK9 Inhibitors

The FOURIER trial was a randomized, double-blind, placebo-controlled trial of evolocumab vs. placebo in 27,564 patients with ASCVD and an LDL-C level greater than 70 mg/dL who were on statin therapy [118]. Approximately 40% of the patients had diabetes [119]. The primary end point was cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization and the key secondary end point was cardiovascular death, myocardial infarction, or stroke. The median duration of follow-up was 2.2 years. Baseline LDL-C levels were 92 mg/dL and evolocumab resulted in a 59% decrease in LDL-C levels (LDL-C level on treatment approximately 30 mg/dL). Evolocumab treatment significantly reduced the risk of the primary end point (hazard ratio 0.85; $p < 0.001$) and the key secondary end point (hazard ratio 0.80; $p < 0.001$). The results were consistent across key subgroups, including the subgroup of patients in the lowest quartile for baseline LDL-C levels (median, 74 mg/dL). Of note, a similar decrease in cardiovascular events occurred in patients with diabetes treated with evolocumab and glycemic control was not altered [106]. Further analysis has shown that in the small number of patients with a baseline LDL-C level less than 70 mg/dL, evolocumab reduced cardiovascular events to a similar degree as in the patients with an

LDL-C greater than 70 mg/dL [120]. Finally, the lower the on-treatment LDL-C levels (down to levels below 20 mg/dL), the lower the cardiovascular event rate, suggesting that greater reductions in LDL-C levels will result in greater decreases in cardiovascular disease [121].

The ODYSSEY trial was a multicenter, randomized, double-blind, placebo-controlled trial involving 18,924 patients who had an acute coronary syndrome 1–12 months earlier, an LDL-C level of at least 70 mg/dL, a non-HDL-C level of at least 100 mg/dL, or an apolipoprotein B level of at least 80 mg/dL while on high-intensity statin therapy or the maximum tolerated statin dose [122]. Approximately 29% of the patients had diabetes. Patients were randomly assigned to receive alirocumab 75 mg every 2 weeks or matching placebo. The dose of alirocumab was adjusted to target an LDL-C level of 25–50 mg/dL. The primary end point was a composite of death from coronary heart disease, non-fatal myocardial infarction, fatal or non-fatal ischemic stroke, or unstable angina requiring hospitalization. During the trial, LDL-C levels in the placebo group was 93–103 mg/dL, while in the alirocumab group LDL-C levels were 40 mg/dL at 4 months, 48 mg/dL at 12 months, and 66 mg/dL at 48 months (the increase with time was due to discontinuation of alirocumab or a decrease in dose). The primary end point was reduced by 15% in the alirocumab group (HR 0.85; $p < 0.001$). In addition, total mortality was reduced by 15% in the alirocumab group (HR 0.85; 95% CI 0.73–0.98). The absolute benefit of alirocumab was greatest in patients with a baseline LDL-C level >100 mg/dL. In patients with an LDL-C level >100 mg/dL the number needed to treat with alirocumab to prevent an event was only 16. Similar relative risk reductions were observed in patients with diabetes. It should be noted that similar to the other PCSK9 outcome trials the duration of this trial was very short (median follow-up 2.8 years) which may have minimized the beneficial effects. Additionally, because alirocumab 75 mg every 2 weeks was stopped if the LDL-C level was <15 mg/dL on two consecutive measurements the beneficial effects may have been blunted (7.7% of patients randomized to alirocumab were switched to placebo).

The duration of the PCSK9 outcome trials were relatively short and it is well recognized from previous statin trials that the beneficial effects of lowering LDL-C levels takes time with only modest effects observed during the first year of treatment. In the FOURIER trial the reduction of cardiovascular death, myocardial infarction, or stroke was 16% during the first year but was 25% beyond 12 months. In the ODYSSEY trial the occurrence of cardiovascular events was similar in the alirocumab and placebo group during the first year of the study with benefits of alirocumab appearing after year 1. Thus, the long-term benefits of treatment with a PCSK9 inhibitor may be greater than that observed during these relatively short-term studies.

The results of the ezetimibe and PCSK9 trials have several important implications. First, it demonstrates that combination therapy has benefits above and beyond statin therapy alone. Second, it provides further support for the hypothesis that lowering LDL per se will reduce cardiovascular events (i.e., the beneficial effects of statins on cardiovascular disease are predominantly due to lowering LDL-C). Third,

it suggests that lowering LDL levels to much lower levels will have significant added benefits. These results have implications for determining the goals of therapy and have influenced the recent treatment guidelines.

Statins + Low-Dose Omega-3-Fatty Acids

ORIGIN was a double-blind study in 12,536 patients at high risk for cardiovascular disease who had impaired fasting glucose, impaired glucose tolerance, or diabetes [123]. Patients were randomized to receive a 1-g capsule containing at least 900 mg of ethyl esters of omega-3 fatty acids (EPA 465 mg and DHA 375 mg) or olive oil placebo for approximately 6 years. Greater than 50% of the patients were on statin therapy. The primary outcome was death from cardiovascular causes. Triglyceride levels were decreased by 14.5 mg/dL in the group receiving omega-3-fatty acids compared to the placebo group ($p < 0.001$), without a significant effect on other lipids. The primary outcome was not significantly decreased among patients receiving omega-3-fatty acids as compared with those receiving placebo. The use of omega-3-fatty acids also had no significant effect on the rates of major vascular events, death from any cause, or death from arrhythmia.

A Study of Cardiovascular Events in Diabetes (ASCEND) was a randomized, placebo-controlled, double-blind trial of 1-g omega-3-fattys acids (400 mg EPA and 300 mg DHA ethyl esters) vs. olive oil placebo in 15,480 patients with diabetes without a history of cardiovascular disease (primary prevention trial) [124]. Approximately 75% of patients were on statin therapy. The primary end point was non-fatal myocardial infarction, non-fatal stroke, transient ischemic attack, or vascular death. Total cholesterol, HDL-C, and non-HDL-C levels were not significantly altered by omega-3-fatty acid treatment (changes in triglyceride levels were not reported). After a mean follow-up of 7.4 years the composite outcome of a serious vascular event or revascularization occurred in 11.4% on omega-3-fatty acids and 11.5% on placebo. Serious adverse events were similar in placebo and omega-3-fatty acid-treated groups.

Taken together these studies indicate that low-dose omega-3-fatty acids do not reduce cardiovascular events in patients with diabetes. Studies in non-diabetics have also found little effect of consuming low-dose fish oil on ASCVD [125].

Statins + High-Dose Omega-3-Fatty Acids

The Japan EPA Lipid Intervention Study (JELIS) was an open-label, non-placebo-controlled study in patients with baseline total cholesterol levels >251 mg/dL with cardiovascular disease ($n = 3664$) or without cardiovascular disease ($n = 14{,}981$). All were placed on statin therapy and randomly assigned to be treated with 1800 mg of EPA (Vascepa) + the statin ($n = 9326$) or statin alone ($n = 9319$) with a 5-year follow-up [126]. Approximately 16% of the patients had diabetes. The mean baseline triglyceride level was 153 mg/dL. The primary end point was any major coronary

event, including sudden cardiac death, fatal and non-fatal myocardial infarction, and other non-fatal events, including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting. On treatment, total cholesterol, LDL-C, and HDL-C levels were similar in the two groups but plasma triglycerides were modestly decreased in the EPA-treated group (5% decrease in EPA group; $p = 0.0001$). In the EPA + statin group the primary end point occurred in 2.8% of the patients vs. 3.5% of the patients in the statin-alone group (19% decrease; $p = 0.011$). Unstable angina and non-fatal coronary events were also significantly reduced in the EPA group but in this study sudden cardiac death and coronary death did not differ between groups. Unstable angina was the main component contributing to the primary end point and this is a more subjective end point than other end points, such as a myocardial infarction, stroke, or cardiovascular death. A subjective end point has the potential to be an unreliable end point in an open-label study which therefore is a limitation of the JELIS Study. The reduction in events was similar in the subgroup of patients with diabetes. In patients with triglyceride levels >150 mg/dL and HDL-C levels <40 mg/dL there was a 53% decrease in events [127]. In the EPA group, small increases in the occurrence of bleeding (1.1% vs. 0.6%, $p = 0.0006$), gastrointestinal disturbance (3.8%% vs. 1.7%, $p < 0.0001$), and skin abnormalities (1.7% vs. 0.7%, $p < 0.0001$) were seen. Pain was slightly decreased with EPA (1.6% vs. 2.0%, $p = 0.04$).

The Reduction of Cardiovascular Events with EPA–Intervention Trial (REDUCE-IT) was a randomized, double-blind trial of 2 g twice per day of EPA ethyl ester (icosapent ethyl) (Vascepa) vs. placebo (mineral oil) in 8179 patients with hypertriglyceridemia (135–499 mg/dL) and established cardiovascular disease or high cardiovascular disease risk (diabetes plus one risk factor) who were on stable statin therapy [128]. Approximately 60% of the patients in this trial had diabetes. The primary end point was a composite of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, coronary revascularization, or unstable angina. The key secondary end point was a composite of cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke. At baseline, the median LDL-C level was 75.0 mg/dL, HDL-C level was 40.0 mg/dL, and triglyceride level was 216.0 mg/dL. The median change in triglyceride level from baseline to 1 year was a decrease of 18.3% (−39.0 mg/dL) in the EPA group and an increase of 2.2% (4.5 mg/dL) in the placebo group. After a median of 4.9 years the primary end-point occurred in 17.2% of the patients in the EPA group vs. 22.0% of the patients in the placebo group (hazard ratio, 0.75; $p < 0.001$), indicating a 25% decrease in events. The beneficial effects were similar in patients with and without diabetes. The number needed to treat to avoid one primary end-point event was 21. The reduction in cardiovascular events was noted after approximately 2 years of EPA treatment. Additionally, cardiovascular deaths were decreased by 20% in the EPA group (4.3% vs. 5.2%; hazard ratio, 0.80; 95% CI, 0.66–0.98; $p = 0.03$). The cardiovascular benefits of EPA were similar across baseline levels of triglycerides (<150, ≥150 to <200, and ≥200 mg/dL). Moreover, the cardiovascular benefits of EPA appeared to occur irrespective of the attained triglyceride level at 1 year (≥150 or <150 mg/dL), suggesting that the cardiovascular risk reduction was not associated with attainment of a normal triglyceride level. An increase in hospitalization for atrial fibrillation or flutter (3.1% vs. 2.1%, $p = 0.004$) occurred in the EPA group. In addition, serious

bleeding events occurred in 2.7% of the patients in the EPA group and in 2.1% in the placebo group ($p = 0.06$). There were no fatal bleeding events in either group and the rates of hemorrhagic stroke, serious central nervous system bleeding, and serious gastrointestinal bleeding were not significantly higher in the EPA group than in the placebo group.

These results demonstrate that EPA treatment reduces cardiovascular disease events. Of note the reduction in TG levels is relatively modest and would not be expected to result in the magnitude of the decrease in cardiovascular disease observed in the JELIS and REDUCE-IT trials. Other actions of EPA, such as decreasing platelet function, anti-inflammation, decreasing lipid oxidation, and stabilizing membranes, could account for or contribute to the reduction in cardiovascular events [129]. It is likely that the beneficial effects of EPA seen in the JELIS and REDUCE-IT trials are multifactorial.

The Statin Residual Risk Reduction with Epanova in High Risk Patients with Hypertriglyceridemia (STRENGTH) trial is a randomized, placebo-controlled, double-blind trial of 4 g/day of omega-3-fatty acids (Epanova, a mixture of 75% EPA and 25% DHA fatty acids) vs. placebo in over 13,000 patients on statins with hypertriglyceridemia (180–500 mg/dL), optimal LDL-C levels (<100 mg/dL or on maximal statin therapy), low HDL-C (<42 mg/dL in men and <47 mg/dL in women), and either cardiovascular disease or high risk for cardiovascular disease [130]. The primary outcome of the STRENGTH Trial is major atherosclerotic cardiovascular events (cardiovascular death, myocardial infarction, stroke, coronary revascularization, or hospitalization for unstable angina). The primary end point occurred in 785 patients (12.0%) treated with omega-3 CA vs. 795 (12.2%) treated with corn oil (hazard ratio, 0.99 [95% CI, 0.90–1.09]; $p = 0.84$) [131]. Thus, in contrast to EPA alone this omega-3-fatty acid formulation failed to show benefits.

Current Guidelines for Serum Lipids

There are several different guidelines for treating lipids in patients with diabetes. While there are many similarities between the guidelines there are also key differences. Of particular note some guidelines provide specific LDL-C goals, while other guidelines do not.

American Diabetes Association

The 2020 American Diabetes Association (ADA) recommends that adult patients with diabetes have their lipid profile determined at the time of diabetes diagnosis and at least every 5 years thereafter or more frequently if indicated [132]. This profile includes total cholesterol, HDL-C, triglycerides, and calculated LDL-C. A lipid panel should be obtained immediately prior to initiating statin therapy. Once a patient is on statin therapy, testing should be carried out 4–12 weeks after initiating

therapy and annually thereafter to monitor efficacy and adherence. Lifestyle modification, including a reduction in saturated fat, trans fat, and cholesterol intake, weight loss if indicated, an increase in omega-3-fatty acids, viscous fiber, and plant stanols/sterol intake, and increased physical activity, is indicated in all patients with diabetes. A focus on a Mediterranean style diet or Dietary Approaches to Stop Hypertension (DASH) diet should be encouraged. In patients with elevated triglyceride levels glycemic control is beneficial and dietary changes and lifestyle changes, including weight loss and abstinence from alcohol should be undertaken. Secondary disorders and medications that raise triglyceride levels should be looked for. The recommendations for lipid-lowering therapy are shown in Table 14.4. If one follows these recommendations almost all patients with diabetes over the age of 40 will be on statin therapy and many under the age of 40 will also be treated with statins. The addition of ezetimibe should be considered to further lower LDL-C levels in high-risk primary prevention patients. In very high-risk patients with ASCVD, if the LDL-C level on statin therapy is greater than 70 mg/dL the use of ezetimibe or a PCSK9 inhibitor should be considered. The use of fibrates or niacin with statins was generally not recommended. However, in patients with ASCVD or other cardiovascular risk factors on a statin with controlled LDL-C but elevated triglyceride levels (135–499 mg/dL) the addition of icosapent ethyl (EPA; Vascepa) can be considered. Finally, in patients with fasting triglyceride levels greater than 500 mg/dL an evaluation for secondary causes of hypertriglyceridemia should be undertaken and drug therapy to reduce the risk of pancreatitis should be considered.

Table 14.4 ADA recommendations for lipid-lowering therapy

Primary prevention
Age 20–39: With additional risk factors may be reasonable to initiate statin therapy
Age 40–75: Moderate-intensity statin therapy[a]
Age >75: Moderate-intensity statin therapy is reasonable after discussion
Patients at high risk: Multiple risk factors[b] or age 50–70 it is reasonable to use high-intensity statin therapy[c]
Patients with 10-year risk >20%: reasonable to add ezetimibe to maximally tolerated statin to reduce LDL by >50%
Secondary prevention
All ages <75: High-intensity statin therapy/maximally tolerated stain
Age >75: Reasonable to continue statin therapy or initiate statin therapy after discussion.
Very High Risk: If LDL >70 mg/dL on maximally tolerated statin consider adding ezetimibe or PCSK9 inhibitor

Table from reference [41] with permission

[a]Moderate-intensity statin–atorvastatin 10–20 mg, rosuvastatin 5–10 mg, simvastatin 20–40 mg, pravastatin 40–80 mg, lovastatin 40 mg, Fluvastatin XL 80 mg, pitavastatin 3–4 mg

[b]Risk factors include LDL-C >100 mg/dL, high blood pressure, smoking, chronic kidney disease, albuminuria, and family history of premature ASCVD

[c]High-Intensity statin–atorvastatin 40–80 mg, rosuvastatin 20–40 mg

American College of Cardiology and American Heart Association

The 2018 American College of Cardiology and American Heart Association (ACC/AHA) guidelines are similar to the ADA guidelines described above and recommend the following [133]. "In patients 40–75 years of age with diabetes mellitus and LDL-C ≥70 mg/dL (≥1.8 mmol/L), start moderate-intensity statin therapy without calculating 10-year ASCVD risk. In patients with diabetes mellitus at higher risk, especially those with multiple risk factors or those 50–75 years of age, it is reasonable to use a high-intensity statin to reduce the LDL-C level by ≥50%." Furthermore, with diabetes they recommend that "In patients with clinical ASCVD, reduce low-density lipoprotein cholesterol (LDL-C) with high-intensity statin therapy or maximally tolerated statin therapy. The more LDL-C is reduced on statin therapy, the greater will be subsequent risk reduction. Use a maximally tolerated statin to lower LDLC levels by ≥50%. In very high-risk ASCVD, use an LDL-C threshold of 70 mg/dL (1.8 mmol/L) to consider addition of non-statins to statin therapy. Very high-risk includes a history of multiple major ASCVD events or one major ASCVD event and multiple high-risk conditions. In very high-risk ASCVD patients, it is reasonable to add ezetimibe to maximally tolerated statin therapy when the LDL-C level remains ≥70 mg/dL (≥1.8 mmol/L). In patients at very high risk whose LDL-C level remains ≥70 mg/dL (≥1.8 mmol/L) on maximally tolerated statin and ezetimibe therapy, adding a PCSK9 inhibitor is reasonable, although the long-term safety (>3 years) is uncertain and cost effectiveness is low at mid-2018 list prices." With regard to testing, they recommend "Assess adherence and percentage response to LDL-C-lowering medications and lifestyle changes with repeat lipid measurement 4–12 weeks after statin initiation or dose adjustment, repeated every 3–12 months as needed." Finally, there are several diabetes-specific risk enhancers that are independent of other risk factors that should be considered in evaluating risk of cardiovascular events and level of treatment (Table 14.5).

Table 14.5 Diabetes-specific risk enhancers that are independent of other risk factors in diabetes

Long duration (≥10 years for type 2 diabetes mellitus or ≥20 years for type 1 diabetes mellitus)
Albuminuria ≥30 μg of albumin/mg creatinine
eGFR <60 mL/min/1.73 m^2
Retinopathy
Neuropathy
ABI < 0.9

ABI ankle-brachial index
Table from reference [41] with permission

Table 14.6 National lipid association recommendations

Diabetes with 0–1 risk factors[a] and no end organ damage[b]	LDL-C < 100 mg/dL; Non-HDL-C < 130 mg/dL
Diabetes with pre-existing ASCVD, 2 or more risk factors[a] or end organ damage[b]	LDL-C < 70 mg/dL; Non-HDL-C < 100 mg/dL

Table from reference [41] with permission

[a]Risk factors—age > 45 for males, >55 for females; family history of early coronary heart disease; current cigarette smoking; high blood pressure >140/>90 mmHg; or low HDL < 40 mg/dL males, <50 mg/dL females

[b]End Organ Damage—retinopathy, albumin/creatinine ratio >30 mg/g, or chronic kidney disease

National Lipid Association

The National Lipid Association (NLA) has treatment goals for patients with diabetes [134]. In patients with type 1 or type 2 diabetes with pre-existing ASCVD, two or more risk factors for ASCVD, or evidence of end organ damage, the goal LDL is <70 mg/dL and the goal non-HDL-C is <100 mg/dL (Table 14.6). In patients with diabetes with 0–1 risk factors and no end organ damage, the LDL goal is <100 mg/dL and the non-HDL-C goal is <130 mg/dL. The NLA guidelines recommend considering drug therapy if a patient with diabetes is not at goal.

American Association of Clinical Endocrinologists/American College of Endocrinology

The American Association of Clinical Endocrinologists and American College of Endocrinology guidelines consider individuals with type 2 diabetes to be at high, very high, or extreme risk for ASCVD [135, 136]. Patients with type 1 diabetes and a duration of diabetes of more than 15 years or two or more risk factors, poorly controlled A1c, or insulin resistance with metabolic syndrome should be considered to have an equivalent risk to patients with type 2 diabetes. The recommended treatment goals are shown in Table 14.7.

European Society of Cardiology and European Atherosclerosis Society

Finally, the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) has guidelines for the treatment of lipids in patients with diabetes [137]. These guidelines classify patients with diabetes as very high risk, high risk, or moderate risk (Table 14.8). The recommended goals of therapy based on risk classification are shown in Table 14.9. As with other guidelines, intensification of statin therapy should be considered before the introduction of combination therapy. If the goal is not reached, statin combination with ezetimibe should be considered next.

Table 14.7 ASCVD risk categories and treatment goals

Risk category	Risk factors/10-year risk	LDL-C (mg/dL)	Non-HDL-C (mg/dL)	Apo B (mg/dL)	TG (mg/dL)
Extreme risk	Diabetes and clinical cardiovascular disease	<55	<80	<70	<150
Very high risk	Diabetes with one or more risk factors[a]	<70	<100	<80	<150
High risk	Diabetes and no other risk factors	<100	<130	<90	<150

Table from reference [41] with permission

[a]Risk factors are high LDL-C, polycystic ovary syndrome, cigarette smoking, hypertension (blood pressure ≥140/90 mmHg or on hypertensive medication), low HDL-C (<40 mg/dL), family history of coronary artery disease (in male, first-degree relative younger than 55 years; in female, first-degree relative younger than 65 years), chronic renal disease (CKD) stage 3/4, evidence of coronary artery calcification and age (men ≥45; women ≥55 years). Subtract 1 risk factor if the person has high HDL-C

Table 14.8 ESC/EAS classification of risk in patients with diabetes

Very high risk—target organ damage, or at least three major risk factors, or early onset of T1DM of long duration (>20 years)
High risk—without target organ damage, with DM duration >10 years or another additional risk factor
Moderate risk—Young patients (T1DM < 35 years; T2DM < 50 years) with DM duration <10 years, without other risk factors. Calculated SCORE >1% and <5% for 10-year risk of fatal CVD

Table from reference [41] with permission

Table 14.9 ESC/EAS goals of therapy in patients with diabetes

	LDL-C	Non-HDL-C (mg/dL)	Apo B (mg/dL)
Very high risk	>50% reduction and <55 mg/dL (<1.4 mmol/L)	<85	<65
High risk	>50% reduction and <70 mg/dL (<1.8 mmol/L)	<100	<80
Moderate risk	<100 mg/dL	<130	<100

Table from reference [41] with permission

Our Recommendations for Guidelines

Thus, different organizations have proposed somewhat different recommendations for the treatment of lipids in patients with diabetes. Despite these differences it is clear that the vast majority of patients with diabetes will need to be treated with statins regardless of which guidelines one elects to follow.

The approach we use is to combine these recommendations (Tables 14.10 and 14.11). In patients with diabetes who have pre-existing ASCVD we initiate intensive statin therapy. Given the extensive data showing that the lower the LDL-C the greater the reduction in cardiovascular events most secondary prevention patients would benefit from the addition of ezetimibe to maximize LDL-C lowering without markedly increasing costs [123]. We prefer LDL or non-HDL-C goals over

Table 14.10 ASCVD risk categories and treatment goals

Risk category	Risk factors/10-year risk	LDL-C (mg/dL)	Non-HDL-C (mg/dL)
Very high risk	Diabetes and clinical cardiovascular disease or multiple risk factors	<55	<80
High risk	Diabetes with one or more risk factors	<70	<100
Moderate risk	Diabetes and no other risk factors	<100	<130

Table from reference [41] with permission

Table 14.11 Drug therapy according to risk category that is typically required

Very high risk	Intensive statin therapy + ezetimibe. Add PCSK9 if not close to goal
High risk	Intensive statin therapy. Add ezetimibe if not at goal
Moderate risk	Moderate statin therapy. Increase to intensive statin therapy if not at goal

Table from reference [41] with permission

percent reduction. In patients with diabetes 40–75 years of age without pre-existing cardiovascular disease we calculate the 10-year risk of developing cardiovascular disease (http://www.cvriskcalculator.com/) and identify risk factors and risk-enhancing factors (Table 14.5). We initiate intensive statin therapy if the 10-year risk is >7.5% or if there are multiple risk factors or risk enhancers. We initiate moderate statin therapy if the risk is <7.5% without multiple risk factors or risk enhancers. Four to 12 weeks after initiating statin therapy we obtain a lipid panel to determine if the LDL and non-HDL-C levels are at goal. In patients with pre-existing cardiovascular disease or multiple risk factors/enhancers (i.e., very high-risk patients) our goal is an LDL-C <55 mg/dL and a non-HDL-C <80 mg/dL. In patients who are at high risk our goal is an LDL-C <70 mg/dL and a non-HDL-C <100 mg/dL. In patients with moderate risk an LDL-C goal of <100 mg/dL and a non-HDL c <130 mg/dL is appropriate. With therapy, if the levels are not at goal, we first adjust the statin dose until the patient is taking the maximally tolerated statin dose and then consider adding additional medications. In patients with diabetes who are less than 40 years of age we initiate statin therapy if the patient has overt cardiovascular disease, long-standing diabetes, or risk factors/enhancers for cardiovascular disease and the LDL and non-HDL-C levels are not at goal. In patients over 75 years of age with a reasonable life expectancy we begin moderate statin therapy and adjust based on response. When there is difficulty classifying a patient's risk, we will obtain a coronary calcium score (CAC) and use the score to help stratify the patient's risk. In all cases the benefits and risks of lipid-lowering therapy needs to be discussed with patients and the patient's personnel preferences taken into account.

Treatment of Lipid Abnormalities in Patient with Diabetes

Life Style Changes

Initial treatment of lipid disorders should focus on lifestyle changes [138]. There is little debate that exercise, both aerobic and resistance, is beneficial and that all patients with diabetes should, if possible, exercise for at least 150 min/week (for example, 30 min 5 times per week). Exercise increases fitness and improves insulin resistance even with limited weight loss; reductions in obesity are even more beneficial. Exercise will decrease serum triglyceride levels and increase HDL-C levels (an increase in HDL-C requires vigorous exercise) [34, 138]. It should be noted that many patients with diabetes may have substantial barriers to participating in exercise programs, such as comorbidities that limit exercise tolerance, risk of hypoglycemia, and presence of microvascular complications (visual impairment, neuropathy) that make exercise difficult.

The ideal diet is controversial and for detailed information on nutrition therapy for adults with diabetes see the consensus report by the American Diabetes Association [139]. Everyone agrees that weight loss in obese patients is essential [34, 138]. But how this can be achieved is hotly debated with many different "experts" advocating different approaches. The wide diversity of approach is likely due to the failure of any approach (even those that work in the short term) to be effective in the long term for the majority of obese patients with diabetes. Ultimately no weight loss diet will be successful if the patient cannot follow the diet for the long term, and therefore, the diet needs to be tailored to the specific preferences of the patient. If successful, weight loss will decrease serum triglyceride levels, increase HDL-C levels, and modestly reduce LDL-C [34, 138].

To reduce LDL-C levels, it is important that the diet decrease saturated fat, trans fatty acids, and cholesterol and increase soluble fiber [138]. To reduce triglyceride levels, it is important to decrease intake of simple sugars, particularly fructose, and ethanol [138]. High carbohydrate diets may increase serum triglyceride levels in some patients and if the amount of fat in the diet is markedly reduced serum HDL-C levels may decrease. Very high levels of triglycerides require diets that are very low in fat.

Bariatric surgery can have profound effects on weight and can result in marked improvements in lipid profiles with a decrease in triglycerides and LDL-C and an increase in HDL-C [34, 138]. Additionally, observational studies have shown a decrease in cardiovascular events following bariatric surgery in patients with and without diabetes [140–144].

Table 14.12 Effect of lipid-lowering drugs

	LDL-C	HDL-C	Triglycerides
Statins	↓ 20–60%	↑ 5–15%	↓ 0–35%[a]
Bile acid sequestrants	↓ 10–30%	↑ 0–10%	↑ 0–10%[b]
Fibrates	↓ 0–15%[c]	↑ 5–15%	↓ 20–50%
Niacin	↓ 10–25%	↑ 10–30%	↓ 20–50%
Ezetimibe	↓ 15–25%	↑ 1–3%	↓ 10–20%
PCSK9 Inhibitors	↓ 50–60%	↑ 5–15%	↓ 5–20%
Bempedoic Acid	↓ 15–25%	↓ 5–6%	No change
High-Dose Fish Oil	↑ 0–50%[c]	↑ 4–9%	↓ 20–50%[a]

Table from reference [41] with permission
[a]Patients with elevated TG have largest decrease
[b]In patients with high TG may cause marked increase
[c]In patients with high TG may increase LDL

Drug Therapy

The effect of statins, fibrates, niacin, ezetimibe, omega-3-fatty acids, bile acid sequestrants, bempedoic acid, and PCSK9 inhibitors on lipid levels in patients with diabetes is virtually identical to that seen in the non-diabetic patients (Table 14.12). Below we will highlight issues particularly relevant to the use of these drugs in patients with diabetes. For detailed information on lipid-lowering drugs see the following reviews on Triglyceride-Lowering Drugs and Cholesterol-Lowering Drugs [45, 125]. Most interventions reach maximal effect within 4–6 weeks allowing one to quickly adjust therapy to achieve goals.

Statins

Statins are easy to use and generally well tolerated by patients with diabetes. However, statins can adversely affect glucose homeostasis. In patients without diabetes the risk of developing diabetes is increased by approximately 10%, with higher doses of statin causing a greater risk than more moderate doses [145, 146]. The mechanism for this adverse effect is unknown but older, obese patients with higher baseline glucose levels are at greatest risk. In patients with diabetes, an analysis of nine studies with over 9000 patients with diabetes reported that the patients randomized to statin therapy had a 0.12% higher A1c than the placebo group, indicating that statin therapy is associated with only a very small increase in A1c levels in patients with diabetes, which is unlikely to be clinically significant [147]. Individual studies, such as CARDS and the Heart Protection Study, have also shown only a very modest effect of statins on A1c levels in patients with diabetes [87, 90, 148]. Muscle symptoms occur in patients with diabetes similar to what is observed in patients without diabetes.

Ezetimibe

Ezetimibe is easy to use and generally well tolerated by patients with diabetes.

Fibrates

Fibrates are easy to use and generally well tolerated by patients with diabetes. When combining fibrates with statin therapy it is essential to use fenofibrate as the risk of inducing myositis is much less than when statins are used in combination with gemfibrozil, which can inhibit statin metabolism [149]. In the ACCORD-LIPID Trial the incidence of muscle disorders was not increased in the statin + fenofibrate group compared to the statin-alone group [110]. The dose of fenofibrate needs to be adjusted in patients with renal disease and fenofibrate itself can induce a reversible increase in serum creatinine levels. It should be noted that marked reductions in HDL-C levels can occur in some patients treated with both fenofibrate and a thiazolidinedione [150]. The mechanism for this marked decrease in HDL-C is unknown.

Diabetic Retinopathy: Fenofibrate has been shown to have beneficial effects on diabetic eye disease. The FIELD study, described earlier, was a randomized trial of fenofibrate vs. placebo in patients with type 2 diabetes. Laser treatment for retinopathy was significantly reduced in the fenofibrate group compared to the placebo group (3.4% patients on fenofibrate vs. 4.9% on placebo; $p = 0.0002$) [151]. Fenofibrate therapy reduced the need for laser therapy to a similar extent for maculopathy (31% decrease) and for proliferative retinopathy (30% decrease). In the ophthalmology sub-study ($n = 1012$), the difference in the primary end point of two-step progression of retinopathy grade did not reach significance between the fenofibrate and control groups (9.6% patients on fenofibrate vs. 12.3% on placebo; $p = 0.19$). In patients without pre-existing retinopathy there was no difference in progression (11.4% vs. 11.7%; $p = 0.87$). However, in patients with pre-existing retinopathy, significantly fewer patients on fenofibrate had a two-step progression than did those on placebo (3.1% patients vs. 14.6%; $p = 0.004$). A composite end point of two-step progression of retinopathy grade, macular edema, or laser treatments was also significantly reduced in the fenofibrate group (HR 0.66; $p = 0.022$).

In the ACCORD Study a subgroup of participants were evaluated for the progression of diabetic retinopathy by three or more steps on the Early Treatment Diabetic Retinopathy Study Severity Scale or the development of diabetic retinopathy necessitating laser photocoagulation or vitrectomy over a 4-year period [152]. At 4 years, the rates of progression of diabetic retinopathy were 6.5% with fenofibrate therapy ($n = 806$) vs. 10.2% with placebo ($n = 787$) (adjusted odds ratio, 0.60; $p = 0.006$). Of note, this reduction in the progression of diabetic retinopathy was of a similar magnitude as intensive glycemic treatment vs. standard therapy.

Taken together these results indicate that fibrates have beneficial effects on the progression of diabetic retinopathy. The mechanisms by which fibrates decrease diabetic retinopathy are unknown.

Diabetic Nephropathy: The Diabetes Atherosclerosis Intervention Study (DAIS) evaluated the effect of fenofibrate therapy (n = 155) vs. placebo (n = 159) on changes in urinary albumin excretion in patients with type 2 diabetes [153]. Fenofibrate significantly reduced the worsening of albumin excretion (fenofibrate 8% vs. placebo 18%; $p < 0.05$). This effect was primarily due to reduced progression from normal albumin excretion to microalbuminuria (fenofibrate 3% vs. 18% placebo; $p < 0.001$).

In the FIELD trial, fenofibrate reduced urine albumin/creatinine ratio by 24% vs. 11% in placebo group ($p < 0.001$), with 14% less progression and 18% more albuminuria regression ($p < 0.001$) in the fenofibrate group than in participants on placebo [154]. As expected, fenofibrate therapy acutely increased plasma creatinine levels and decreased eGFR but over the long term, the increase in plasma creatinine was decreased in the fenofibrate group compared to the placebo group (14% decrease; $p = 0.01$). Similarly, there was a slower annual decrease in eGFR in the fenofibrate group (1.19 mL/min/1.73 m^2 vs. 2.03 mL/min/1.73 m^2 annually, $p < 0.001$). The effect of fenofibrate on kidney function was greater in those with higher triglycerides and lower HDL-C. End-stage renal disease, dialysis, renal transplant, and renal death were similar in the fenofibrate and placebo groups, but the incidence was low.

In the ACCORD-LIPID trial the post-randomization incidence of microalbuminuria was 38.2% in the fenofibrate group and 41.6% in the placebo group ($p = 0.01$) and post-randomization incidence of macroalbuminuria was 10.5% in the fibrate group and 12.3% in the placebo group ($p = 0.04$), indicating a modest reduction in the development of proteinuria in patients treated with fenofibrate [110]. There was no significant difference in the incidence of end-stage renal disease or need for dialysis between the fenofibrate group and the placebo group.

These studies suggest that fibrates may have a beneficial effect on diabetic kidney disease. One should recognize that reducing proteinuria is a surrogate marker and may not indicate a reduction in the development of end-stage renal disease. The mechanisms accounting for decreased in proteinuria are unknown.

Amputations: In the FIELD study the risks of first amputation were decreased by 36% ($p = 0.02$) and minor amputation events without known large-vessel disease by 47% ($p = 0.027$) in the fenofibrate-treated group [155]. The reduction in amputations was independent of glucose control or dyslipidemia. No difference between the risks of major amputations was seen in the placebo and fenofibrate groups. The basis for this reduction in amputations is unknown.

Do fibrates have an independent effect on microvascular disease? Multiple studies cited above have now shown that fenofibrate decreases retinopathy, nephropathy, and amputation. The effects are independent of blood glucose control. Given that there also was no effect of fenofibrate on cardiovascular (macrovascular) disease, these results may suggest that fenofibrate has an independent effect on microvascular disease. Further studies are warranted, but these results should be taken into account when considering treatment of hypertriglyceridemia in patients with diabetes who are on statins.

Bile Acid Sequestrants

Bile acid sequestrants are relatively difficult to take due to GI toxicity (mainly constipation) [50]. Diabetic subjects have an increased prevalence of constipation, which may be exacerbated by the use of bile acid sequestrants. On the other hand, in diabetic patients with diarrhea, the use of bile acid sequestrants may be advantageous. Bile acid sequestrants may also increase serum triglyceride levels, which can be a problem in patients with diabetes who are already hypertriglyceridemic [45]. An additional difficulty in using bile acid sequestrants is their potential for binding other drugs [45]. Many drugs should be taken either 2 h before or 4 h after taking bile acid sequestrants to avoid the potential of decreased drug absorption. Patients with diabetes are frequently on multiple drugs for glycemic control, hypertension, etc., and it can sometimes be difficult to time the ingestion of bile resin sequestrants to avoid these other drugs. Colesevelam (Welchol) is a bile acid sequestrant that comes in pill, powder, or chewable bars and causes fewer side effects and has fewer interactions with other drugs than other preparations [156]. The usual dose is 3.75 g/day and can be given as tablets (take 6 tablets once daily or 3 tablets twice daily), oral suspension (take one packet once daily), or chewable bars (take 1 bar once daily). Of particular note is that a number of studies have shown that colesevelam improves glycemic control in patients with diabetes, resulting in an approximately 0.5% decrease in A1c levels, and colesevelam has therefore been approved by the FDA for the lowering of glucose levels [46, 157].

Niacin

Niacin is well known to cause skin flushing and itching and GI upset [158]. Additionally, niacin reduces insulin sensitivity (i.e., causes insulin resistance), which can worsen glycemic control [158]. Studies have shown that niacin is usually well tolerated in diabetic subjects who are in good glycemic control [159, 160]. In patients with poor glycemic control, niacin is more likely to adversely impact glucose levels. In the HPS2-Thrive trial, niacin therapy significantly worsened glycemic control in patients with diabetes and induced new onset diabetes in 1.3% of subjects who were non-diabetic [114]. High doses of niacin are more likely to adversely affect glycemic control. Niacin can also increase serum uric acid levels and induce gout, both of which are already common in obese patients with type 2 diabetes [158]. Additionally, recent trials have reported an increased incidence of infection and bleeding with niacin therapy [158]. However, niacin is the most effective drug in increasing HDL-C levels, which are frequently low in patients with diabetes but whether this will reduce cardiovascular events has not been demonstrated.

Omega-3-Fatty Acids

A Cochrane review of fish oil in patients with diabetes have demonstrated that this is a safe approach and does not result in worsening of glycemic control in patients with diabetes [161]. Fish oil effectively lowers triglyceride levels but, in some

patients, particularly those with significant hypertriglyceridemia, high-dose fish oil increases LDL-C levels [161]. It should be noted that fish oil products that contain just EPA (Vascepa) do not adversely affect LDL-C levels [162]. When using fish oil to lower serum triglyceride levels it is important to recognize that one is aiming to provide 3–4 g of DHA/EPA per day. The quantity of these active omega-3-fatty acids can vary greatly from product to product. Prescription fish oil products contain large amounts of these active ingredients, whereas the amount of DHA/EPA in food supplements can vary greatly and, in some supplements, levels are very low. Additionally, while prescription omega-3-fatty acid preparations have high levels of quality control, omega-3-fish oil food supplements may have contaminants and the dosage may not be precisely controlled. As discussed earlier only high-dose EPA has been shown to decrease cardiovascular events.

PCSK9 Inhibitors

Two monoclonal antibodies that inhibit PCSK9 (proprotein convertase subtilisin/kexin type 9) are approved for the lowering of LDL-C levels: Alirocumab (Praluent) and evolocumab (Repatha). Alirocumab is administered as either 75 or 150 mg subcutaneously every 2 weeks or 300 mg subcutaneously every 4 weeks, while evolocumab is administered as either 70 mg subcutaneously every 2 weeks or 420 mg subcutaneously once a month [45]. A meta-analysis of three trials with 413 patients with type 2 diabetes found that evolocumab caused a 60% decrease in LDL-C compared to placebo and a 39% decrease in LDL-C compared to ezetimibe treatment [163]. In addition, in patients with type 2 diabetes, evolocumab decreased non-HDL-C (55% vs. placebo and 34% vs. ezetimibe) and Lp(a) (31% vs. placebo and 26% vs. ezetimibe). These beneficial effects were not affected by glycemic control, insulin use, renal function, and cardiovascular disease status. Thus, PCSK9 inhibitors are effective therapy in patients with type 2 diabetes and the beneficial effects on pro-atherogenic lipoproteins are similar to what is observed in non-diabetic patients. Additionally, except for local reactions at the injection sites PCSK9 inhibitors do not seem to cause major side effects.

Bempedoic Acid

The effects of bempedoic acid on LDL-C levels in patients with diabetes are similar to the decreases seen in non-diabetics. Patients with type 2 diabetes often have elevated uric acid levels and increases in uric acid and an increased risk of gouty attacks are major side effects of bempedoic acid [45]. In clinical trials, 26% of bempedoic acid-treated patients with normal baseline uric acid values experienced hyperuricemia one or more times versus 9.5% in the placebo group. Elevations in blood uric acid levels may lead to the development of gout and gout was reported in 1.5% of patients treated with bempedoic acid vs. 0.4% of patients treated with placebo. The risk for gout attacks were higher in patients with a prior history of gout

(11.2% for bempedoic acid treatment vs. 1.7% in the placebo group). In patients with no prior history of gout only 1% of patients treated with bempedoic acid and 0.3% of the placebo group had a gouty attack. Tendon rupture is another rare but important side effect of bempedoic acid therapy but whether this is more common in patients with diabetes is unknown. Tendon rupture occurred in 0.5% of patients treated with bempedoic acid vs. 0% of placebo-treated patients [45]. In meta-analyses, bempedoic acid therapy was associated with a decrease in the onset of diabetes and worsening of diabetes (RR 0.65, $p = 0.03$) [164]. In a review focusing solely on the development of new onset diabetes it was reported that new-onset diabetes/hyperglycemia occurred less frequently with bempedoic acid vs. placebo (4.7/100 vs 6.4/100 patient-years) [165].

Therapeutic Approach

First Priority: LDL-C

The first priority in treating lipid disorders in patients with diabetes is to lower the LDL-C levels to goal, unless triglycerides are markedly elevated (>500–1000 mg/dL), which increases the risk of pancreatitis. LDL-C is the first priority because the database linking lowering LDL-C with reducing cardiovascular disease is extremely strong and we now have the ability to markedly decrease LDL-C levels. Dietary therapy is the initial step but, in almost all patients, will not be sufficient to achieve the desired LDL-C goals. If patients are willing and able to make major changes in their diet it is possible to achieve significant reductions in LDL-C levels but this seldom occurs in clinical practice [166].

Statins are the first-choice drugs to lower LDL-C levels and the vast majority of diabetic patients will require statin therapy. There are several statins currently available in the US and they are available as generic drugs and therefore relatively inexpensive. The particular statin used may be driven by price, ability to lower LDL-C levels, and potential drug interactions. Patients with ASCVD (secondary prevention patients) should be started on intensive statin therapy (atorvastatin 40–80 mg/day or rosuvastatin 20–40 mg/day). Given the extensive data showing that the lower the LDL-C the greater the reduction in ASCVD events, most secondary prevention patients would benefit from the addition of ezetimibe to maximize LDL-C lowering. Ezetimibe is now a generic drug, and therefore, this strategy will not markedly increase costs. Similarly, primary prevention patients who are at high risk for cardiovascular events will also benefit from the use of high-intensity statin therapy in combination with ezetimibe. Primary prevention patients at moderate risk can be started on moderate-intensity statin therapy.

If a patient is unable to tolerate statins or statins as monotherapy are not sufficient to lower LDL-C to goal the second-choice drug is either ezetimibe or a PCSK9 inhibitor. Ezetimibe can be added to any statin. PCSK9 inhibitors can also be added to any statin and are the drug of choice if a large decrease in LDL-C is required to

reach goal (PCSK9 inhibitors will lower LDL-C levels by 50–60% when added to a statin, whereas ezetimibe will only lower LDL-C by approximately 20%). Bile acid sequestrants and bempedoic acid are alternatives with the use of a bile acid sequestrant particularly useful if a reduction in A1c level is also needed. Ezetimibe, PCSK9 inhibitors, bempedoic acid, and bile acid sequestrants additively lower LDL-C levels when used in combination with a statin, because these drugs increase hepatic LDL receptor levels by different mechanisms, thereby resulting in a reduction in serum LDL-C levels [45]. Niacin and the fibrates also lower LDL-C levels but are not usually employed to lower LDL-C levels.

Second Priority: Non-HDL-C and Triglycerides

The second priority should be non-HDL-C (non-HDL-C = total cholesterol − HDL-C), which is particularly important in patients with elevated triglyceride levels (>150 mg/dL). Non-HDL-C is a measure of all the pro-atherogenic apolipoprotein B containing particles. Numerous studies have shown that non-HDL-C is a strong risk factor for the development of cardiovascular disease [167] and the ASCVD calculators use non-HDL-C. The non-HDL-C goals are approximately 30 mg/dL greater than the LDL-C goals. For example, if the LDL goal is <100 mg/dL then the non-HDL-C goal would be <130 mg/dL. Drugs that reduce either LDL-C or triglyceride levels will reduce non-HDL-C levels. To lower triglyceride levels initial therapy should focus on glycemic control and lifestyle changes, including a decrease in simple sugars and ethanol intake. Additionally, if possible, discontinue medications that increase triglyceride levels. If drugs are needed fibrates and omega-3-fatty acids reduce triglyceride levels. As discussed above, studies with the omega-3-fatty acid icosapent ethyl (EPA; Vascepa) added to statin therapy have reduced the risk of cardiovascular events. The National Lipid Association has recommended "that for patients aged ≥45 years with clinical ASCVD, or aged ≥50 years with diabetes mellitus requiring medication plus ≥1 additional risk factor, with fasting TGs 135–499 mg/dL on high-intensity or maximally tolerated statin therapy (±ezetimibe), treatment with icosapent ethyl is recommended for ASCVD risk reduction" [168]. Alternatively, one could use fenofibrate. As discussed earlier, in the ACCORD-LIPID trial there was a suggestion of benefit with fenofibrate therapy in the patients in whom the baseline triglyceride levels were elevated (>204 mg/dL) and HDL cholesterol levels decreased (<34 mg/dL) [110]. This may be an ideal treatment option in certain patients with diabetes as fenofibrate has also been shown to reduce the risk and/or progression of microvascular disease as discussed in detail earlier.

Very High Triglycerides

Patients with very high triglyceride levels (>500–1000 mg/dL) are at risk of pancreatitis and therefore lifestyle and triglyceride-lowering drug therapy should be initiated early. Initial treatment is a low-fat diet and glycemic control. Treating secondary

disorders that raise triglyceride levels and when possible, stopping drugs that increase triglyceride levels is essential. If the triglyceride levels remain above 500 mg/dL the addition of fenofibrate and/or omega-3-fatty acids is indicated [125].

Low HDL-C

While there is strong epidemiologic data linking low HDL-C levels with cardiovascular disease, there is no clinical trials demonstrating that increasing HDL-C levels reduce cardiovascular disease. Thus, the use of drugs such as niacin to raise HDL-C levels cannot therefore be recommended.

Conclusion

Patients with diabetes, particularly type 2 diabetes, often have dyslipidemia. Because of the high risk of ASCVD in patients with diabetes modern therapy demands that we aggressively treat lipids to reduce the high risk of cardiovascular disease in this susceptible population and in those with very high triglycerides to reduce the risk of pancreatitis.

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Part III
Diabetic Microvascular Disease

Chapter 15
Diabetic Retinopathy

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Abbreviations

CSME	Clinically significant diabetic macular edema
DCCT	Diabetes Control and Complications Trial
DME	Diabetic macular edema
DR	Diabetic retinopathy
EDIC	Epidemiology of Diabetes Interventions and Complications study
H/Ma	Hemorrhages and microaneurysms
IRMA	Intraretinal microvascular abnormalities
NPDR	Nonproliferative diabetic retinopathy
NVD	New vessels on the disc
NVE	New vessels elsewhere
PDR	Proliferative diabetic retinopathy
PKC	Protein kinase C
PPV	Pars plana vitrectomy
PRP	Panretinal photocoagulation (scatter laser treatment)
UKPDS	United Kingdom Prospective Diabetes Study
VCAB	Venous caliber abnormalities
VEGF	Vascular endothelial growth factor
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy

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Introduction

Diabetic retinopathy (DR) is a microvascular complication that afflicts virtually all patients who have had diabetes mellitus for more than a decade [1]. Despite many years of research, there is presently no known cure or means of preventing DR, and DR remains the leading cause of new-onset blindness in working-aged Americans [2]. Nationwide clinical trials in the 1980s and 1990s demonstrated that scatter (panretinal) laser photocoagulation reduces the 5-year risk of severe vision loss (i.e., best corrected visual acuity of 5/200 or worse) from proliferative DR (PDR) from as high as 60% to less than 4%. In addition, these trials demonstrated the efficacy of focal/grid macular laser for treatment of patients with diabetic macular edema (DME). Beginning in the early 2000s, availability of intravitreally delivered vascular endothelial growth factor (VEGF) inhibitors (anti-VEGF) and steroid therapies further improved visual acuity outcomes in patients with DME. Anti-VEGF injections have become the primary therapy for center involving DME with vision loss and also provided an alternative for patients with PDR, as well as vitreous hemorrhages secondary to neovascularization from PDR. Vitrectomy surgery, with endolaser photocoagulation as indicated, can frequently prevent further vision loss or restore useful vision in eyes that have nonresolving vitreous hemorrhage or traction retinal detachment threatening central vision. Although numerous new therapies are currently in development, until a prevention or cure for diabetes and diabetic retinopathy is discovered, the keys to preventing vision loss from DR are regular eye examinations to determine the need for timely laser photocoagulation or anti-VEGF intervention, and rigorous control of blood glucose and any accompanying systemic medical conditions, such as hypertension, renal disease, and dyslipidemias (Fig. 15.1).

This chapter reviews the current understanding of the etiology and pathophysiology of DR, the clinical manifestations of the disease, and current guidelines for appropriate disease management and future treatment strategies.

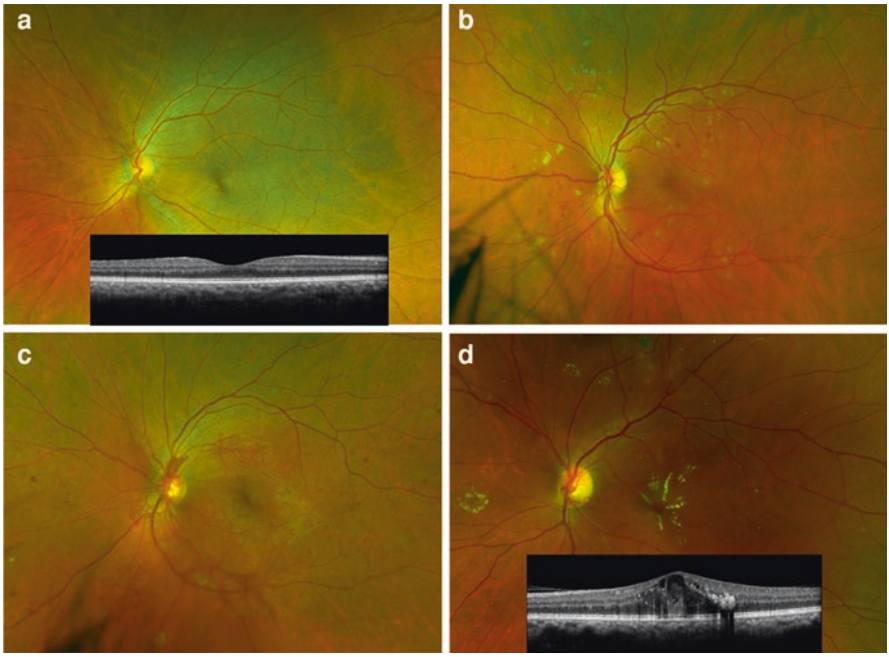

Fig. 15.1 Different diabetic retinopathy (DR) severity levels. (Panel **a**) An eye with no DR and no macular edema on OCT. (Panel **b**) Moderate–severe NPDR. The eye has extensive cotton wool spots, hemorrhages/microaneurysms, and intraretinal microvascular abnormalities (IRMA). (Panel **c**) An eye with high-risk proliferative diabetic retinopathy (PDR). Large areas of neovascularization on the disc (NVD) and elsewhere (NVE) along the superotemporal and inferotemporal arcades are seen. (Panel **d**) An eye with mild NPDR and diabetic macular edema. The macular area has areas of retinal swelling and extensive hard exudate (yellow spots) deposition. An OCT B-scan of the foveal area shows fluid cysts and loss of normal foveal contour

Pathophysiology of Diabetic Retinopathy

Early Studies

Diabetic retinopathy is a highly specific retinal vascular complication of both type 1 and type 2 diabetes. Initial studies [3–5] of DR concentrated on retinal microaneurysms, an early clinical sign of retinal disease. Cogan, Toussaint, and Kuwabara pioneered many of these early investigations to elucidate the pathophysiology of DR. [3] Microaneurysms were shown to develop bordering areas of occluded capillaries with either normal or hyperplastic endothelial linings [5]. Additionally, a loss of mural cells in the diabetic vessels resulted in outpouchings of the capillary walls. The retinal microaneurysms appeared to develop from these areas that were deficient in mural cells.

The relative retinal ischemia common to DR and numerous other retinal vascular disorders is thought to underlie the development of retinal neovascularization and edema [6, 7]. In 1948, Michaelson postulated that retinal ischemia initiated the release of a vasoproliferative factor [6]. This putative vasoproliferative factor resulted in new vessel growth at the optic disc and other areas of the retina and iris, and might account for the increased vascular permeability associated with these disorders. As discussed below, recent studies have greatly increased our understanding of several now-identified vasoproliferative factors.

Studies using experimentally induced diabetes in dogs demonstrated that hyperglycemia, characterized by deficient insulin activity, is capable of eliciting DR, even in animals that do not have hereditary forms of diabetes [8–12]. Engerman's studies of alloxan-induced diabetic dogs showed that progression of DR is related to the level of glycemic control, further underscoring the role of hyperglycemia as the underlying etiology of DR.

Present Understanding

Multiple investigations of DR have focused on the biochemical basis of the disease. Studies of numerous biochemical pathways, including the sorbitol pathway, advanced glycation end products (AGEs), and the protein kinase C (PKC) pathway, demonstrate that biochemical changes occur in the retina long before clinically evident abnormalities are observed. These studies suggest that if appropriate novel therapeutic interventions can be identified, early intervention might prevent or reverse the microvascular abnormalities associated with diabetic retinopathy.

Numerous studies have focused on the polyol pathway due to the increased flux through this pathway in the diabetic condition. Aldose reductase is present in the pericytes of the retinal capillaries and since damage to the pericytes occurs early in the evolution of DR, the role of aldose reductase in the pathogenesis of DR has been extensively evaluated. Furthermore, aldose reductase is present in nerve tissue and induces depletion of myoinositol, leading to a decrease in nerve conduction velocity in diabetic neuropathy. Inhibitors of aldose reductase have been effective in preventing damage to the lens, in preventing thickening of retinal capillary basement membranes in diabetic animals, and in improving nerve conduction velocity in patients with diabetic neuropathy. Thus, it has been postulated that aldose reductase inhibitors may also be able to prevent, delay, or halt the development or progression of DR. Unfortunately, clinical trials of the aldose reductase inhibitor sorbinil have not proved clinically effective in preventing the progression of DR. [13, 14]

Additional studies have evaluated advanced glycation end products (AGEs). The presence of high concentrations of glucose can result in the glycation of numerous proteins, especially albumin [15]. These glycated proteins adversely affect cellular and capillary function, structure, and metabolism. Exposure to glycated proteins induces changes in the glomerulus similar to those observed in diabetes, as well as changes in the nerves resembling diabetic neuropathy. The effect of AGE in the eye

is being actively studied. AGE can affect both the neuronal and vascular components of the eye, as well as induce numerous growth factors. As such, it may play a role in the progression of diabetic retinopathy.

Other studies have concentrated on the hyperglycemia-induced activation of protein kinase C (PKC), which can affect a wide range of vascular functions, including vascular permeability, contractility, retinal blood flow, and growth factor expression and signal transduction [16, 17]. Hyperglycemia is known to increase the level of diacylglycerol (DAG), which is the physiologic activator of PKC. Much of the vascular dysfunction associated with diabetes is thought to be mediated through this increased action of PKC. There are numerous isoforms of PKC; however, in the retina, PKC α, β, and δ are primarily expressed. Investigations have suggested that the β isoform of PKC is principally associated with the pathology associated with the diabetic state [18]. In laboratory animals, PKC β selective inhibitors have been shown to ameliorate renal dysfunction, retinal blood flow abnormalities, vascular permeability [19], and neovascularization associated with diabetes and diabetes-like models [20]. In addition, activation of PKC is partially involved in the expression of critical growth factors, such as vascular endothelial growth factor (VEGF) [14], which mediates much of the neovascularization and vascular permeability in the eye. Thus, inhibition of the β isoform PKC may possibly block numerous pathological processes in the diabetic condition that result in the vascular dysfunction and ocular complications associated with DR. Since a PKC β selective inhibitor has been shown to be well tolerated in animals and ameliorates many of the abnormalities associated with diabetes, these molecules have also been evaluated in clinical trials. These studies have demonstrated that although PKC beta inhibition using ruboxistaurin does not prevent the progression of diabetic retinopathy, it may have a beneficial effect on macular edema and on reducing vision loss and need for laser therapy for diabetic macular edema [21, 22]. The magnitude of the effect however, did not support further use in the clinic.

The 50-Year Medalist study at the Joslin Diabetes Center has evaluated over 1000 patients who have survived 50 or more years of insulin-dependent diabetes [23, 24]. The study has identified a potential factor associated with protection from advanced DR in this cohort. Retinol binding protein 3 (RBP3) was found at higher concentrations in retina and vitreous samples from Medalist patients with no to mild NPDR versus PDR [25]. Data from preclinical studies have since suggested that this photoreceptor-secreted protein may have effects on the vascular and neural retina mediated by its binding to GLUT1 receptors and secondary decreased expression of vascular endothelial growth factor (VEGF).

Genetic Risk Factors

From early concordant twin studies it has been postulated that genetic risk factors exist between onset, severity, and progression of DR. [26] There has been recent focus on possibly elucidating these genetic risk factors which may delay or hasten

the progression to severe PDR. There are published reports on two unique cohorts of patients with type 1 DM of more than 50 years. A possible genetically determined protective effect against the development of diabetic nephropathy and large vessel disease was seen in the Golden Years cohort in the UK [24]. The Joslin Diabetes Center's 50-year Medalist cohort has observed that only approximately 50% of type 1 diabetic patients with extreme duration diabetes have developed PDR despite decades of hyperglycemia [27]. These two cohorts point to a possible genetic susceptibility or resistance to the development of PDR in these unique cohorts with extreme durations of diabetes. In addition, common genetic factors may be involved in the development of PDR and end-stage renal disease among diabetic patients due to the high degree of concordance of these two complications [28].

Candidate gene approaches look at specific allele or gene variants associated with disease mechanism. These studies have evaluated a large number of potential genetic associations with diabetic eye disease but have not yielded consistent of reproducible results. The best known and most studied is the VEGF gene [29–31]. The most common polymorphism, rs2010963, has yielded inconsistent results with only one out of four large meta-analyses confirming its relationship with advanced diabetic retinopathy [32–35]. Studies have identified a specific SNP at the promoter of the erythropoietin gene, located at 7q21, that is associated with higher rates of development of severe diabetic eye and kidney complications [36]. Erythropoietin has previously been shown to be angiogenic in the eye [37]. The promoter polymorphism identified [36] results in the formation of an AP1 transcription binding site with 25-fold increase in promoter activity. Indeed, patients with this polymorphism have 7.5-fold increased erythropoietin concentration in the vitreous of the eye. One of the largest studies to look at candidate genes in DR was the Candidate gene Association Resource (CARe) [38]. Upon evaluating 2691 participants with type 2 DM, the study did not find an association between the commonly studied candidate genes and DR.

In contrast to the candidate gene approach, genome-wide association studies (GWAS) explore the entire genome, offering an unbiased approach to all potential genetic associations [39, 40]. The GWAS approach has been used in many populations, but results have been variable and most identified variants have not been reproduced in other populations or independent cohorts [41–44]. Possible reasons could include relatively small sample sizes in these studies, failure to account for potential confounders, such as diabetes duration, and a frequent lack of clear definitions for cases and controls [45].

Whole exome sequencing (WES) attempts to overcome some of the limitations in GWAS by sequencing only the protein coding regions (exons), thereby not including non-coding regions [46]. There have only been a few studies that have implemented this technique and future studies with larger cohorts will demonstrate if an association between specific gene variants and DR can be found using this approach [47, 48].

Natural History and Clinical Features of Diabetic Retinopathy

Epidemiology

In 2019, it was estimated that 463 million people worldwide have diabetes [49, 50]. This number is projected to increase to 700 million by 2045. A recent meta-analysis that included data from 22,896 patients with diabetes found that the overall prevalence was 34.6% for any DR, 6.81% for DME, and 10.2% for vision threatening diabetic retinopathy (combination of moderate NPDR or worse and DME) [51].

Early data from the 1980s suggested that 25% of patients with type 1 and 15.5% with type 2 DM developed advanced proliferative diabetic retinopathy (PDR) after 15 years of diabetes [52, 53]. Of note, these numbers were reported prior to landmark clinical trials establishing the importance of intensive glycemic control on limiting DM complications both systemically and within the eye. The change in trend was reflected in a large meta-analysis looking at 27,120 diabetic patients with 10 years or more of follow-up and determined that the 4 and 10 year risk of progression to PDR was substantially lower in the 1986–2008 cohort compared to the 1975–1985 one [54].

DR is the most frequent cause of new-onset blindness among American adults aged 20–74 years. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, approximately 4% of younger-onset patients (aged <30 years at diabetes diagnosis) and nearly 2% of older-onset patients (aged ≥30 years at diabetes diagnosis) were legally blind. In the younger-onset group, 86% of blindness was attributable to DR. In the older-onset group, where other eye diseases were also common, 33% of the cases of legal blindness were due to DR. [55, 56] Rates of blindness and visual impairment from DR have decreased in some developed countries in the modern era due to improvements in DR screening programs, patient education, systemic control, and advances in treatment. However, diabetes-related blindness is still a common cause of vision loss globally and results in health care costs in the United States in excess of $500 million annually [57].

Duration of diabetes is closely associated with the onset and severity of DR. Clinical signs of DR are rare in prepubescent patients with type 1 diabetes, but nearly all patients with type 1 diabetes and more than 60 percent of patients with type 2 diabetes will develop some degree of DR after 20 years [55, 56]. In patients with type 2 diabetes, approximately 20% will have DR at the time of diabetes diagnosis, and most will develop some degree of DR over subsequent decades.

Level of glycemic control is another significant risk factor for the onset and progression of DR. [58–65] Both the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) Study demonstrated a clear and sustained relationship between hyperglycemia and diabetic microvascular complications, including retinopathy, nephropathy, and

neuropathy, among type 1 diabetic patients [58–65] In the DCCT, 1441 patients with type 1 diabetes who had either no retinopathy at baseline (primary prevention cohort) or minimal-to-moderate nonproliferative diabetic retinopathy (NPDR) (secondary progression cohort) were treated by either conventional diabetes therapy (i.e., one or two injections of insulin daily) or intensive diabetes management (i.e., three or more daily insulin injections or a continuous subcutaneous insulin infusion.) The patients were followed for 4–9 years. The DCCT showed that intensive insulin therapy reduced or prevented the development of DR by 27% as compared with conventional therapy. Additionally, intensive therapy reduced the progression of DR by 34–76% and had a substantial beneficial effect over the entire range of DR. This improvement was achieved with an average 10% reduction in HbA_{1c} from 8 to 7.2%. The EDIC study has followed patients enrolled in the DCCT study for nearly three decades after their original DCCT participation. Participants who had been assigned to intensive treatment were encouraged to continue, and participants originally assigned to conventional treatment were advised to change to intensive treatment. The risk reductions observed in the DCCT between the rates of microvascular complications in the intensive compared to conventional treatment were sustained throughout 18 years of follow-up [60, 66]. These beneficial effects were achieved despite a continuously narrowing difference in HbA_{1c} between groups which was not statistically significant by 5 years of follow-up [60]. Furthermore, over a median follow-up of 23 years, intensive glycemic control was associated with a 48% risk reduction in diabetes-related ocular surgeries and a 37% risk reduction in all ocular procedures [67]. This finding underscores the need for intensive diabetes management as soon as it is safely possible which should be sustained with a target HbA_{1c} level of 7.0% or less. Although intensive therapy does not prevent DR completely, when begun early before microvascular complications are present; it is effective in significantly reducing the risk of development and progression of DR.

The United Kingdom Prospective Diabetes Study (UKPDS) found similar results for patients with type 2 diabetes. In the UKPDS, 4209 patients with newly diagnosed type 2 diabetes who had either no DR at baseline (primary prevention cohort) or minimal-to-moderate NPDR (secondary progression cohort) were randomly assigned to conventional or intensive blood glucose control, using sulfonylureas and/or insulin. The UKPDS showed that intensive therapy reduced the risk of all microvascular endpoints, including vitreous hemorrhage, retinopathy requiring laser photocoagulation, and renal failure by 25%. Overall, intensive control resulted in a 29% reduction in need for laser photocoagulation, a 17% reduction in a two-step progression of DR, a 24% reduction in the need for cataract extraction, a 23% reduction in vitreous hemorrhage, and a 16% reduction in legal blindness. This improvement was achieved with an average 10% reduction in HbA_{1c} from 7.9% to 7.0% [68, 69].

Renal disease, as manifested by microalbuminuria and proteinuria, is yet another significant risk factor for onset and progression of DR. [70, 71] Similarly, hypertension has been associated with PDR in some studies and may be a risk factor for the development of macular edema [72, 73]. Both renal retinopathy and hypertensive retinopathy can be superimposed on DR. Additionally, elevated serum lipid levels are associated with lipid deposits in the retina (hard exudates) and visual loss [37, 74, 75]. Thus, systemic control of blood pressure, renal disease, and serum lipids are

critically important components in the management of DR. [76] In addition, several studies suggest that pregnancy in patients with type 1 diabetes patients may aggravate DR. [77–79]

Clinical Findings in Diabetic Retinopathy

Clinical findings associated with early and progressing DR include hemorrhages and/or microaneurysms (H/Ma), cotton wool spots (CWS), hard exudates (HE), intraretinal microvascular abnormalities (IRMA), and venous caliber abnormalities (VCAB), including venous loops, venous tortuosity, and venous beading. Microaneurysms are saccular outpouchings of the capillary walls. These microaneurysms can leak fluid, causing areas of hyperfluorescence on a fluorescein angiogram. Ruptured microaneurysms, as well as leaking capillaries and intraretinal microvascular abnormalities, result in intraretinal hemorrhages. These intraretinal hemorrhages can be "flame shaped" or spot-like in appearance, reflecting the architecture of the layer of the retina in which they occur. Flame-shaped hemorrhages are generally in the nerve fiber layer of the retina, which runs parallel to the retinal surface. Dot or pinpoint hemorrhages are deeper in the retina, reflecting cells that are arranged perpendicular to the retinal surface.

Intraretinal microvascular abnormalities are abnormal vessels located within the retina itself. They may represent either localized intraretinal new vessel growth or shunting vessels through areas of poor vascular perfusion. It is common for IRMA to be found adjacent to cotton wool spots, which are feathery lesions in the nerve fiber layer of the retina resembling the fluffy appearance of cotton. Cotton wool spots are caused by microinfarcts in the nerve fiber layer. Cotton wool spots in a ring or partial ring surrounding the optic nerve head are frequently signs of severe renal disease or hypertension.

Venous caliber abnormalities are a sign of severe retinal hypoxia. Venous caliber abnormalities can be associated with any of the lesions of NPDR; however, in many cases of severe retinal hypoxia, distal retinal areas may be free of nonproliferative lesions due to the extensive vascular loss present. Such "lesion free" areas are termed "featureless retina."

Vision loss from DR can result from persistent, non-clearing vitreous hemorrhage, traction retinal detachment, retina ischemia, and/or diabetic macular edema. Neovascularization and contraction of the accompanying fibrous tissues can distort the retina and lead to traction retinal detachment. If a traction retinal detachment involves or threatens the macula, irreversible severe vision loss may result. Also, the new vessels may bleed, causing preretinal or vitreous hemorrhage. Pars plana vitrectomy can relieve the traction in cases where vision is threatened and can remove persistent vitreous hemorrhage, often restoring useful vision. The most common cause of vision loss from diabetes, however, is macular disease and macular edema. Macular edema is more likely to occur in patients with type 2 diabetes, which represents 90% or more of the diabetic population. In diabetic macular disease, macular edema or non-perfusion of the capillaries in the macular area results in the loss of central vision.

Classification of Diabetic Retinopathy

DR is broadly classified as nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). The lesions of NPDR include dot and blot hemorrhages and/or microaneurysms, cotton wool spots, hard exudates, venous caliber abnormalities, and intraretinal microvascular abnormalities. Based on the presence and extent of these retinal lesions, NPDR is further classified as mild, moderate, severe, or very severe NPDR. (Table 15.1) PDR is characterized by new vessels on the optic disc (NVD), new vessels elsewhere on the retina (NVE),

Table 15.1 Levels of diabetic retinopathy

Non-proliferative Diabetic Retinopathy (NPDR)	Characteristics
Mild NPDR	• At least one microaneurysm • Characteristics not met for more severe DR
Moderate NPDR	• Hemorrhages &/or microaneurysms (H/ma) of a moderate degree (i.e., ≥ standard photograph 2A[1*]) and/or • Soft exudates (cotton wool spots), venous beading (VB), or intraretinal microvascular abnormalities (IRMA) definitely presentand • Characteristics not met for more severe DR
Severe NPDR	One of the following: • H/Ma ≥ standard 2A in 4 retinal quadrants • Venous beading in ≥ 2 retinal quadrants (see standard photo 6B) • IRMA in ≥ 1 retinal quadrant ≥ standard photo 8A • Characteristics not met for more severe DR
Very severe NPDR	• Two or more lesions of severe NPDR • No retinal neovascularization
Proliferative Diabetic Retinopathy (PDR)	*Characteristics*
Early PDR	• New vessels definitely present • Characteristics not met for more severe DR
High-risk PDR	One or more of the following: • Neovascularization on the optic disc (NVD) ≥ standard photo 10 A (i.e., ≥1/4 to 1/3 disc area) • Any NVD with vitreous or preretinal hemorrhage • Neovascularization elsewhere on the retina (NVE) ≥1/2 disc area with vitreous or preretinal hemorrhage
Clinically significant macular edema (CSME)	
Any one of the following lesions: • Retinal thickening at or within 500 microns (1/3 disc diameter) from the center of the macula • Hard exudates at or within 500 microns from the center of the macula with thickening of the adjacent retina • A zone or zones of retinal thickening ≥ 1 disc area in size, any portion of which is at or within 1 disc diameter from the center of the macula	

[1*]Standard photographs refer to the Modified Airlee House Classification of Diabetic Retinopathy (see reference ETDRS report # 12)

preretinal hemorrhage (PRH), vitreous hemorrhage (VH), and/or fibrous tissue proliferation (FP). Based on the presence or absence of proliferative lesions, their severity, and their location, PDR is classified as early PDR or high-risk PDR. (Table 15.1) Diabetic macular edema (DME) can be present with any level of diabetic retinopathy and needs to be evaluated in addition to the level of DR. DME that involves or threatens the center of the macula is termed clinically significant macular edema (CSME). (Table 15.1) The level of NPDR establishes the risk of progression to sight-threatening retinopathy and appropriate clinical management as specifically detailed in Table 15.2.

In elucidating the natural history of DR, the Early Treatment Diabetic Retinopathy Study (ETDRS) evaluated the risks of progression from no or minimal DR to sight-threatening PDR. Importantly, the ETDRS showed that certain nonproliferative lesions, particularly venous beading, intraretinal microvascular abnormalities, and hemorrhages and/or microaneurysms are significant prognosticators for the development of proliferative disease within a 12-month period. Pregnancy, puberty, and cataract surgery can accelerate these changes.

Table 15.2 Recommended general management of diabetic retinopathy

	Risk of Progression to		
Level of DR	PDR 1 year	High-Risk PDR-5 year	F/U (mos)
Mild NPDR • No DME • Non-ciDME • ciDME	5%	15%	12 3–6 1
Moderate NPDR • No DME • Non-ciDME • ciDME	12–27%	33%	6–12 3–6 1
Severe NPDR • No DME • Non-ciDME • ciDME	52%	60%	3–4 2–4 1
Very severe NPDR • No DME • Non-ciDME • ciDME	75%	75%	3–4 2–4 1
PDR < high risk • No DME • Non-ciDME • ciDME		75%	3–4 2–4 1
High-risk PDR • No DME • Non-ciDME • ciDME			2–4 2–4 1

NPDR Non-proliferative diabetic retinopathy, *PDR* Proliferative diabetic retinopathy, *DME* Diabetic macular edema, *ciDME* Center-involved diabetic macular edema, *mos* months, *Occ* Occasionally, *OccAF* Occasionally after focal

Table 15.3 International clinical DR and DME disease severity scales [80]

Diabetic Retinopathy Disease Severity	
No apparent DR	No abnormalities
Mild NPDR	Microaneurysm (ma) only
Moderate NPDR	More than ma only but less than severe NPDR
Severe NPDR	Any of the following and no PDR: >20 intraretinal hemorrhages in each 4 quadrants Definite VB in 2+ quadrants Prominent IRMA in 1 quadrant
PDR	One or more of: NV, VH, PRH
Diabetic macular edema disease severity	
DME apparently absent	No apparent retinal thickening or HE in posterior pole
DME apparently present	Some apparent retinal thickening or HE in posterior pole
Mild DME—Some retinal thickening or HE in posterior pole but distant from center of the macula	
Moderate DME—Retinal thickening or HE approaching the center of the macula but not involving the center	
Severe DME—Retinal thickening or HE involving the center of the macula	

In an effort to standardized classification of DR across international borders and among different health care providers, leaders from various groups and nations (the Global Diabetic Retinopathy Project Group) established and promulgated the Proposed International Classification of Diabetic Retinopathy [80]. (Table 15.3) This classification identifies three levels of NPDR and one level of PDR. With regard to macular edema, two major categories—macular edema present and macular edema absent—are identified. If macular edema is present, three categories are defined: macular edema not threatening the center of the macula, macula edema threatening the center of the macula, and macula edema involving the center of the macula.

Recently, optical coherence tomography (OCT) has become the benchmark to diagnose and monitor diabetic macular edema. OCT has also been accompanied by widespread use of intravitreal anti-VEGF and steroid medications to treat DME. OCT has been used to monitor patient response to injections and drive therapy. Clinical trials exploring the effects of anti-VEGF as well as novel therapeutics routinely use OCT. As such, current diabetic macular edema classification is based on central subfield thickness (CST), which is the average thickness of a circular area 1 mm in diameter centered around the center point [81]. A large multicenter study looking at CST OCT measurements in patients with mild or no DR proposed values of ≥320 μm for males and 305 μm for females (approximately 2 SDs above the average of the normative cohort) to be used to determine the presence or absence of DME [82]. Of note, these measurements are for the Heidelberg OCT machine and values are different on other devices [83]. These cut-off values are routinely used in clinical trials to enroll, treat, and monitor patients. If a patient has CST values

greater than the cut-off they are deemed to have center involved macular edema (ciDME), while if edema is present but CST values are below threshold they are graded as having non-ciDME.

While CST values are diagnostic for ciDME, they are not the only determinant for treatment. Post hoc analysis by the DRCR Retina Network has found only a moderate correlation between CST thickness and visual acuity [84]. As such, there are patients with extensive thickening and excellent VA, while others have mild thickening and poor VA. Therefore, in current treatment algorithms, both VA and CST are used to determine initiation, continuance, and deferral of therapy.

Treatment of Diabetic Retinopathy

Overview

Appropriate clinical management of DR was initially defined by five major, randomized, multicentered clinical trials: the Diabetic Retinopathy Study (DRS) [85–95], the Early Treatment Diabetic Retinopathy Study (ETDRS) [73, 74, 96–103], the Diabetic Retinopathy Vitrectomy Study (DRVS) [104–108], the Diabetes Control and Complications Trial (DCCT) [58, 61–65], and the United Kingdom Prospective Diabetes Study (UKPDS) [68, 109]. These studies elucidated delivery and proper timing for laser photocoagulation surgery for the treatment of both DR and DME [110–115]. They also established guidelines for vitrectomy surgery. The DRS demonstrated that scatter (panretinal) laser photocoagulation was effective in reducing the risk of severe vision loss from PDR by 50% or more.

The ETDRS was a multicenter, randomized clinical study designed to test [1] whether 650 mg of aspirin per day had any effect on the progression of diabetic retinopathy, [2] whether focal laser photocoagulation for macular edema reduced the risk of moderate vision loss (i.e., a doubling of the visual angle, e.g., 20/20 reduced to 20/40), and [3] whether scatter laser photocoagulation was more beneficial in reducing the risk of severe vision loss (i.e., best corrected visual acuity of 5/200 or worse) when applied prior to the development of high-risk PDR, as defined below. The ETDRS enrolled 1377 patients at 22 centers nationwide. Major conclusions of the ETDRS were as follows: [1] a daily dose of 650 mg of aspirin does not prevent the development of high-risk proliferative retinopathy and does not reduce the risk of visual loss, nor does it increase the risk of vitreous hemorrhage; [2] focal laser photocoagulation for diabetic macular edema reduces the risk of moderate visual loss by at least 50%, from nearly 30% to less than 15%; and [3] both early scatter laser photocoagulation and photocoagulation at the time of reaching high-risk PDR result in significant reduction in the risk of severe visual loss to less than 4%, although some groups, including those with type 2 diabetes or type 1 diabetes of long duration, have a greater benefit from early treatment [116]. As mentioned

above, the ETDRS also clarified the natural history of DR and the risk of progression of DR based on the baseline level of retinopathy [110–113].(Table 15.1) Finally, the ETDRS identified specific lesions that placed an eye at high risk for visual loss [111]. These lesions include H/Ma, VCAB, and IRMA as detailed in Table 15.1. Based on ETDRS findings, proper diagnosis of the level of DR (Table 15.2) determines appropriate timing of follow-up evaluation and when to initiate laser photocoagulation.

The DRVS demonstrated that early vitrectomy was useful in restoring vision for some persons who have severe vision loss due to vitreous hemorrhage. In addition, the DRVS demonstrated that persons with severe fibrovascular proliferation were more likely to obtain better vision, and less likely to have poor vision, when PPV as performed early. The DRVS demonstrated the value of vitrectomy in restoring useful vision, particularly in patients with type 1 diabetes. The treatment benefits demonstrated in the DRVS, which was completed in 1989, are not totally applicable today due to dramatic advances in surgical techniques, the advent of endolaser photocoagulation, and the use of anti-VEGF therapy.

The understanding of the role of growth factors in DR and DME has grown significantly over the past decade [29, 117]. Multiple growth factors mediate both the neovascularization of PDR and the increased permeability associated with DME. VEGF is believed to be one of the fundamental growth factors involved in these processes in the eye. This central role of VEGF in the pathogenesis of retinal neovascularization and vascular leakage has triggered a new paradigm in the management of retinal disease with pharmacologic agents. Intravitreal injections of anti-VEGF agents have been shown to inhibit the development of choroidal and retinal neovascularization and decrease the amount of vascular leakage. These compounds are injected into the vitreous cavity of the eye on a repetitive basis and have robust and consistent clinical data to demonstrate beneficial activity. The marked beneficial effect induced by anti-VEGF agents in eyes with severe neovascularization of the retina and anterior segment has dramatically improved the treatment of those cases where the severity of the condition precluded laser treatment [118, 119]. Ranibizumab and aflibercept have been FDA approved for the treatment indications of DR and DME.

Many of the clinical studies and trials that have shaped clinical care for DR and DME in the modern era have been performed by the DRCR Retina Network. The DRCR Retina Network is a National Institutes of Health-sponsored collaborative network dedicated to multicenter clinical trial research of retinal diseases, including DR, DME, and associated disorders. Results of phase 3 clinical trials initiated and completed by the Network have established anti-VEGF therapy as the standard of care in the management of DME. They have also established its safety and efficacy in the management of eyes with PDR. These studies have also defined treatment algorithms for the administration of intravitreal medications in both DME and PDR as well as how to incorporate retinal imaging in the management and care of patients.

Proliferative Diabetic Retinopathy

Panretinal Photocoagulation

Both the DRS and the ETDRS demonstrated the value of scatter (panretinal) laser photocoagulation for treating PDR. In scatter laser photocoagulation, 1200 to 1800 laser burns are applied to the peripheral retinal tissue, focused at the level of the retinal pigment epithelium. Large vessels are avoided, as are areas of preretinal hemorrhage. It has been previously thought that treatment should be divided into two or more sessions, spaced one to two weeks apart with follow-up after 3 months from completion of the treatment. Evidence from a multicenter prospective, nonrandomized trial has shown that single session PRP may not be as detrimental as previously thought, and may be the same or even better long term than divided treatment [120].

The response to scatter laser photocoagulation varies. The most desirable effect is to see a regression of the new vessels. In some cases, there may be a stabilization of the neovascularization, with no further growth. This response may be acceptable, with careful clinical monitoring. In some cases, new vessels continue to proliferate, requiring additional scatter laser photocoagulation. In cases where neovascularization continues and does not respond to further laser photocoagulation, vitreous hemorrhage and/or traction retinal detachment may occur, possibly requiring surgical intervention with pars plana vitrectomy if vision is threatened. Eyes with high-risk PDR should receive prompt scatter laser photocoagulation. Eyes approaching high-risk characteristics (i.e., eyes with PDR less than high risk, and eyes with severe or very severe NPDR) may also be candidates for scatter laser photocoagulation. Recent progression of the eye disease, status of the fellow eye, compliance with follow-up, concurrent health concerns, such as hypertension or kidney disease, and other factors must be considered in determining if laser surgery should be performed in these patients. In particular, patients with type 2 diabetes should be considered for panretinal photocoagulation prior to the development of high-risk PDR since the risk of severe visual loss and the need for PPV can be reduced by 50% in these patients by early scatter treatment, especially when macular edema is present [116].

Anti-VEGF Therapy

The DRCR Retina Network Protocol S compared the use of intravitreal anti-VEGF (ranibizumab) to PRP in the management of PDR. The study demonstrated that ranibizumab therapy was non-inferior to PRP with regard to visual acuity (VA) outcomes at 2 and 5 years [121]. Moreover, this trial found that eyes treated with anti-VEGF had less visual field loss, less need for vitrectomy, and less frequent development of DME than eyes that received PRP. The effectiveness of intravitreal

anti-VEGF therapy for PDR was further confirmed by the CLARITY study, which compared the safety and efficacy of intravitreal aflibercept versus PRP in patients with active PDR [122]. This trial found that aflibercept treatment resulted in superior VA outcomes at 1 year compared to PRP.

Patients in DRCR Protocol S were followed for a total of 5 years. Although, average VA at year 5 was excellent (mean 20/25 in both the ranibizumab and PRP groups), most patients still required at least 1 injection during year 5 [123]. On average, patients received 2.9 injections in both years 4 and 5, with a mean total of 19.2 injections over the 5 years [123]. Therefore, the large number of visits and increased cost associated with anti-VEGF therapy as well as the need for close continued monitoring to manage recurrences are important factors to consider prior to initiating therapy [124].

Diabetic Macular Edema

Focal Laser Photocoagulation

Focal laser for CSME has been shown to be effective in reducing the risk of moderate visual loss [125]. In focal laser photocoagulation, lesions from 300 microns to 3000 microns from the center of the macula that are contributing to thickening of the macula area are directly photocoagulated. These lesions are generally identified by fluorescein angiography and consist primarily of leaking microaneurysms. Although fluorescein angiography is generally used to identify treatable lesions for focal laser photocoagulation, fluorescein angiography is not necessary for the diagnosis of CSME.

Follow-up evaluation following focal laser surgery generally occurs after 3 or 4 months. In the cases where macular edema persists, further treatment may be necessary. Macular laser has a diminished role for treatment of DME in the era of anti-VEGF therapy, but is still of use in eyes that are not candidates for anti-VEGF or as adjunctive therapy when edema persists despite intravitreal anti-VEGF treatment.

Steroid Therapy

Both periocular and intravitreal steroids have been used for the treatment of DME. To validate the initial findings from small case series and uncontrolled clinical trials, two multicenter randomized prospective clinical trials were undertaken to address both the effectiveness and safety of both routes of steroid administration. Peribulbar steroid injections were found to have no significant benefit for the treatment of DME and further study of the approach has been currently abandoned [126]. The results of a 2-year trial comparing intravitreal steroids to focal laser have shown that despite an initial rapid reduction in retinal thickness and improvement in vision with the intravitreal steroid injection, by 1 year the results were no better than

laser photocoagulation, and after 2 years, the steroid was inferior to the laser treatment in both visual outcome and retinal thickness. In addition, there was an approximately fourfold increase in the rate of intraocular pressure complications and a fourfold increase in need for cataract surgery in the steroid-treated group [125].

Another large multicenter study compared intravitreal anti-VEGF to macular laser and intravitreal steroid therapy (Triamcinolone) and demonstrated that although intravitreal steroids were associated with initial VA gains, this was followed by a decrease in mean VA after week 24 [127, 128]. At years 1 and 2, VA gains were not significantly different between the steroid group and the laser group (+1.1 letters and −1.5 letters mean difference at 1 and 2 years, respectively) [127, 128]. These findings may have been due in part to the development of cataracts or the negative impact of cataract surgery on macular edema in triamcinolone group eyes. A subgroup analysis in triamcinolone-treated eyes that were pseudophakic at baseline demonstrated that the visual acuity results were substantially better than for phakic eyes. Approximately 50% of eyes in the triamcinolone group had an intraocular pressure (IOP) elevation >10 mmHg from baseline, IOP >30, or initiation of IOP lowering medications at 1 or more visits during 2 years of follow-up. In addition, 59% required cataract surgery during the 2 years of follow-up. Given these results, intravitreal steroid therapy is generally regarded as second-line treatment for most eyes with DME unless they are not candidates for anti-VEGF injections.

Anti-VEGF Therapy

The DRCR Retina Network Protocol I was one the first phase 3 study to compare the following treatments for ci-DME: intravitreal ranibizumab, intravitreal triamcinolone (TA), and macular laser. The study helped establish anti-VEGF as the current standard of care in the management of patients with ci-DME. This trial showed that ranibizumab therapy was highly effective in the treatment of ci-DME, with patients gaining on average 8 or 9 letters compared to only 3 letters in the laser group at 1 year [127, 128]. Furthermore, the visual acuity (VA) gains achieved in the first year with ranibizumab were maintained all through the 5-year follow-up with a decreasing frequency of injections reaching a median of 0–1 injections in the fourth and fifth year [129].

The DRCR Retina Network also sought to compare available anti-VEGF medications and in a large multicenter study (Protocol T) compared the three available treatments; bevacizumab, ranibizumab, and aflibercept [130]. The study demonstrated that in patients with a baseline VA of 20/32–20/40, visual outcomes with all three anti-VEGF medications were similar [130]. In contrast, in patients with a VA of 20/50 or worse at baseline, aflibercept was associated with significantly more VA gains and DME resolution compared to both ranibizumab and bevacizumab at 1 year. By the second year, although the differences between aflibercept and ranibizumab decreased and were no longer significant, aflibercept was still superior to bevacizumab [131].

Because the inclusion criteria for most initial studies of anti-VEGF for DME required a baseline VA of 20/32 or worse, there were no clear guidelines for patients

with a VA of 20/25 or better and ci-DME [132]. The DRCR Retina Network Protocol V explored a strategy of initial observation with anti-VEGF if needed for visual worsening during follow-up, macular laser with anti-VEGF treatment if needed for vision loss or initial intravitreal aflibercept (anti-VEGF) at baseline in patients with a VA of 20/25 or better and ci-DME on OCT. The study concluded that the rates of visual loss were similar in all three groups and at 2 years the mean visual acuity was 20/20 in each group. These findings suggest that for most eyes with good baseline vision despite ciDME, a strategy of initial observation with subsequent initiation of anti-VEGF if VA were to decrease is a viable strategy. The subsequent Protocol AC study demonstrated that a specific form of step therapy using bevacizumab first, followed by aflibercept when outcomes were suboptimal, resulted in similar visual acuity outcomes to aflibercept monotherapy in eyes with moderate visual impairment from CI-DME.

Vitrectomy for Advanced PDR

In cases with vitreous hemorrhage secondary to PDR, a recent randomized controlled trial compared a strategy of intravitreal aflibercept versus immediate vitrectomy with PRP [133]. The study reported there were no significant differences in mean VA score between both groups at 24 weeks. Eyes assigned to vitrectomy had faster visual recovery and greater clearance of vitreous hemorrhage over the first 4 weeks of the study. The study thus suggests that both treatment strategies are reasonable approaches to cases of vitreous hemorrhage from PDR that are uncomplicated by macula-threatening vitreoretinal traction. However, vitrectomy still remains the only treatment available to relieve traction that involves or threatens the center of the macula.

Novel Treatments

Numerous recent advances in our understanding of the basic mechanisms underlying the progression of DR have raised the possibility of novel therapies against the progression of NPDR, PDR, and DME.

Fenofibrate

Two phase 3 studies of the peroxisome proliferator-activated receptor α medication, fenofibrate, have suggested that this oral agent may prevent worsening of early stage DR. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study randomized 9795 study participants with type 2 diabetes to fenofibrate versus placebo [134]. The need for laser treatment for DR or DME was significantly lower in the fenofibrate group (HR 0.69, 95% CI 0.56–0.84, P = 0.0002) [135]. In

addition, eyes with existing DR at baseline demonstrated a significant reduction in DR worsening with fenofibrate treatment (3.1% versus 14.6%, $P = 0.004$). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial subsequently evaluated ocular-specific outcomes in participants randomized within a 2x2 factorial design to simvastatin in combination with either fenofibrate or placebo [136]. At 4 years, DR worsening was significantly less frequent in the fenofibrate group (6.5% versus 10.2%, adjusted OR, 0.60, 95% CI, 0.42–0.86, $P = 0.0056$). The DRCR Retina Network is currently conducting a phase 3 trial of fenofibrate versus placebo for prevention of DR worsening in eyes with mild to moderately severe NPDR.

Plasma Kallikrein Inhibitors (PKI)

Preclinical data has demonstrated that plasma kallikrein contributes to DME development through both VEGF-dependent and -independent mechanisms [137, 138]. Although there are several PKI in development, KVD001 is the furthest one along. A phase 2 clinical trial compared two doses of intravitreal KVD001 (6 μg and 3 μg) with sham injections in patients with ci-DME [139]. There were no significant differences between VA gains in the treatment groups vs sham. However, the group treated with KVD001 6 μg had less vision loss than that given sham injections (32.5% vs. 54.5%, $p = 0.042$) at 16 weeks. Larger clinical trials are needed to definitively establish efficacy or lack thereof for the indication of DME.

Ang-Tie2 Targeting Drugs

The Tie2 signaling pathway is specific to vascular endothelial cells. Tie2 signaling is responsible for the maintenance of vascular health, promoting endothelial cell survival and stability. Angiopoietin 1 (Ang1) is a Tie2 agonist, while Ang2 is predominantly a Tie2 inhibitor. Under conditions of hypoxia and hyperglycemia, Ang2 is upregulated and in turn potentiates the action of VEGF [140–142]. The inhibition of Tie2 receptors by Ang2 disrupts its pro-vascular stabilizing effects, in turn resulting in increased vascular permeability and disruption of vascular structure [143, 144].

There have been many drugs targeting this signaling pathway but the only one currently in phase 3 clinical trials for DME is faricimab. Faricimab is a bispecific antibody that binds to both VEGF and Ang2. Recent results from the phase 2 BOULEVARD trial demonstrated that the 6.0 mg faricimab dose demonstrated a statistically significant gain of vision compared to ranibizumab 0.3 mg (+3.6 letters, $p = 0.03$) at 24 weeks [145]. In addition, an observation period from week 24 to week 36 showed that eyes treated with faricimab had a longer time to re-treatment compared to ranibizumab. Phase 3 results from the YOSEMITE and RHINE studies were recently reported as showing non-inferior visual outcomes of intravitreal faricimab to aflibercept treatment after 1 year of treatment. Extended dosing with treatment intervals of 16 weeks were achieved by over 50% of study participants treated with Faricimab [146].

Photobiomodulation

Photobiomodulation (PBM), or irradiation by light in the far-red (FR) to near-infrared (NIR) light spectrum (630–900 nm), has recently been explored for the treatment of DME. In the retina of diabetic mice PBM inhibited the generation of superoxide and decreased the expression of iNOS and MnSOD [147, 148]. In addition, PBM decreased vascular leakage and capillary degeneration [149]. Preliminary human studies enrolling 4 to 10 patients with DME were consistent with a favorable anatomic response with improvement in edema in PBM-treated eyes and an acceptable safety profile [150, 151]. However, the DRCR Retina Network Protocol AE, a phase 2 randomized trial did not find any clinical benefit from PBM versus placebo in the eyes of patients with ciDME and vision of 20/25 or better.

Conclusions/Summary

Appropriate management of DR and diabetic eye disease requires a thorough knowledge of both diabetes mellitus and the findings from key multicentered, randomized clinical trials, such as the DRS, ETDRS, DRVS, DCCT, UKPDS, and the recent trials by the DRCR Retina Network. Accurate diagnosis of DR severity level is essential to determine appropriate care and follow-up schedules and to assess the need for timely laser photocoagulation for PDR and DME. Since DR usually causes no symptoms when it is most amenable to treatment, strategies to reduce the risk of vision loss must stress the need for regular eye examination, even in patients with no ocular complaints [152]. (Table 15.4) Currently, patients with type 1 diabetes ten years of age and older are encouraged to have a comprehensive, dilated retinal eye examination within three to five years of diagnosis, and at least annually thereafter. Patients with type 2 diabetes are encouraged to have a comprehensive, dilated eye examination at the time of diagnosis, and at least annually thereafter. Patients contemplating pregnancy should have their eyes examined prior to conception whenever possible and pregnant women should have their eyes examined early in the first trimester and each trimester thereafter. In all cases, abnormal findings may require

Table 15.4 Suggested frequency of eye examination

Type of Diabetes	Recommended first Examination	Routine Minimal Follow-Up
Type 1 DM	Older than 10 years: 3–5 years after onset of diabetes or at puberty	Yearly
Type 2 M	Upon diagnosis of diabetes	Yearly
During pregnancy	• Prior to conception for counseling • Early in first trimester	• Each trimester • More frequently as indicated • 1–2 months post-partum

accelerated examination schedules, and the presence of concurrent medical conditions, such as hypertension and renal disease, may also require more frequent ocular examination and should be aggressively controlled in conjunction with the patient's internist or diabetologist.

Our understanding of DR has expanded dramatically in the past 30 years. Treatment modalities that can substantially reduce visual loss have been developed and extensively validated; however, these therapies are not yet ideal and active research is continuing into methods of curing or preventing DR. Until these milestones are reached, current strategies must continue to address the critical need for regular eye examination, optimal systemic control and prompt, appropriate laser photocoagulation, intravitreal anti-VEGF therapy, and/or vitrectomy when indicated.

ADA Guidelines [116, 152]

1. Patients >10 years of age with type 1diabetes should have an initial dilated and comprehensive eye examination by an ophthalmologist or optometrist within 3–5 years after the onset of diabetes. In general, screening for diabetic eye disease is not necessary before 10 years of age. Patients with type 2 diabetes should have an initial dilated and comprehensive eye examination by an ophthalmologist or optometrist shortly after the diagnosis of diabetes is made.
2. Subsequent examinations for both type 1 and type 2 diabetic patients should be repeated annually by an ophthalmologist or optometrist who is knowledgeable and experienced in diagnosing the presence of diabetic retinopathy and is aware of its management. Examinations will be required more frequently if retinopathy is progressing.
3. When planning pregnancy, women with preexisting diabetes should have a comprehensive eye examination and should be counseled on the risk of development and/or progression of diabetic retinopathy. Women with diabetes who become pregnant should have a comprehensive eye examination in the first trimester and close follow-up throughout pregnancy. This guideline does not apply to women who develop gestational diabetes because such individuals are not at increased risk for diabetic retinopathy.
4. Patients with any level of macular edema, severe NPDR, or any PDR require the prompt care of an ophthalmologist who is knowledgeable and experienced in the management and treatment of diabetic retinopathy. Referral to an ophthalmologist should not be delayed until PDR has developed in patients who are known to have severe non-proliferative or more advanced retinopathy. Early referral to an ophthalmologist is particularly important for patients with type 2 diabetes and severe NPDR, since laser treatment at this stage is associated with a 50% reduction in the risk of severe visual loss and vitrectomy.
5. Patients who experience vision loss from diabetes should be encouraged to pursue visual rehabilitation with an ophthalmologist or optometrist who is trained or experienced in low-vision care.

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Chapter 16
Microcirculation of the Diabetic Foot

Ying Zhang, Ikram Mezghani, and Aristidis Veves

Introduction

Diabetic foot problems are major contributors to health care costs and hospitalizations. A complete understanding of how the disease process works is essential in learning how to best prevent and treat these complications. Foot ulceration affects 6% of diabetic patients per year and can lead to lower extremity amputation, a major risk for death [1, 2], and is one of the costliest complications of diabetes [3]. DFU is more prominent in middle-age male patients but it also affects a large number of the older patients and, as the morbidity improves in the diabetic population, mainly due to reduction in cardiovascular disease, the percentage of the affected older population is increasing [4, 5].

Abnormalities of the microcirculation are generally accepted as early changes in diabetes [6–12]. Eventual manifestations of altered microcirculation, such as retinopathy, nephropathy, and neuropathy, are related to the duration and severity of diabetes [13–15]. Intensive glycemic control was found in the Diabetes Control and Complications Trial (DCCT) to significantly delay the development and progression

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of these microvascular complications in type 1 diabetic patients, with similar results reported in type 2 diabetic patients in the United Kingdom Prospective Diabetes Study (UKPDS) [15–18]. The capillary microcirculation to foot skin is no exception and has shown signs of significant impairment in diabetic patients, especially when metabolic control is poor [19]. This chapter will focus on the changes that occur in the microcirculation of the diabetic foot and the different methods used for their evaluation.

Anatomy of Skin Morphology and Microcirculation

The morphological structure of the skin consists of two layers: the outer epidermis layer and the inner dermis layer. The epidermis layer contains keratin, has no blood supply, and is fed by the dermal papilla layer. The dermis is composed of papillary and reticular layers of collagen and elastic fibers. It consists of a network of microcirculation that provides nutrients to tissues and removes waste [20]_ENREF_20. The skin microcirculation consists of nutrient capillary blood flow and body temperature regulatory arteriovenous shunt. It is organized into two horizontal plexuses: the superior–inferior papillary plexus, and the inferior cutaneous plexus. The entire vascular network of the skin varies greatly from one area to another. It is estimated that in the normal foot, a large amount of the total cutaneous blood flow circulates through the arteriovenous shunt, while the remaining blood passes through the further vegetative capillary bed [20]. The nutrient capillaries are organized into successful energy units, and each dermal nipple is supplied by a capillary ring [21]. Since the exchange of nutrients and metabolites between blood and tissues occurs at the capillary level, the integrity of the capillary circulation has a significant impact on the health of the entire skin.

Regulation of Skin Blood Flow

Skin blood flow (SBF) regulation plays an important role in ensuring a consistent blood supply to the various cells and tissues. A balanced blood flow in the skin's microcirculatory system maintains nutrient supply, removes biological and cellular waste products, plays a role in regulating blood pressure and thermoregulation, and is involved with effective wound healing [22]. Local SBF is regulated by the sympathetic nervous system, which controls the vasoconstriction and vasodilation of the blood vessels by the central neural reflex, the nerve axon reflex, and the local sympathetic venoarteriolar axon reflex.

The arteriovenous anatomic structure has thick walls and low resistance, which enables the blood to flow directly from the arterioles to the venules at a high speed. In glabrous skin, which is skin that does not contain hair follicles and is mainly found at the palm of the hands and plantar aspect of the feet, there is a large amount of arteriovenous anatomy heavily innervated by sympathetic vasoconstrictor. In

contrast, there is little to no arteriovenous anastomoses in non-glabrous skin that is innervated by sympathetic vasoconstrictors [23].

Thermoregulation in the glabrous skin is achieved by numerous arteriovenous anastomoses, which allow direct communication between arterioles and venous plexuses and cause the diversion of large amounts of blood from the skin capillaries, resulting in heat loss and bypassing nutrients from the capillaries [24]. As discussed later, this can be harmful in the contexts of diabetic skin nutrition [25].

The Concept of "Small Vessel Disease"

For the purpose of clarity in discussing microcirculation, the concept of "small vessel disease" should be defined. Early retrospective pathological studies in diabetic patients who underwent amputation led to the misconception of occlusive lesions in the foot medium and/or small arteries, the so-called "small vessel disease" [26]. It was hypothesized that occlusive "small vessel disease" occurs even in the absence of any lower extremity macrovascular occlusive disease and contributes to impaired DFU healing [26]. This concept originated from the histological existence of periodic acid-Schiff-positive material occluding the medium-sized or small-sized arteries in amputated limb specimens [26]. However, subsequent studies demonstrated the absence of such occlusive lesions in medium or small-sized arteries [27–29]. It should be emphasized that the term "small vessel disease" initially referred to medium or small-sized arteries and not to the microcirculation, which is composed of arterioles, capillaries, and venules. Therefore, the lack of any occlusive lesions in medium or small foot arteries does not imply that there are no abnormalities in the foot microcirculation.

Structural Changes of the Foot Microcirculation

Metabolic alterations in diabetes cause both structural and functional changes in multiple areas within the arteriolar and capillary systems [30, 31]. The most characteristic structural changes of the capillary circulation in diabetic patients are a reduction in the capillary size and thickening of the basement membrane [32, 33]. Skin capillary density in diabetics, on the other hand, does not differ from that of healthy subjects [34]. These changes in capillary size and basement membrane thickness are more pronounced in the legs. This phenomenon is most likely due to the higher hydrostatic pressure in the lower extremities, especially in diabetic patients with poorly controlled blood sugar levels [35].

It is believed that increased hydrostatic pressure and shear force in the microcirculation evoke an injury response in the microvascular endothelium. This injury may result in an increased elaboration of the extravascular matrix proteins leading to capillary basement membrane thickening and arteriolar hyalinosis [36, 37]. Thickened membranes impair the migration of leukocytes and hamper the

hyperemic response to injury, increasing the susceptibility of the diabetic foot to infection [29, 30, 38, 39]. These structural modifications also decrease the elastic properties of the capillary vessel walls, limiting their capacity for vasodilatation, and may eventually result in a significant loss of the autoregulatory capacity [40]. It is of interest that these changes do not result in narrowing or occlusion of the capillary lumen; on the contrary, some investigators initially reported that the arteriolar blood flow may be normal or even increased [41]. Nonetheless, more recent studies reported reduced capillary density, reduced lumen area due to remodeling, and increased arteriolar occlusion in the presence of critical limb ischemia [42]. However, it is doubtful whether these changes have any pathophysiological consequences in the lower extremity skin capillary blood flow [21, 43].

Functional Changes of the Microcirculation

In addition to the structural changes wrought by diabetes on the microcirculation, techniques that allow the measurement of skin blood flow have highlighted functional disturbances as well. Using these techniques, researchers have observed that diabetic patients have reduced maximal hyperemic response to heat, even in the early stages of the disease [37, 44]. The idea that impaired capillary microcirculation could be a major contributing factor in the development of diabetic foot pathology has encouraged more in-depth research in this direction [29, 38]. Further development of new techniques to evaluate the microcirculation to peripheral tissues has expanded the understanding of these functional changes and their role in altering the microvascular blood flow. Before discussing the changes in vascular reactivity, it would be of particular importance to review the different techniques currently used for evaluating the microcirculation.

Methods of Evaluating the Microcirculation of the Feet

Measurements of Capillary Blood Flow Using Laser Doppler Flowmetry

Currently, this method is the most widely accepted technique for evaluating blood flow in the skin microcirculation. Basically, it measures the capillary flux, which is a combination of velocity and the number of moving red cells. This is achieved by employing red laser light that is transmitted to the skin through a fiber-optic cable. The frequency shift of light backscattered from the moving red cells beneath the probe tip is computed to give a measure of the superficial microvascular perfusion [39].

Laser Doppler flowmetry (LDF) employs a single-point laser probe, consisting of a transmitting and a receiving optical fiber, and can provide continuous measurements of flux that can detect fast changes. However, the variability is quite high as

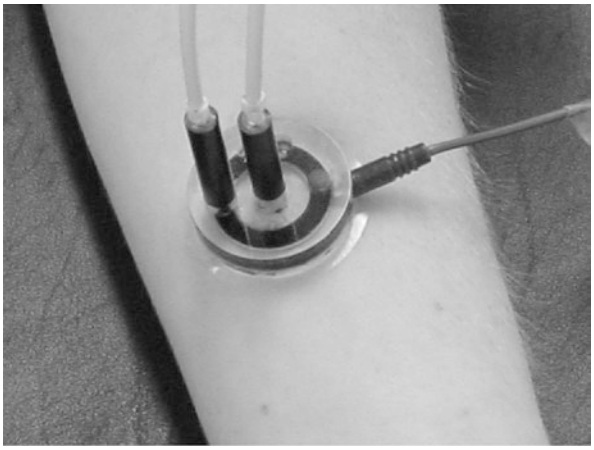


Fig. 16.1 Laser Doppler flowmetry: Measurements of direct and indirect effect of vasoactive substance using single-point laser probes: One probe is used in direct contact with the iontophoresis solution chamber (colored ring) and measures the direct response. The center probe measures the indirect response (nerve axon-related effect). A small quantity (<1 ml) of 1% acetylcholine chloride solution or 1% SNP solution is placed in the iontophoresis. A constant current of 200 mA is applied for 60 s achieving a dose of 6 mC/cm^{-2} between the iontophoresis chamber and a second non-active electrode placed 10–15 cm proximal to the chamber (black strap around the wrist). This current causes a movement of solution to be iontophoresed toward the skin

it is affected by non-uniform skin blood flow and movement artifacts. In our unit, LDF is used mainly for evaluating the hyperemic response to a heat stimulus, or for evaluating the nerve axon-related hyperemic response. To assess heat-related hyperemic response, the baseline blood flow measurements are made first. The skin is then heated to 44 °C for 20 min using a small brass heater, following which the maximum blood flow is measured to evaluate the magnitude of change from baseline. To measure nerve axon-related hyperemic response, two single-point laser probes are applied (Fig. 16.1). One probe measures the blood flow to an area of skin, which is exposed directly to acetylcholine (Ach). The second probe, placed in close proximity (5 mm), measures the indirect effect of applied Ach. This indirect effect results from stimulation of C-nociceptive nerve fibers in the area and reflects the integrity of the nerve axon-related reactive hyperemia.

In contrast, Laser Doppler Imaging (LDI) scans large skin areas, which considerably reduces variability but fails to detect rapid flux changes [45]. Our unit employs this technique to evaluate the endothelium-dependent microvascular reactivity, which evaluates the magnitude of change in blood flow in response to Ach admitted to the skin through the iontophoresis technique and the endothelium-independent microvascular reactivity, which evaluates the magnitude of change in blood flow in response to sodium nitroprusside (SNP).

The iontophoresis technique is used to apply these vasoactive substances to a localized area of the skin. In this technique, a delivery vehicle device is attached firmly to the skin with double-sided adhesive tape. The device contains two

chambers that accommodate two single-point laser probes. A small quantity of less than 1 ml of 1% Ach solution or 1% of SNP solution is placed in the iontophoresis chamber and a constant current of 200 mA is applied for 60 s, achieving a dose of 6 mC/cm^{-2} between the iontophoresis chamber and a second non-active electrode placed 10–15 cm proximal to the chamber. This current causes a movement of solution to be iontophoresed toward the skin, resulting in vasodilatation.

After the adhesive device has been removed, the localized area exposed to either of the vasoactive substances is scanned. The laser Doppler perfusion imager sequentially scans an area of skin and produces a color-coded image of skin erythrocyte flux on a computer monitor (Fig. 16.2). This technique is best suited for studying the

Fig. 16.2 Laser Doppler imaging: A laser beam is emitted from the laser source to sequentially scan a chosen skin area. We employ the scanner to evaluate the area in which hyperemia is produced by the iontophoresed vasoactive substance on the volar surface of the forearm

relative changes in flow induced by a variety of physiological maneuvers or pharmaceutical intervention procedures.

Laser Speckle Contrast Imaging (LSCI) is often employed as an alternative to LDI. LSCI is based on the quantification of the speckle pattern, which represents dynamic changes in backscattered light as a result of interaction with red blood cells. Its main advantage is that it combines the spatial variability of LDI with the good temporal variability of LDF [46]. However, when the iontophoresis technique is used, maximal vasodilation lasts for several minutes and as a result, temporal variability is not a major problem.

Measurements of Transcutaneous Oxygen Tension ($TcPO_2$)

The technique of measuring oxygen tension transcutaneously is based on the fact that oxygen is capable of diffusing through tissue and skin. Although the rate of diffusion is very low at normal surface body temperature, the application of heat to a localized area sufficiently enhances the flow of oxygen through the dermis to allow non-invasive measurement of capillary oxygen level. The measurements are affected by the affinity of blood for oxygen and the tissue properties, as well as the change in skin temperature. These factors may influence, to some extent, the accuracy of these measurements.

Capillaroscopy

This is one of the most sensitive and accurate methods of assessing skin microcirculation at the micro-level by visualizing the density, morphology, and blood flow in capillaries using real-time video technology in vivo. With good spatial resolution, it can be directed to a single capillary and allows the measurement of the capillary's structure and blood flow. Furthermore, a particular capillary area can be measured for its diameter, length, and density. However, it has measuring limitations when applied to the nail bed and other peripheral skin areas, it can only measure the nutrient capillaries, it needs high image quality to give effective measurements, and it is relatively complex and time consuming [47].

Measurements and Quantifying Tissue Oxygenation Using Hyperspectral Imaging

Hyperspectral imaging (HSI) is based on optical spectroscopy. Since the absorption spectra of many tissue chromophores are known, the characteristics of tissues can be measured according to the content of tissue chromophores [48–50]. This measures the dermal blood volume and oxygen saturation. Deoxyhemoglobin has an absorption peak near 554 nm, while oxyhemoglobin has two absorption peaks near 542 nm and 578 nm [51]. Based on these differences, spectral measurements can be

employed to evaluate local oxygen saturation in tissues. HSI has shown early promise and may be important in monitoring the healing and development of DFUs and in predicting ulcer risk [52, 53]. However, conflicting results have also been obtained, possibly due to the technique measuring oxygenation in total skin blood flow [50]. Nonetheless, given its non-invasive nature and easy to use, this is one of the most promising new techniques and further standardization and improvement have the potential to be widely adopted in clinical practice [54].

Photoacoustic Imaging

Photoacoustic Imaging (PAI), also known as optoacoustic imaging, is an imaging technique based on optically induced ultrasound imaging of tissue. When tissue is illuminated by a short laser pulse, typically between 5 and 100 ns, locally absorbed light generates a temperature rise. As this occurs within a very short period of time, the local mechanical stress generates a thermoelastic effect, which leads to the emission of sound waves [55, 56]. These sound waves can be detected as ultrasonic waves, and they can be used in conjunction with reconstruction algorithms to visualize where light absorption occurs. Because the light absorption coefficients of different tissue types depending on the wavelength of the laser used, an appropriately selected wavelength can be used to target a specific type of tissue, such as oxyhemoglobin or deoxyhemoglobin, melanin, or water. For example, by measuring ultrasonic responses to light pulses of different wavelengths, it is possible to locate blood vessels with high oxygen hemoglobin or high deoxyhemoglobin levels and to distinguish between arteries and veins.

Changes in Vascular Reactivity

The classic description of the diabetic neuropathic foot as warm and red with palpable pulses and distended veins points to a possibility of increased blood flow in the affected limb. Studies to explore this presentation found that the blood flow in the nutritional skin microcirculation is stable or even reduced, indicating a functional ischemia of the skin microcirculation and maldistribution of blood flow to the foot [19, 57]. It was also suggested that both structural and functional changes in the skin microcirculation result in a significant shifting of the blood flow away from nutritional capillaries to subpapillary arteriovenous shunts of a much lower resistance. As these shunts are innervated by sympathetic nerves, coexisting autonomic neuropathy and sympathetic denervation, which occur in diabetic patients with severe neuropathy, may lead to an opening of these shunts, augmentation of the maldistribution of blood between the nutritional capillaries and subpapillary vessels, and consequent aggravation of microvascular ischemia

[58, 59]. Studies using venous occlusion plethysmography, Doppler sonography and venous oxygen tension measurements support this concept [60]. These disturbances in nutritive microcirculation may be of importance in the development of diabetic foot complications and may help explain why the diabetic foot is more susceptible to the effect of pressure and has an impaired ulcer healing process.

Functional Changes

Measuring capillary blood flow by laser Doppler flowmetry has enabled the evaluation of endothelial function in diabetic limbs more precisely. Early application of this technique showed a reduced hyperemic response to heat stimulus and pointed to the role of endothelial dysfunction as the cause of the impaired vascular reactivity at microcirculatory level [44, 58]. Such dysfunction was shown to occur early in the course of diabetes and may even predict diabetic micro- and macrovascular complications [61, 62]. Endothelial dysfunction was also reported in patients with impaired glucose tolerance and in relatives of type 2 diabetic patients [63].

To evaluate the relation between changes in microcirculation and neuropathy in the presence or absence of peripheral vascular disease, the skin microcirculation of foot was thoroughly investigated using both single-point laser imaging and laser scanning techniques in five groups [43]. The first group included diabetic patients with neuropathy (DN), the second group included diabetic patients with both neuropathy and peripheral vascular disease (DI), the third group included diabetic patients with Charcot arthropathy (DA), the fourth group included diabetic patients without complications (DC), and the fifth group included healthy control subjects (C). As shown in Fig. 16.3a, the percentage of increase in blood flow over baseline in response to heating the skin to 44 °C was reduced in the diabetic neuropathic and ischemic patients (DN, DI), whereas no difference existed among the remaining three groups. On the other hand, the endothelium-dependent vasodilatation (response to iontophoresis of Ach) was reduced in diabetic patients with neuropathy, vascular disease, and arthropathy. The endothelium-independent vasodilatation (response to iontophoresis of SNP) was more severely reduced in the ischemic-neuropathic patients compared with other groups and was reduced in the neuropathic groups with or without Charcot disease compared to the controls (Fig. 16.3b). These findings pointed to the close association between diabetic neuropathy and microcirculatory impairment in the form of reduced endothelium-dependent and endothelium-independent vasodilation at the foot level even in the absence of large vessel peripheral vascular disease. They also implied that the presence of neuropathy may be an important contributing factor as the coexistence of neuropathy and peripheral vascular disease did not result in a greater decrease in endothelium-dependent vasodilation than that due to neuropathy alone.

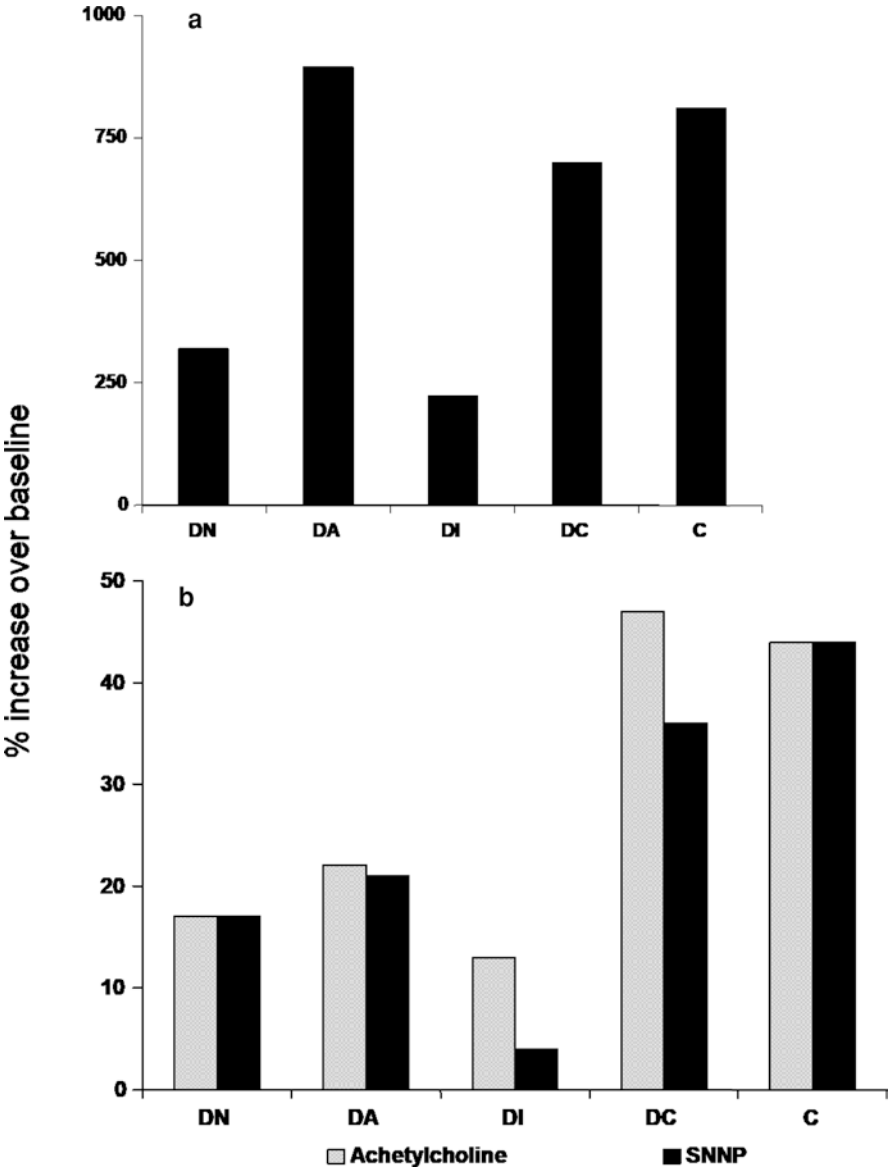


Fig. 16.3 (**a**) The maximal hyperemic response to heating of foot skin at 44 °C for at least 20 min (expressed as the percentage of increase over baseline flow measured by a single-point laser probe) is reduced in the diabetic with neuropathy (DN) and in diabetic patients with neuropathy and peripheral vascular disease (DI) when compared with diabetic patients with Charcot arthropathy (DA), diabetic patients without complications (DC), and normal control subjects (C) ($p < 0.001$). (**b**) The response to iontophoresis of acetylcholine and SNP (expressed as the percentage of increase over baseline flow measured by laser scanner). The response to acetylcholine is equally reduced in the DN, DI, and DA groups when compared with the DC and C groups ($p < 0.001$). The response to SNP was more pronounced in the DI group and also reduced in the DN and DA groups compared with the DC and C groups ($p < 0.001$)

The Role of the Nerve Axon Reflex in Vasodilation

In healthy subjects, the ability to increase blood flow depends on the existence of normal neurogenic vascular response. The normal neurovascular response is conducted through the C-nociceptive nerve fibers. Stimulation of these nerve fibers leads to antidromic stimulation of adjacent C-fibers, which secrete substance P, calcitonin gene-related peptide (CGRP), and histamine, causing vasodilatation and increased blood flow to the injured tissues, thereby promoting wound healing; Lewis' triple flare response (Fig. 16.4). In cases of diabetic neuropathy, this neurovascular response is impaired, leading to a significant reduction of blood flow under conditions of stress, such as injury or infection, and increasing the vulnerability of the neuropathic limb to severe diabetic foot problems [64, 65].

Evidence that diabetic neuropathy contributes to vasodilatory impairment is provided by studies in our lab that used the previously described single-point laser probe technique to evaluate the nerve axon-related vasodilatory response. We found that the indirect response to iontophoresis of Ach was significantly reduced in diabetic patients with neuropathy, diabetic patients with neuropathy and peripheral vascular disease, and diabetic patients with Charcot arthropathy, when compared with healthy subjects or diabetic patients without complications [66, 67] (Fig. 16.5). Further evidence is provided by a study designed to evaluate the role of the

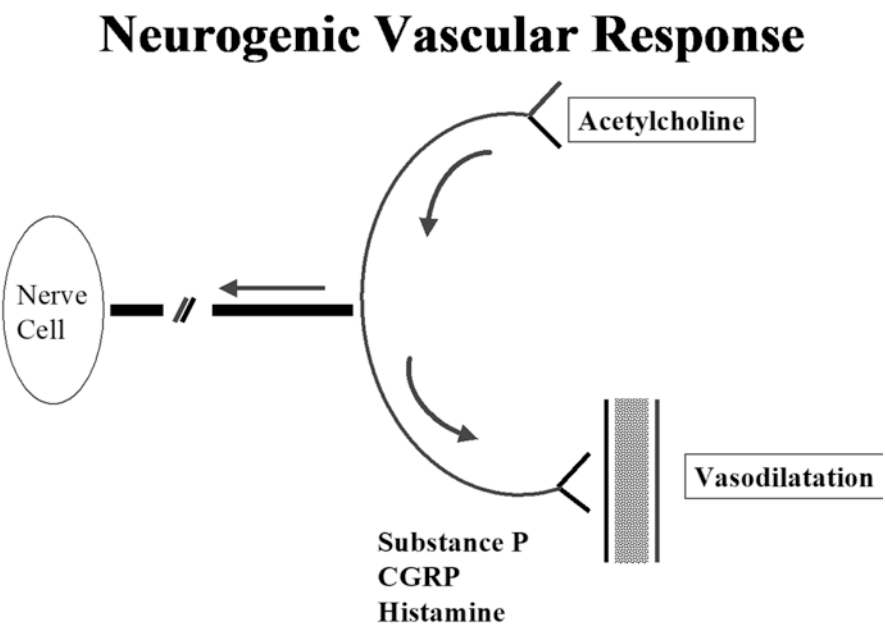


Fig. 16.4 Stimulation of the C-nociceptive nerve fibers leads to antidromic stimulation of the adjacent C fibers, which secrete substance P, CGRP, and histamine that cause vasodilatation and increased blood flow

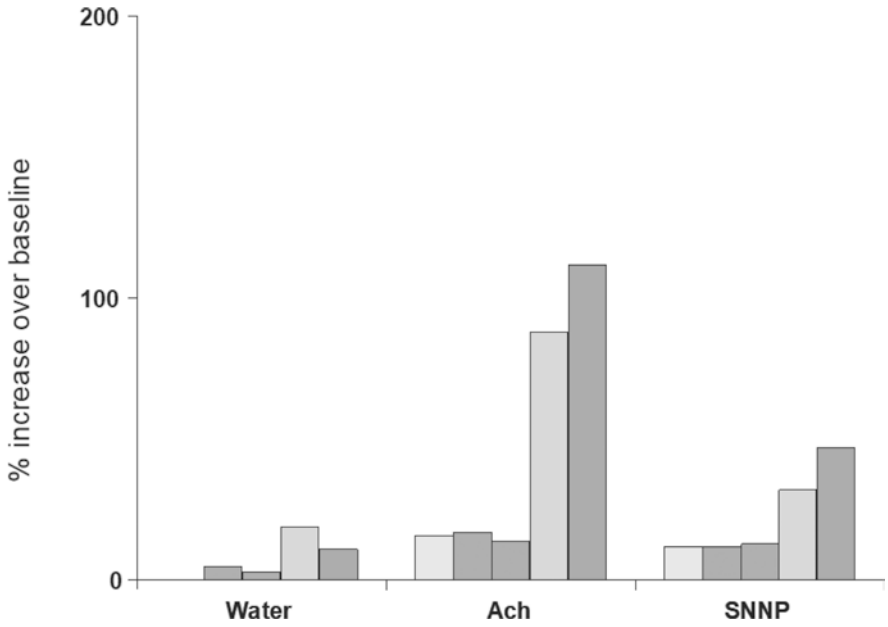


Fig. 16.5 The response of blood flow (expressed as the percentage of increase over baseline flow measured by a single-point laser probe) in a skin area adjacent to, but not in direct contact with, the iontophoresis solution. During the iontophoresis of deionized water, a mild response is observed in all groups. In contrast, during iontophoresis of acetylcholine (Ach), the response is reduced in diabetic patients with neuropathy (DN), diabetic patients with neuropathy and peripheral vascular disease (DI), and diabetic patients with Charcot arthropathy (DA), when compared diabetic patients without complications (DC) and normal control subjects (C) ($p < 0.001$). *A similar response is observed during iontophoresis of SNP, but is less than half when compared with the response achieved with Ach*

C-nociceptive nerve fibers in nerve axon reflex-related vasodilation. In this study, nerve axon reflex-related vasodilation was measured in three groups: diabetic neuropathic, diabetic non-neuropathic, and healthy control. Measurements were first taken on the forearm and the foot of each subject. Then, after blocking the C-nociceptive nerve fibers with dermal anesthesia, measurements were repeated. A clear reduction in nerve axon reflex-related vasodilation occurred in all three groups on the forearm but only in the two non-neuropathic groups on the foot, indicating that C-nociceptive fiber function is the main factor that influences nerve axon reflex-related vasodilation [68] (Fig. 16.6).

The contribution of the nerve axon reflex-related vasodilatation response to the total endothelium-dependent and endothelium-independent vasodilation was also studied in a group of diabetic patients versus a control group at both forearm and foot level [69]. The nerve axon-related response in healthy subjects was found to be 35% of the total response at the forearm level and 29% at the foot level (Fig. 16.7). In contrast the response to SNP, a substance that does not specifically excite the C-nociceptive fibers was 13% and 12%, respectively, indicating that the presence of

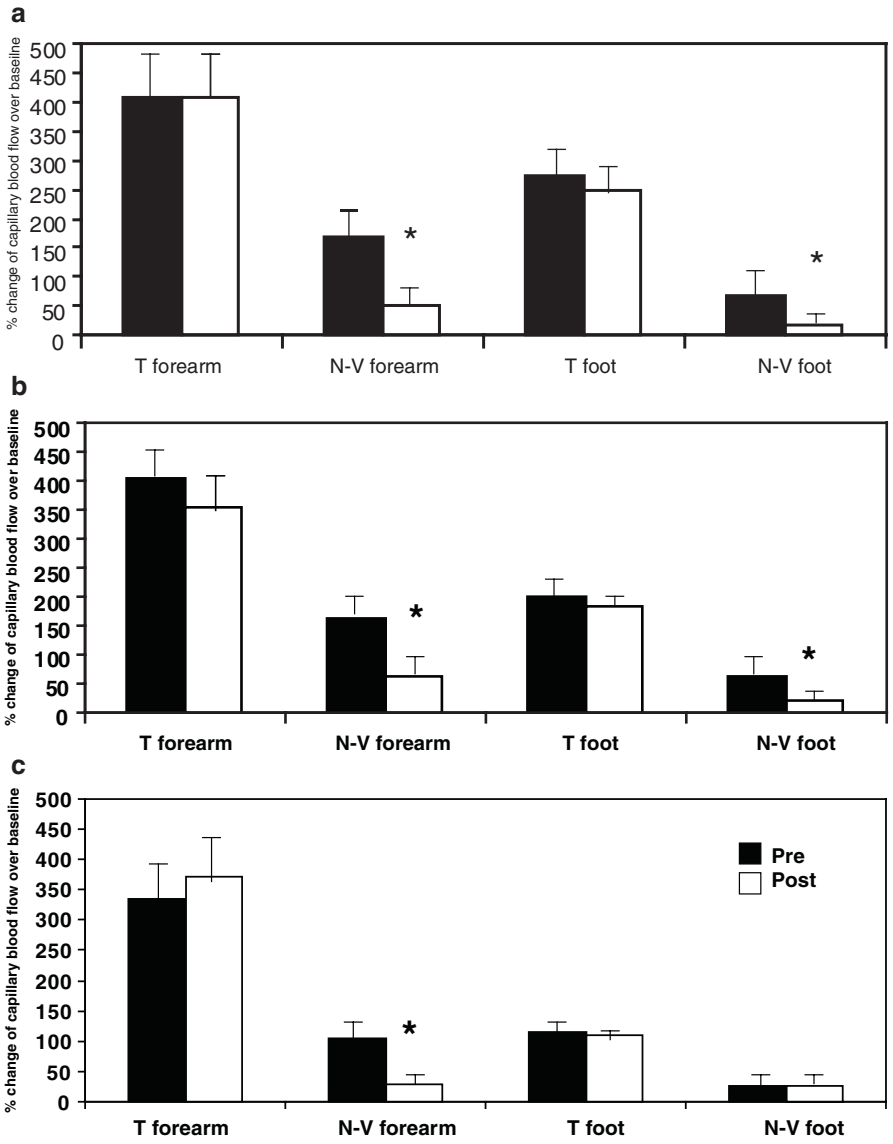


Fig. 16.6 Total and nerve axon reflex-related vasodilatory response to acetylcholine before (black columns) and after (white columns) the application of local anesthesia in healthy subjects (**a**), non-neuropathic diabetic patients (**b**), and diabetic neuropathic patients (**c**)

a non-specific galvanic response may also be implicated (Fig. 16.8). In the presence of neuropathy, the response significantly reduced at a level of only 8% of the total response. These findings indicate that although the neurovascular response is an

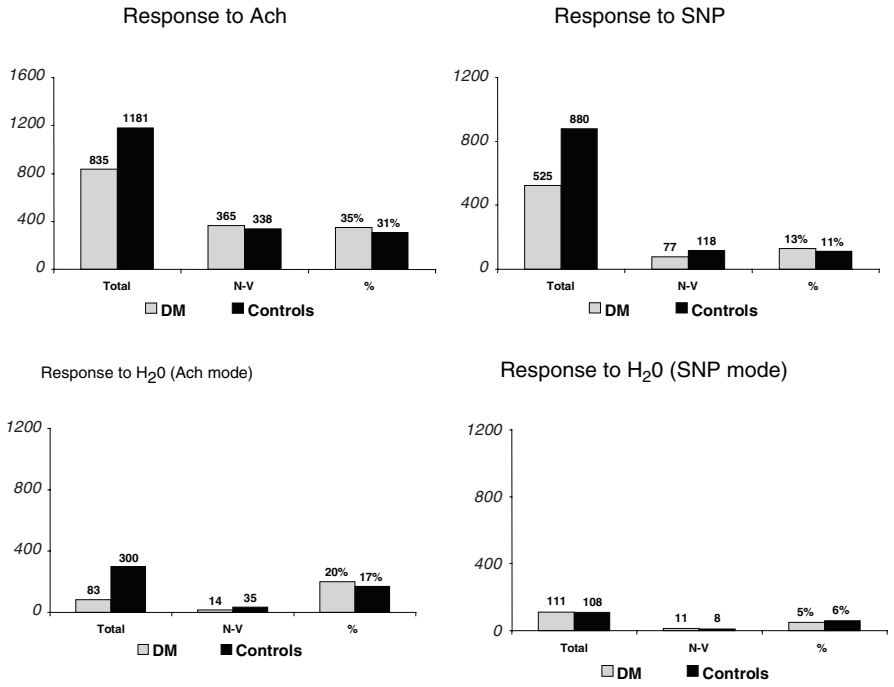


Fig. 16.7 The total and the nerve axon-related vasodilatation in the upper extremities in response to acetylcholine (Ach), SNP and deionized water (H_2O) in a group of diabetic patients versus a control group of healthy subjects. The contribution of nerve axon-related response to the total response to Ach is 35% in diabetic patients and 31% in control group ($p > 0.05$) *and the contribution of nerve axon-related response to the total response to SNP is 13% in diabetic patients and 11% in control group* ($p > 0.05$)

important factor in skin microcirculation function, it is not the sole or dominant pathway through which vasodilation is achieved [64].

The abnormality in nerve axon-related vascular reactivity is believed to further aggravate the abnormalities in the microcirculation and contribute to a vicious cycle of injury [51]. It becomes apparent that involvement of C-nociceptive fibers in diabetes not only leads to impaired pain perception but also to impaired vasodilation under condition of stress, such as injury or inflammation.

Differences Between Forearm and Foot Microcirculation

As mentioned previously, erect posture may lead to differences in the microcirculation at the foot level when compared to other parts of the body that are closer to the heart and therefore have a reduced hydrostatic pressure. In order to test this hypothesis, we have examined the differences in the foot and forearm skin microcirculation

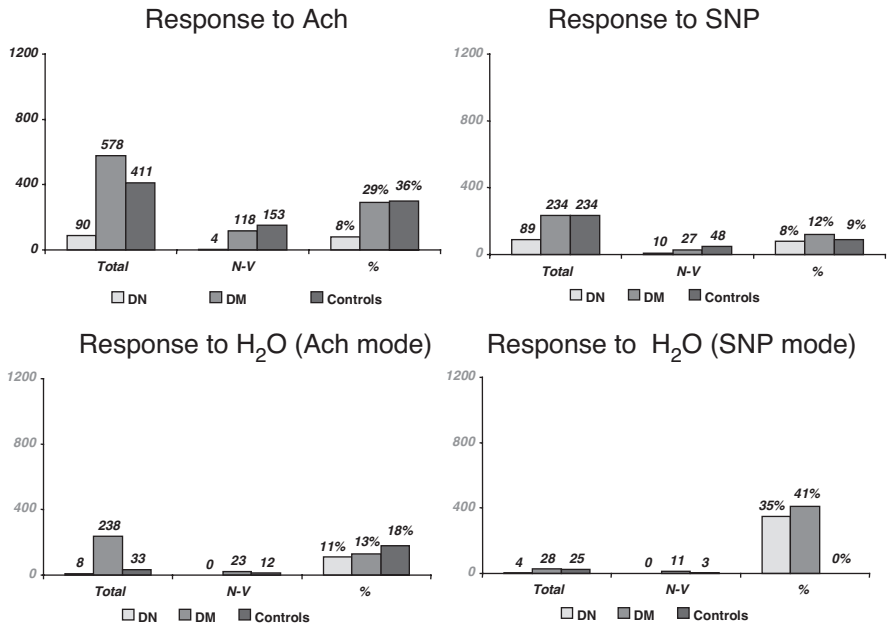


Fig. 16.8 The total and the nerve axon-related vasodilatation in the lower extremities in response to acetylcholine (Ach), SNP and deionized water (H_2O) in a group of diabetic patients with (DN) or without (DM) neuropathy versus a control group of healthy subjects. The contribution of nerve axon-related response to the total response to Ach is 8% in DN, 29% in DM, and 36% in the control group (*$p < 0.001$ between DN* versus *DM and controls) and the contribution of nerve axon-related response to the total response to SNP is 8% in DN, 12% in DM, and 9% in the control group ($p > 0.05$ between DN* versus *DM and controls)*

in diabetic patients with or without neuropathy and healthy subjects [66] [54]. No differences were found in the maximal hyperemic response between forearm and foot level, although the response in the neuropathic group was significantly lower at both levels in comparison to the diabetic non-neuropathic and the healthy control subjects (Fig. 16.9). The endothelium-dependent and endothelium-independent vasodilatation was significantly lower at the foot level when compared to the forearm level in both healthy controls and in diabetic patients with or without neuropathy (Fig. 16.10). In addition, the neuropathic group showed a significantly lower response at both forearm and foot levels when compared to the non-neuropathic and control groups. Evaluation of the nerve axon-mediated vasodilatation response also revealed a significantly lower response at the foot level versus the forearm level in the three groups (Fig. 16.11). These results indicate that the microcirculation at the foot level is compromised even in healthy subjects when compared to the forearm level. The presence of diabetes may further compromise the microcirculation to a level that creates a hypoxic environment and allows the development of neuropathic changes. These factors may also explain why neuropathy initially occurs in the lower extremities of diabetic patients [58, 59].

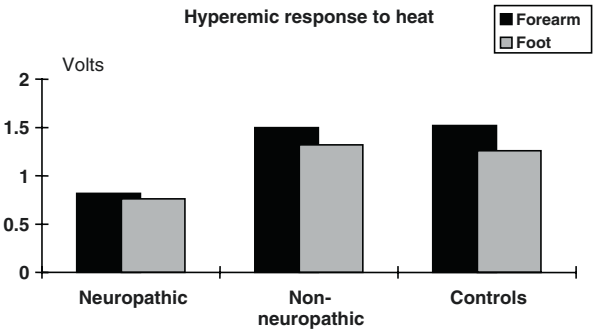
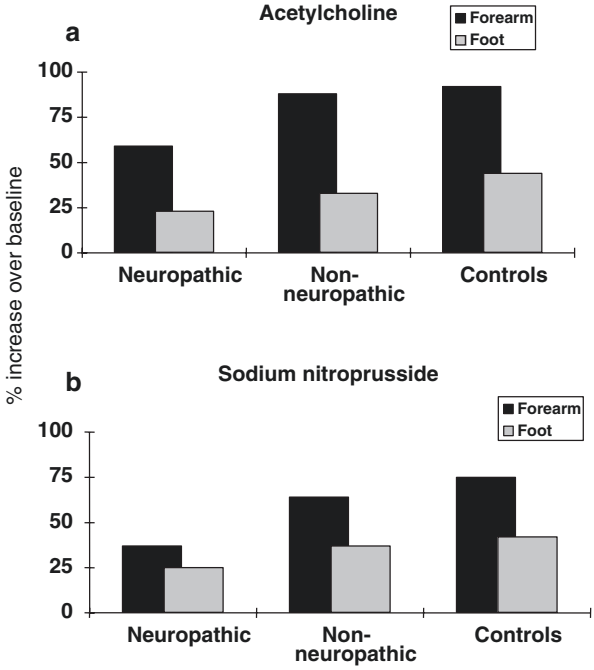


Fig. 16.9 The hyperemic response to heat stimulus (expressed as the percentage of increase over baseline flow measured by single-point laser probe) at forearm versus foot level in diabetic patients with or without neuropathy and in healthy control subjects. No difference was observed between the forearm and foot in any of the three groups. The response in neuropathic group is significantly lower compared with the other two groups at both forearm and foot level ($p < 0.001$)

Fig. 16.10 The response to iontophoresis of acetylcholine (**a**) and SNP (**b**) (expressed as the percentage of increase over baseline flow measured by laser scanner) at forearm versus foot level in diabetic patients with or without neuropathy and in healthy control subjects. The response at the foot level is significantly lower than that of the forearm in all groups ($p < 0.01$). *The response in neuropathic group is significantly lower compared with the other two groups at both forearm and foot level* ($p < 0.05$)



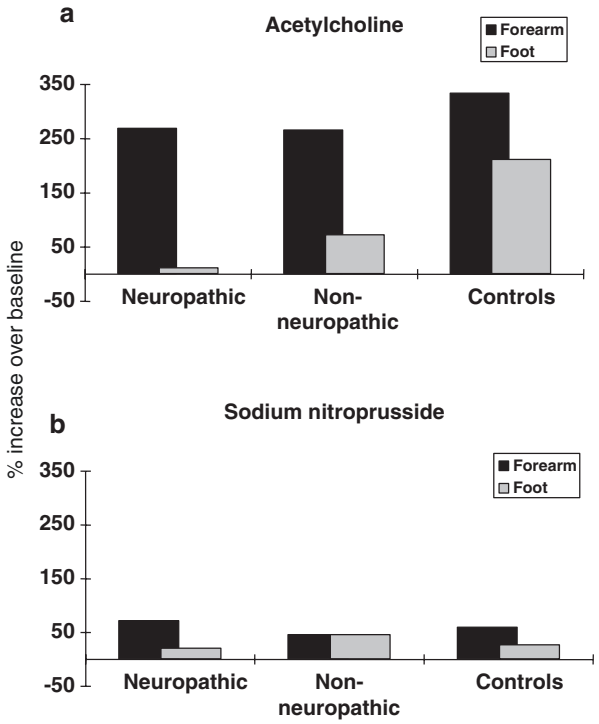


Fig. 16.11 The axon reflex-mediated vasodilatation related to the C-nociceptive fibers (expressed as the percentage of increase over baseline flow measured by two single-point laser probe) at forearm versus foot level in diabetic patients with or without neuropathy and in healthy control subjects. The response to acetylcholine, which directly stimulates the C fibers, is lowest in the neuropathic group, but is also reduced in non-neuropathic group ($p < 0.001$), *while no differences are found at the forearm level. A much smaller response is observed during the iontophoresis of SNP (non-specific stimulus) at both foot and forearm level and is smaller in all three groups*

Microvascular Changes in Diabetic Foot with Charcot Arthropathy

The diagnosis of Charcot neuroarthropathy is made when gross destruction of the joints of the mid-foot results in significant foot deformity. The skin temperature of Charcot feet is usually higher due to increased blood flow in arteriovenous shunts. Although the endothelial-dependent and endothelial-independent vasodilatation is impaired in Charcot patients, the maximal hyperemic response to heat is preserved

[43] (Fig. 16.4). These findings indicate that the hyperemic response in Charcot disease is present but is probably unregulated and results in excessive bone resorption. The final results of these changes are complete joint destruction and gross deformity of the foot shape. These findings are consistent with clinical observations that the development of Charcot neuroarthropathy is extremely rare in the presence of peripheral vascular disease. Poor blood flow to the extremity would prevent much of a hyperemic response, protecting the foot from bone resorption and deformation, though certainly contributing to other microcirculatory derangement.

Conclusions

Microcirculation to the diabetic foot suffers multiple significant structural and functional changes. Nerve axon-related microvascular reactivity is clearly impaired in the diabetic population. There is a growing belief that both the failure of the dysfunctional vessels to dilate and the impairment of the nerve axon reflex are major causes for impaired wound healing in diabetic patients. Further studies are required to clarify the precise etiology of observed endothelial dysfunction in diabetic patients and to identify the possible potential therapeutic interventions to prevent it or to retard its progression. Studies are also required to examine the vascular changes in the peripheral nerves, rather than in the skin.

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Chapter 17
Diabetic Nephropathy

Jennifer Kelly and Richard Solomon

Introduction

The prevalence of diabetes around the world is expected to reach 642 million people by 2040. About 40% of people with diabetes will develop chronic kidney disease (CKD), including a significant number who will develop end-stage kidney disease (ESKD) [1]. In contrast with the major developments in cardiovascular therapeutics, an entire generation has gone by with no new treatments to effectively stem the rising tide of kidney failure in people with diabetes [2]. The terms diabetic nephropathy (DN) and diabetic kidney disease (DKD) are often used interchangeably. DN is the classic term used for disease caused by hyperglycemia affecting the glomerulus, while DKD can be thought of as a more generic and widely encompassing term that includes disease outside of the glomerulus [3]. DKD is undoubtedly a worldwide medical catastrophe, with features of high prevalence, multifactorial pathogenesis, and lack of effective strategies in the treatment and management [4]**.** People with diabetes frequently die or become disabled due to micro- and macrovascular complications of the disease. Persistent microalbuminuria is an early sign of DKD as well as a prognostic marker for cardiovascular morbidity and mortality.

Much of our initial understanding about the progression of DKD comes from studying people with type 1 diabetes. However, once proteinuria develops in a person with diabetes, the course and progression of kidney disease are similar in both

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types [5]**.** Recent advancements in pathophysiology have led to a better understanding of the linkage between proteinuria, cardiovascular disease and DN. This improved knowledge helps to formulate a multidisciplinary cardio-reno-protective approach. Specific treatment of patients with diabetic nephropathy can be divided into four major areas: cardiovascular risk reduction, glycemic control, BP control, and inhibition of the renin–angiotensin–aldosterone system (RAAS). Newer classes of medications used to treat Type 2 Diabetes (T2D) have been shown to also lower both cardiac and renal complications [6]. A major advance over the past three years has been the identification of impressive renoprotective actions with some of the newer glucose-lowering agents. In this chapter, we will discuss the key factors involved in the pathophysiology of diabetic kidney disease and new developments in its management.

Pathogenesis

Diabetes is associated with a variety of microvascular and macrovascular complications. Kidney disease is a microvascular complication occurring in up to 40% of all patients with diabetes [7]. Diabetes is the leading cause of chronic kidney and diabetes is present in approximately 40% of patients reaching end-stage kidney disease. Despite the prevalence of this condition, therapies to mitigate kidney function loss are limited and will be reviewed below. A necessary precursor for future therapeutic interventions is an understanding of the pathogenesis of diabetic kidney disease (DKD).

First, it is well accepted that the development of DKD involves the interplay of many factors, some related to control of glycemia and some related to genetic disposition. This is why only 40% of patients with diabetes develop DKD despite a wide spectrum of glycemic control and why despite excellent glycemic control, some patient will nevertheless progress to end-stage kidney disease.

The relationship to glycemic control has been documented in seminal clinical trials of glycemic control in both Type 1 and Type 2 diabetes [8, 9]. It follows from these therapeutic trials that hyperglycemia must play a key permissive if not causative role. A number of pathways impacted by hyperglycemia have been suggested as mediating this relationship, including activation of the tubuloglomerular feedback (TGF) within the kidney resulting in upregulation of the renin–angiotensin–aldosterone system (RAAS), increases in advanced glycation end-products (AGE) interacting with the receptor for AGEs (RAGE), upregulation of inflammatory pathways, metabolic disturbances within mitochondria contributing to ischemia, and alterations in the coagulation system. Given the multiplicity of pathways contributing to DKD, it is accepted that a multitargeted approach to prevention of DKD is necessary, i.e., one size does not fit all. However, despite this compelling evidence, not all patients with diabetes develop DKD. It is in this domain that the role of genetic factors comes into play. A number of candidate gene polymorphism have

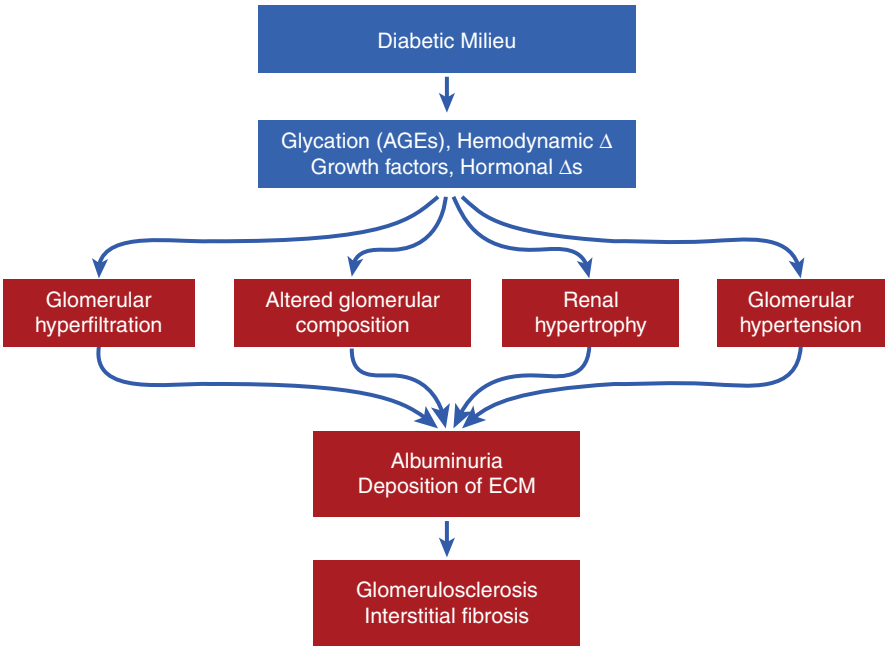

Fig. 17.1 Pathophysiology of diabetic kidney disease

been identified as associated with DKD, including polymorphisms involving the ACE enzyme and nitric oxide synthase (vide infra).

These pathophysiologic pathways (Fig. 17.1) must account for both the gross and microscopic histologic characteristics of DKD that include: enlarged kidney size, large glomeruli, glomerular basement membrane thickening, increased mesangial expansion with non-cellular material, and interstitial fibrosis. These pathologic characteristics in turn are related to the functional abnormalities that allow for clinical diagnosis of DKD: hyperfiltration, albuminuria, proteinuria, and progressive decline in glomerular filtration rate. In this review, we will attempt to make the connection among hyperglycemia, pathologic changes in the kidney, and clinical markers of kidney disease.

Tubuloglomerular Feedback [10, 11]

The kidney is able to control delivery of solute (sodium and chloride primarily) to the distal part of the nephron where the kidney "fine tunes" reabsorption to maintain salt and water homeostasis. A critical mechanism in this ability is tubuloglomerular feedback (TGF) in which the amount of sodium and chloride delivered to the macula densa (MD) at the beginning of the distal tubule is a determinant of the rate of

glomerular filtration in that same nephron. When an increase in delivery occurs, the cells of the MD release adenosine to constrict the afferent arteriole entering the glomerulus resulting in a decrease in glomerular hydrostatic pressure and GFR within that glomerulus. This reduces the solute delivery to the MD. When delivery is reduced, the afferent arteriole dilates and efferent arteriole constricts (a result of generation of angiotensin II), increasing glomerular hydrostatic pressure and GFR within that glomerulus. In the setting of hyperglycemia, more glucose is filtered by the glomerulus. This stimulates active uptake of sodium through the sodium–glucose cotransporters (SGLT1 and SGLT2) located on the luminal side of the proximal tubule. Chloride follows passively resulting in a decrease in the delivery of sodium and chloride to the MD. This activates TGF resulting in an increase afferent arteriole vasodilation, efferent vasoconstriction, rise in glomerular hydrostatic pressure, and an increase in GFR. On the whole kidney scale, this results in hyperfiltration (usually GFR >140 mL/min), mediated by a whole kidney rise in glomerular pressure (so-called glomerular hypertension). Hyperfiltration is thus an early marker of the effect of hyperglycemia on the kidney. It occurs in both Type 1 and Type 2 diabetes although because Type 2 diabetes is diagnosed much later in the course of the disease, it is often missed in that population. The sustained increase in capillary pressure within the glomerulus contributes to remodeling of these vessels with thickening of the basement membrane, expansion of the size of the capillary network, and glomerulomegaly.

Advanced Glycation End-Products (AGE) [12]

Perhaps one of the most studied of metabolic mechanisms in diabetes is the accumulation of AGEs. We use glycosylated hemoglobin to diagnose and follow the course of diabetes but many other proteins also bind glucose altering their metabolism. Structural proteins such a collagen and fibronectin become more resistant to degradation in the glycosylated state. This finding may account for the accumulation of structural proteins within the mesangium of the kidney and the basement membrane of the glomerulus that are characteristics of DKD.

AGE–RAGE Interaction [13]

The receptor for AGE (RAGE) is widely expressed and helps to clear AGEs from the body. All cells of the kidney express RAGE. The activation of the receptor stimulates a number of downstream pathways involving inflammation, fibrosis, and oxidative stress. The AGE–RAGE axis may play a direct role in promoting albuminuria. Activation of the AGE–RAGE axis results in an increase in heparinase by podocytes

within the glomerulus. This degrades the heparin sulfate which lines both sides of the glomerular basement membrane providing a negative charge barrier that restricts the movement of albumin (negatively charged) across the glomerular barrier. With loss of this negative charge barrier, albumin is able to cross from the capillary into the urinary space of Bowman's capsule and be excreted in the urine. Activation of the AGE–RAGE axis also stimulates the production of NF-kB in the kidney, a pivotal proinflammatory pathway, and profibrotic pathway involving TGF-B, fibronectin, and collagen (review). These pathways contribute to the increased production of matrix proteins that accumulate in the mesangium. AGE–RAGE also results in increase in NAPDH and nitric oxide synthase resulting in an increase in reactive oxygen species. This contributes to alteration in cell function leading to apoptosis. In particular, endothelial cell dysfunction occurs resulting in increased production of endothelin-1 which causes vasoconstriction (favoring hypertension) and promotes podocyte injury, inflammation, and fibrosis. These adverse effects on the endothelium are further magnified by hyperinsulinemia which increases ROS production and proinflammatory signaling mechanisms.

Mitochondrial Dysfunction [14]

Central to many of the signaling mechanisms that are activated in diabetes, dysfunction of the mitochondria plays a key role. Hyperglycemia disrupts the electron chain leading to increase ROS production and apoptosis. The mitochondrial dysfunction shifts metabolism away from aerobic to anaerobic pathways including the polyol pathway and the hexose monophosphate shunt pathway. Both pathways have been implicated in the development of DKD. Hyperglycemia downregulates AMPK, a kinase important for mitochondrial homeosis. Restoration of the function of that kinase is under clinical study for reducing progression of DKD.

Genetic Predisposition [15, 16]

A number of genome-wide association studies (GWAS) have identified insertion/deletion in the ACE gene and a gene for a structural protein (FRMD3) involved in maintaining cell shape in various nephron cells. It has been suggested that this latter protein works in conjunction with bone morphogenetic protein (BMP) which is vital for kidney development and repair from injury. The ACE insertion/deletion results in increased activity of the RAAS. Aldosterone activity is associated with increased fibrosis, oxidative stress, nephrosclerosis, and increase in collagen synthesis and deposition.

Nephropathy Staging (Fig. 17.2 and Table 17.1)

As noted above, the earliest functional change in DKD is hyperfiltration which can be present within weeks of development of hyperglycemia and can be sustained for many years. During this early stage, intermittent microalbuminuria may appear. Sustained microalbuminuria usually develops when GFR has started to fall but may still be in the normal range. By the time microalbuminuria becomes overt proteinuria, GFR has generally fallen further into the chronic kidney disease range (eGFR <60 mL/min). GFR loss progresses over the next years toward end-stage

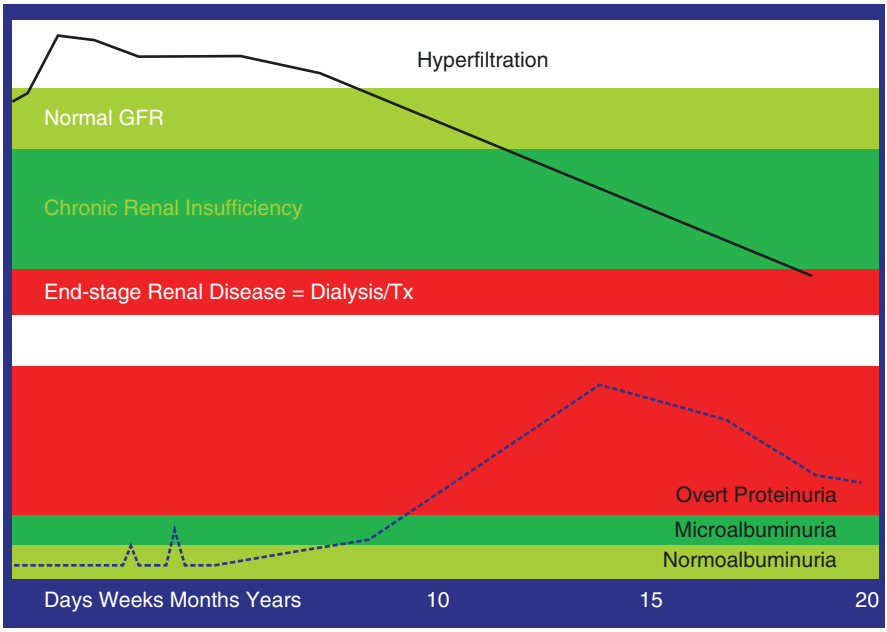

Fig. 17.2 Natural history of diabetic kidney disease (DKD). Top panel: Glomerular filtration rate; bottom panel: microalbuminuria and proteinuria

Table 17.1 Staging of diabetic nephropathy based upon anatomic and functional markers

	Anatomic	Functional
Stage 1	Glomerular basement membrane thickening	0–5 years, hyperfiltration or normal GFR, no albuminuria, increased kidney size (20%)
Stage 2	Mild–severe mesangial expansion	Normal GFR, intermittent albuminuria
Stage 3	Nodular sclerosis	5–10 years, with or without hypertension, microalbuminuria (30–300 mg/day)
Stage 4	Advanced glomerulosclerosis with vascular lesions	Irreversible proteinuria, sustained hypertension, eGFR <60 mL/min/1.73 m^2
Stage 5	End-stage sclerosis	End-stage kidney disease with eGFR <15 mL/min/1.73 m^2

kidney disease. If left untreated and accompanied by uncontrolled hypertension, rates of progression can be as high as 12 mL/min/year. With glycemic and blood pressure control, rates may fall to 3 mL/min/year. Elimination of proteinuria may actually stop progression in some patients.

Treatment to Prevent or Delay Progression of DKD

DKD typically develops after diabetes duration of 10 years in type 1 diabetes but may be present at the diagnosis of type 2 diabetes. DKD can progress to ESKD requiring dialysis or kidney transplantation and is the leading cause of ESKD in the US [17]. A number of key modifiable promoters of DKD have been identified. These include glycemic control, blood pressure, proteinuria, anemia, smoking, and dyslipidemia. Therapy targeted against these promoters for both primary and secondary prevention delays the progression of DKD. Assessing for progression to ESKD is difficult in studies due to its rare occurrence in people with more mild or moderate disease over a short period of time. As a result, composite outcomes including surrogate markers, such as doubling of serum creatinine or (more recently) 40% reductions in eGFR, have been accepted as appropriate outcomes that define the effects of interventions on long-term kidney risk [18]. We will review studies and individual aspects on the effects of optimizing different promoters and parameters along with their impact on preventing the progression of DKD.

Glycemic Control

Prevention of diabetic complications, particularly DKD, by long-term intensive glycemic control from early in the course of diabetes is well established for T1DM and T2DM. However, intensive glucose control after onset of complications or in long-standing diabetes has not been shown to reduce risk of DKD progression or improve overall clinical outcomes [19]. Hence, early intervention is best. Patients with diabetes mellitus (DM) usually have other risk factors for cardiovascular disease, such as hypertension and hyperlipidemia. Nonetheless, the higher rates of cardiovascular morbidity and mortality that are seen in patients with diabetic nephropathy cannot be explained solely by the presence of these traditional risk factors, and CKD has been shown to be an independent risk factor for cardiovascular disease [20]. The American Diabetes Association (ADA) recommends that treatment goals for hyperglycemia should be individualized based on diabetes duration, life expectancy, comorbidities, risk for hypoglycemia, and patient preferences and target an HbA1c of 7% or lower in most patients [21]. Intensive glycemic control with the goal of achieving near-normoglycemia has been shown in large prospective randomized studies to delay the onset and progression of albuminuria and reduced eGFR in patients with type 1 and type 2 diabetes [17].

Insulin alone was used to lower blood glucose in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study of type 1 diabetes, while a variety of agents were used in clinical trials of type 2 diabetes, supporting the conclusion that glycemic control itself helps prevent CKD and its progression [17]. The largest and longest trial performed in patients with type 2 diabetes, the landmark United Kingdom Prospective Diabetes Study (UKPDS), established that the incidence of microvascular complications including nephropathy decreased by 25% in patients with improved glycemic control [22]. This study involved the use of combinations of metformin, sulfonylureas, and insulin for treatment. Multiple studies involving the efficacy of improved glycemic control on long-term complications have since been performed with varying results.

The Action to Control Cardiovascular Risk in Diabetes Study Group (ACCORD) evaluated over 10,000 patients with type 2 diabetes, mean age of 62.2 years, and a median A1c level of 8.1%. Compared with standard therapy, the use of intensive therapy in this study to target normal A1c levels for 3.5 years increased mortality and did not significantly reduce major cardiovascular events, thus identifying a previously unrecognized harm of intensive glucose lowering in high-risk patients with type 2 diabetes [23]. A post-hoc analysis of the long-term ACCORD study (ACCORDION) investigated the impact of intensive BP control, antidiabetic therapy, and fenofibrate on renal and mortality outcomes. Intensive glycemic control reduced the risk of the composite kidney end points, mainly driven by a reduction in incident macroalbuminuria, but randomization to intensive BP control or fenofibrate resulted in an increased risk of the composite kidney outcome, driven entirely by creatinine doubling in both the BP control and fibrate use [18]. In contrast, both intensive BP therapy and fenofibrate were associated with a significantly higher incidence of serum creatinine doubling [20]. This analysis highlighted the imperfections of some of our valued outcome measures.

A large, prospective observational study indicated that intensive glycemic control played an important role in preventing deterioration of diabetic nephropathy. The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial showed that intensive glucose control can reduce development of microalbuminuria and macroalbuminuria [14]. Further analyses from the ADVANCE trial found a > 50% reduction in ESKD in the group randomized to intensive glycemic control that persisted out to 10 years [1]. The ADVANCE-ON study followed a total of 8494 of the ADVANCE participants for an additional median of 5.4 years. Data from the ADVANCE-ON trial suggest that intensive glycemic control (hemoglobin A1c <6.5%) was associated with a long-term reduction in ESKD, without increased risk of cardiovascular events or death, particularly in those with preserved kidney function and well-controlled blood pressure [24]. The ACCORDION study reported similar long-term composite kidney effects overall but found no separate benefit for either doubling of serum creatinine or incident dialysis [18]. Another trial measuring the effect of tight glycemic control on kidney outcomes was the Veterans Affairs Diabetes Trial (VADT) which randomized 1791 veterans with mean duration of type 2 diabetes of 11.5 years,

mean HbA1c of 9.4%, and serum creatinine <1.6 mg/dL to intensive (achieved mean HgA1c of 6.9%) versus standard (achieved mean HgA1c of 8.4%) glucose control [25]. After 5.6 years of follow-up, the intensive control group had a significantly lower rate of progression to micro- and macroalbuminuria and any increase in albuminuria, but no difference was noted between the groups in terms of decline in GFR, rate of major cardiovascular events, death or other microvascular complications [26].

The ADVANCE, ACCORD, and the VADT studies targeted intensive glycemic control with the results being decidedly mixed, with either no benefits on cardiovascular effects ranging to cardiovascular risk in the intensive group and no kidney benefit, with the exception of one trial showing a reduction in albuminuria but no benefit on the preservation of kidney function [6]. The three trials established increased risk for hypoglycemic events related to intensive glycemic control. These trials also revealed that tighter glycemic control prevents microvascular but not macrovascular complications, unlike UKPDS. These results solidify the importance of improving glycemic control overall to prevent complications but not causing hypoglycemia during the process, thereby avoiding immediate dangers. Individual classes of medications used to treat type 2 diabetes will now be reviewed with respect to their efficacy in preventing progression of DKD.

Metformin

It is now commonly accepted that metformin is an important therapeutic option as first-line therapy for type 2 diabetes worldwide [27]. Long-term safety and efficacy of metformin have been well established. Metformin is a biguanide that lowers glucose levels by decreasing gluconeogenesis and improving insulin sensitivity at the tissue level. The UKPDS demonstrated that metformin is associated with a reduced risk of micro- and macrovascular complications in patients with type 2 diabetes. The U.S. Food and Drug Administration (FDA) revised its guidance for the use of metformin in CKD in 2016, recommending use of eGFR instead of serum creatinine to guide treatment and expanding the pool of patients with kidney disease for whom metformin is being considered [17]. By doing so, the renal function exclusionary criteria that was previously present on the use of metformin has been loosened over the past several years. The revised FDA guidance states that metformin is contraindicated in patients with a GFR < 30 mL/min/1.73 m^2; eGFR should be monitored while taking metformin and the benefits and risks of continuing treatment should be reassessed when eGFR falls <45 mL/min/1.73 m^2; metformin should not be initiated for patients with an eGFR of <45 mL/min/1.73 m^2; and metformin should be temporarily discontinued at the time of or before iodinated contrast imaging procedures in patients with eGFR 30–60 mL/min/1.73 m^2 [17].

A series of experimental studies revealed that metformin exerts renoprotective effects via multiple mechanisms [27]. The use of metformin is not recommended in patients with advanced renal impairment as it may increase the risk of lactic

acidosis. Recently, a retrospective cohort study including 10,426 T2D patients with CKD stage 3 (eGFR 30–45 mL/min/1.73 m^2) demonstrated that long-term metformin use was associated with 35% (hazard ratio (HR) 0.65; 95% confidence interval (CI) (0.57–0.73) and 33% (HR 0.67, 95% CI 0.58–0.77) risk reductions in all-cause mortality and progression to ESKD, respectively (median follow-up period: 7.3 ± 4.8 years) [27]. Despite some positive data, further research is required to elucidate the full range of metformin's effects, as the current results are contradictory [28].

Sulfonylureas

These medications lower glucose by stimulating insulin secretion from the beta-cells of the pancreas via the blockage of the ATP-sensitive K+ channels. Medications in this class include glyburide, glimepiride, and glipizide. While effective and typically inexpensive, hypoglycemia remains a major concern with the use of this class. The risk for hypoglycemia increases with diminished kidney function, limiting its use in this population. Despite the fact that these medications can lead to weight gain and higher insulin levels (issues raised as concerns in earlier studies as potentially deleterious to CV health), the UKPDS demonstrated no increased risk for CV events or death with their use [22]. While some studies have reported a reduction in urine albumin excretion in patients with type 2 diabetes using sulfonylureas, further research is needed to explicate the mechanisms of a direct, if any, renoprotective effect of these medications [3, 28].

Thiazolidinediones (TZDs)

The TZD class is used in the treatment of type 2 diabetes as an activator of peroxidase proliferator-activated receptors (PPAR). Their mechanism of action targets insulin resistance, improving insulin action in muscle, adipose and hepatic tissue along with increasing free fatty acid metabolism. While efficacious in lowering A1c, the use of TZDs has been hindered by concerns over developing edema, weight gain, liver abnormalities, and potential cardiac issues. Overall, the available data suggest that TZDs can prevent renal dysfunction and attenuate albuminuria in patient with DM via inhibition of hyperinsulinemia, hyperglycemia, endothelial dysfunction, oxidative stress, and inflammation [28]. Some studies have revealed that rosiglitazone, a type of thiazolidinedione, reduced microalbuminuria independent of the hyperglycemic state [14]. While not as commonly used nowadays, TZDs do remain a viable option in the appropriate patient.

Incretins

The incretins are peptide hormones secreted by the gut in response to food, which increase the secretion of insulin. The response of incretins is reduced in patients with type 2 diabetes and as such has been a useful mechanism of action to target in newer hyperglycemic agents. Circulating glucagon-like peptide-1 (GLP-1) stimulates insulin secretion but is rapidly inactivated (<2 min), primarily by dipeptidyl peptidase-4 (DPP-4). These findings prompted two different strategies to extend and maintain incretin activity in type 2 diabetes: first the use of injectable GLP-1 receptor agonists that are resistant to DPP-4 cleavage and provide supraphysiological concentrations of ligands to the GLP-1 receptor; and second, the use of oral DPP-4 inhibitors, which prevent degradation of endogenously secreted GLP-1 and glucose-dependent insulinotropic polypeptide (also known as gastric inhibitory polypeptide, GIP), another incretin hormone [29]. These two classes will be further reviewed individually.

Dipeptidyl Peptidase-4 (DPP-4) Inhibitors

As incretins are metabolized by DPP-4, inhibition of this enzyme increases the circulation of incretins and improves glycemic control. There are several DPP-4 inhibitors currently available as oral medications for the treatment of type 2 diabetes that can be given at any GFR, some with dose reductions required. The currently available medications in this class include sitagliptin, linagliptin, vildagliptin, alogliptin, and saxagliptin, which have all been reported to decrease albuminuria in patients with type 2 diabetes [28]. The results vary between studies. A post-hoc analysis of 217 patients with type 2 diabetes and micro- or macroalbuminuria on RAAS blockers collected from several phase 3 randomized, placebo-controlled clinical trials found that use of the DPP-4 inhibitor linagliptin for 24 weeks led to a 32% drop in albuminuria, independent of BP or HbA1c values [25]. Alternatively, the Cardiovascular and Renal Microvascular Outcome Study with Linagliptin in Patients with Type 2 Diabetes Mellitus, showed that in 6979 participants with T2DM and high CV and renal risk, linagliptin treatment for 2.2 years was noninferior for CV or renal outcomes compared with placebo across the spectrum of kidney disease [30]. In a pooled analysis of placebo-controlled trials, linagliptin reduced kidney disease events by 16%, driven by an 18% reduction in moderate albuminuria and a 14% reduction in severe albuminuria, with no effects on eGFR [29]. Data has shown that the dipeptidyl peptidase 4 inhibitors linagliptin and saxagliptin can reduce the amount of albuminuria, but the evidence is less clear than with a GLP-1 receptor analog [31]. This other incretin class will be discussed in the next section.

Throughout different studies, DPP-4 inhibitors have demonstrated CV safety but no CV or kidney benefit in people with established CVD [32]. As a class, they have a low side-effect profile and overall good tolerability. Unlike most antihyperglycemic agents, the efficacy of DPP-4 inhibitors is similar regardless of kidney function. These agents are safe and well tolerated even in ESKD patients on dialysis; however, caution should be exercised when these inhibitors are combined with sulfonylureas or insulin in patients with moderate to severe kidney impairment to prevent hypoglycemia [33].

Glucagon-Like Peptide-1 Receptor Agonists (GLP-1RAs)

GLP-1RAs are beneficial in improving glycemic control in patients with type 2 diabetes and may also produce weight loss. Most medications in this class are given as injections either daily or weekly; there is also one oral form available. Recent clinical trials have demonstrated that GLP-1RAs have beneficial effects on renal outcomes, particularly in patients with T2D who are at high risk for CVD. These findings suggest that GLP-1RAs hold great promise in preventing the onset and progression of DKD [34]. The consistency of data across glucagon-like peptide-1 receptor agonists suggests a class effect of protection from DKD. The mechanism of action may be multifactorial and include glycemic control, weight control, and direct effects on the kidney [19]. GLP-1RAs have been shown to prevent renal oxidative stress by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through the activation of PKA and the production of cyclic adenosine monophosphate (cAMP) [34].

The currently available medications in this class include liraglutide, exenatide, dulaglutide, lixisenatide, and semaglutide. A series of clinical trials and experimental studies support the beneficial effects of GLP-1RAs on DKD. Lessons from clinical trials demonstrate these effects are mainly driven by reductions in albuminuria [34]. The Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) study was one of the first major studies performed in this class and assessed the CV outcome of liraglutide compared to placebo. A total of 9340 participants with a high CV risk, who were > 50 years of age, with HbA1c > 7% who at baseline demographics had 20.7% of the patients with an eGFR of 30–59 mL/min/m^2 and 2.4% had an eGFR of <30 mL/min/m^2. Over a median follow-up of 3.8 years, liraglutide treatment resulted in less renal outcomes in comparison to placebo [HR 0.78 (95% CI: 0.67–0.92, $p = 0.03$)], this observation was largely driven by a reduction in new-onset macroalbuminuria in the liraglutide group in comparison to the placebo group [HR 0.74 (95% CI: 0.60–0.91, $p = 0.004$)] [34].

A recent meta-analysis, including the top 5 GLP-1 receptor agonists studies (ELIXA, LEADER, SUSTAIN-6, EXSCEL, and REWIND), showed that treatment with these drugs reduced all-cause mortality by 12% and composite renal outcome (the development of macroalbuminuria, decline in eGFR, progression to ESRD or

death attributable to renal causes) by 17%, mainly due to a reduction in urinary albumin excretion [35]. Although GLP-1 receptor agonists have unaltered pharmacokinetics in advanced kidney disease, their use should be carefully analyzed in this setting, due to the undesirable reduction in appetite and nausea [33]. The ongoing FLOW study is the first dedicated GLP-1RA renal outcome trial in people with T2D, with the aim to determine whether semaglutide reduces adverse renal events in people with T2D and impaired renal function [36]. Renal impairment in this study is defined as either an eGFR 50–75 mL/min/1.73 m^2 and UACR 300–5000 mg/g or an eGFR 25–50 mL/min/1.73 m^2 and UACR 100–5000 mg/g. An estimated 3160 participants are to receive once-weekly subcutaneous semaglutide at a titrated dose for up to 5 years. The primary endpoint is the time to the first occurrence of a composite primary outcome event, defined as a persistent eGFR decline (>50% from baseline), reaching ESKD, renal death, or CV death [34].

Sodium–Glucose Cotransporter-2 (SGLT-2) Inhibitors

SGLT-2 inhibitors normalize plasma glucose levels by inhibiting glucose reuptake in renal proximal tubules, thus inducing glycosuria [28]. SGLT-2 inhibition not only lowers HbA1c by inhibiting tubular glucose intake but also leads to weight loss and a lowering of BP. Because the actions of SGLT-2 inhibitors require filtration through the glomerulus, its beneficial effects may be blunted at lower levels of GFR [25]. In contrast, BP lowering, albuminuria lowering effects, and impact on eGFR are preserved in patients with CKD. These data suggest that SGLT-2 inhibitors may have beneficial kidney effects, even in people with reduced kidney function, where glycemic benefits are limited. Interestingly, a recent study suggested that canagliflozin slows the progression of kidney function decline independently of effects on glycemia [37]. Overall, the findings from a recent meta-analysis strongly support the notion that SGLT2 inhibitors offer kidney protection to a broad range of patients with type 2 diabetes, including those with preserved and low eGFR, ostensibly independent of any glucose-lowering effect (which will probably be minimal in patients with low eGFR) [2].

The evidence from completed trials and meta-analysis shows that SGLT2 inhibitors can reduce the risk of dialysis, transplantation, or death due to kidney disease, with compelling evidence of benefits on a broad range of other clinically important kidney outcomes [38]. The currently available medications in this class include canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin. The unique mechanism of action of these drugs, slowing the progression of kidney function decline independently from glycemic control, raises the hypothesis that their impact could also translate into kidney outcomes in patients without diabetes [37]. Newer data has also shown an impressive reduction in heart failure outcomes with these medications in patients both with and without diabetes. Clinically, the SGLT2 inhibitor empagliflozin is associated with an improvement in cardiovascular outcomes, reduction of the rate of glomerular filtration rate decline, and reduced incidence of

AKI [39]. This is supported further by the findings of the recent Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial, which showed renal benefits of canagliflozin in patients with CKD, including with eGFR as low as 30 mL/min/1.73 m^2. Importantly, renoprotection was achieved across all levels of baseline kidney function, down to an eGFR of 30 mL/min/1.73 m^2, with clear benefits seen even for the subgroup with baseline eGFR between 30 and 45 mL/min/1.73 m^2, for whom these drugs are not currently approved for use in most countries. (S) Also, the CREDENCE trial showed that the SGLT2 inhibitor canagliflozin reduced the risk of kidney failure and cardiovascular outcomes in patients with type 2 diabetes and stages 2 or 3 chronic kidney disease who were already receiving an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker [40]. Currently, SGLT2 inhibitors are not recommended in patients with an eGFR <45 mL/min/1.73 m^2 but given the results of the EMPA-REG OUTCOME trial, such drugs may be used in this patient group in the future for potential end-organ protective effects [41].

Another important recent study, Dapagliflozin in Patients with Chronic Kidney Disease (DAPA-CKD), was stopped early due to overwhelming efficacy. This study showed in patients with CKD, whether or not diabetes was present, the risk of a composite of a sustained declined in the eGFR of at least 50%, ESKD or death from renal or CV causes was significantly lower with dapagliflozin than with placebo. (AB) Based on these results, the eGFR cutoff could potentially be reduced to 25 mL/min/1.73 m^2. As results are comparable in nondiabetic patients with CKD, they also may be recommended for SGLT2 inhibitor treatment [42]. The primary findings of DAPA-CKD showed an extraordinary reduction in risk for the primary outcome (≥50% decline in estimated glomerular filtration rate [eGFR], kidney failure, or death from kidney or cardiovascular causes) of 39% with the SGTL2 inhibitor dapagliflozin, with similar reductions in participants with and without type 2 diabetes. In the overall study population, similar risk reductions were seen across all secondary outcomes, including all-cause mortality [40]. This study further verified the renoprotective efficacy of these agents at lower levels of GFR. In the EMPEROR (EMPagliflozin outcomE tRial in patients with chronic heaRt failure)-Reduced Trial, in addition to observed cardiovascular benefits, empagliflozin slowed the rate of decline in eGFR during double-blind treatment, and the risk of composite renal outcome was lower in the empagliflozin group than in the placebo group [43]. The data available to date further support that the benefits of SGLT2 inhibitors are cumulative with those of RAS blockade and these findings provide the strongest evidence yet that SGLT2 inhibition should be routinely offered to individuals with type 2 diabetes at risk for progressive kidney disease [38]. It will be important to determine if putative beneficial hemodynamic effects of SGLT2i lead to additional kidney or cardiovascular protection when combined with agents that target inflammation, fibrosis, and oxygen metabolism [30]. A new ongoing study titled EMPA-KIDNEY should provide more information on the use of empagliflozin in kidney disease. This is a randomized double-blinded placebo-controlled trial of

empagliflozin versus matching placebo in 6000 people with chronic kidney disease, with or without diabetes. This study will continue for approximately 3–4 years and will assess if empagliflozin reduces the risk of kidney disease progression or cardiovascular death [NCT03594110].

Advanced Kidney Disease

In advanced kidney disease, choices for treatments of type 2 diabetes are limited and insulin remains the mainstay of treatment. With any treatment used during CKD, careful monitoring and potential dose reduction are important. It should be emphasized that in the presence of kidney disease, as long as GFR is >30 mL/min/1.73 m^2, the use of SGLT2 inhibitors or GLP-1 receptor agonists is highly recommended for type 2 DM, since they have proven cardiorenal benefits. Metformin may be used in type 2 DM with GFR >30 mL/min/1.73 m^2. Due to their excretion profile, it is possible to use drugs such as linagliptin, and glipizide in advanced stages of renal disease, although they should be used with caution. When a patient starts dialysis, insulin is the treatment of choice, with special vigilance for the need of dose-reduced titration based on the risks of hypoglycemia. In conclusion, a personalized and evidence-based approach is of utmost importance in treating the hyperglycemia of type 2 DM with advanced kidney disease, ensuring efficacy and safety for the patients [33].

Hypertension

Hypertension is common among people with diabetes. The prevalence of hypertension increases with the duration of diabetes, presence of proteinuria, obesity, and renal insufficiency [44]. High prevalence of hypertension among patients with diabetes cannot be explained by being "essential," a coincidence, or secondary to renal insufficiency. The exact mechanism remains unclear.

For management of hypertension, the Eighth Joint National Committee (JNC-8) recommended the initiation of pharmacologic treatment at a systolic BP > 140 mmHg or diastolic BP >90 mmHg [19]. In the general hypertensive population, including those with diabetes, initial antihypertensive treatment may include a thiazide-type diuretic, a calcium channel blocker, an angiotensin-converting enzyme inhibitor (ACEi), or an angiotensin receptor blocker (ARB). In black patients with diabetes, the JNC-8 recommends initial treatment with a thiazide diuretic or calcium channel blocker. The same BP targets are recommended for those with CKD regardless of diabetes status. In patients who have diabetes with high levels of albuminuria, the medication regimen should include an ACEi or ARB alone on in combination with

medication from another drug class. Per the recent American Diabetes Association guidelines, lower blood pressure targets (e.g., <130/80 mmHg) should be considered for patients based on individual anticipated benefits and risks, which includes patients with CKD who are at increased risk of CKD progression (particularly those with albuminuria) and CVD [11].

Managing blood pressure (BP) in patients with diabetes can be a difficult task for clinicians. Furthermore, the level of BP control is an important determinant of micro- and macrovascular complication among patients with diabetes.

Following the liberalized JNC-8 recommendations, target BP goals have been challenged by the results of the Systolic BP Intervention Trial (SPRINT) [19]. The SPRINT included 9361 nondiabetic participants with hypertension and high CV risk. Participants were randomized to either an intensive (<120 mmHg) or standard (<140 mmHg) systolic BP goal. The trial was terminated early after a median of 3.26 years, because rates of the primary outcome (myocardial infarction, acute coronary syndrome, stroke, heart failure, or death from CV causes) and all-cause mortality were reduced by 25% and 27%, respectively, in the intensively treated group compared with the standard regimen group. The recent Kidney Disease: Improving Global Outcome (KDIGO) guidelines for patients with DKD suggest a target blood pressure of 140/90 mmHg or less for all diabetic patients and 130/80 mmHg or less for microalbuminuric patients with DM. Also, the use of an ACEi or ARB is recommended for patients with DKD and urine albumin excretion of 30 mg per 24 h or more [14].

Historically and currently, the first line of treatment of hypertension should include agents that block the renin–angiotensin–aldosterone system (RAAS). These agents, including ACEis and ARBs, reduce cardiovascular risk, control BP, reduce albuminuria, and slow the progression of renal disease [45]. Non-dihydropyridine calcium channel blockers, such as diltiazem, have been shown to slow the rate of progression of DKD in experimental studies and small clinical trials. These effects are not seen with the dihydropyridine calcium channel blockers such as amlodipine which have variable effects on albumin excretion [3]. The ACCORD study randomized 4733 patients with type 2 diabetes for a mean duration of 11 years and hypertension to tight versus standard systolic BP (mean achieved, 119 versus 134 mmHg) and followed them for an average of 5 years. Aggressive BP control significantly reduced the risk of developing microalbuminuria by 16% but not macroalbuminuria or kidney failure, defined as a serum creatinine >3.3 mg/dL, dialysis or kidney transplantation [25]. Intensive BP control was associated with a reduction in albuminuria but no reduction was seen in end-stage kidney disease. It is important to note that the ACCORD trial was not powered to detect renal events because the trial population was a more general cohort with DM rather than one selected for DKD [6].

The newer terminology used in the endocrine literature focuses on the word albuminuria rather than differentiating between macro and micro. Older terms are still referred to in this text to align with the previous literature. The next section will focus on the differentiation between the presence and absence of proteinuria in DKD.

Proteinuria

In clinical practice, it is well known that albuminuria provides early evidence of DKD in type 2 diabetes. Preventing transition to macroalbuminuria has been crucial as GFR declines at a rate of 5.3 to 5.7 mL/min/year once nephrotic syndrome proteinuria develops [46]. DKD is usually a clinical diagnosis made based on the presence of albuminuria and/or reduced eGFR in the absence of signs or symptoms of other primary causes of kidney damage. The typical presentation of DKD is considered to include a long-standing duration of diabetes, retinopathy, albuminuria without gross hematuria, and gradually progressive loss of eGFR [17]. Albuminuria results from an imbalance between glomerular filtration and tubular resorption of proteins [28]. This phenotype of diabetic kidney disease suggests that there is a dissociation between renal function and level of albuminuria in patients with diabetes and highlights the need for broader understanding of renal function loss apart from those related to an increase in albuminuria [47]. CKD is diagnosed by measuring a reduced eGFR and/or elevated urine ACR on at least two occasions over 90 days [3]. Current guidelines from the ADA and the National Kidney Foundation recommend that patients with T2D be screened annually for albuminuria and eGFR, while patients known DKD should have their eGFR monitored more frequently, every 6 months if eGFR is 45–60 mL/min/1.73 m^2 and every 3 months if eGFR is 30–44 mL/min/1.73 m^2 [21].

Because of variabilities between measurements in urinary albumin excretion, two of three specimens of UACR collected within a 3- to 6-month period should be abnormal before considering a patient to have albuminuria. Exercise within 24 h, infection, fever, congestive heart failure, marked hyperglycemia, menstruation, and marked hypertension may elevate UACR independently of kidney damage [17]. Proteinuria has been considered to be the hallmark of diabetic kidney disease and to precede renal function loss. However, it has become clear that a substantial proportion of patients either with type 1 diabetes or type 2 diabetes have renal function loss without proteinuria, known as non proteinuric diabetic kidney disease [47]. In step with the changing paradigm of the natural history of DKD, emerging evidence suggests that the clinical presentation of DKD is altering [19]. We will now focus on the surfacing data available on the non-proteinuric presentation of DKD.

Non-proteinuria

A comparison of DKD presentation in adults with diabetes during the time periods of 1988 and 1994 and between 2009 and 2014 shows that the prevalence of albuminuria as a manifestation of DKD decreased from 21% to 16%, that low eGFR (<60 mL/min/1.73 m^2) increased from 9% to 14%, and that severely reduced eGFR

(<30 mL/min/1.73 m^2) increased from 1% to 3% [19]. While there are data showing that patients with non-proteinuric diabetic kidney disease carry a lower risk of progression of kidney function loss, compared to those with proteinuric diabetes kidney disease, around 20% of those with nonproteinuric diabetic kidney disease experienced progression to advanced CKD or ESKD in 10 years [47]. In the UKPDS study, of those participants who developed kidney impairment, 61% did not have preceding albuminuria and 39% never developed albuminuria during the study [4]. People who progress to advanced CKD and ESKD tend to have more severe interstitial fibrosis and tubular atrophy, compared to those who did not progress, suggesting that in the absence of proteinuria, tubular damage may play an important role in progression of CKD [47]. The realization of the potential absence of proteinuria in patients with diabetes at risk for DKD is vital to proper screening and management in terms of prevention and treatment.

RAAS

The use of RAAS blockers as first-line BP-lowering agents in patients with DKD is based on high-quality randomized controlled trials throughout the range of type 2 diabetes and DKD [25]. In humans, RAAS inhibition has proved to be the single most effective therapy for slowing the progression of DN; however, 3 randomized, placebo-controlled trials of 256–3326 patients with type 1 diabetes and normoalbuminuria (RASS, EUCLID, AND DIRECT) suggest that early therapy in type 1 diabetes is ineffective in preventing the development of microalbuminuria [31].

While the benefits of RAAS blockade for slowing kidney disease progression with or without diabetes are well established, these agents should be used alone and not in combination. Trials attempting dual RAAS blockade (ONTARGET, ALTITUDE) found increased rates of adverse events (i.e., hyperkalemia and acute kidney injury) [1]. Direct renin inhibition as add-on therapy to ACEi/ARB treatment has been evaluated but eventually discouraged due to lack of effect on kidney-related outcomes and a higher rate of hyperkalemia [25]. It is recommended to not discontinue renin–angiotensin system blockade for minor increases in serum creatinine (<30%) in the absence of volume depletion. Also, the need for annual quantitative assessment of albumin excretion after diagnosis of albuminuria, institution of ACEi or ARB therapy, and achievement of blood pressure control is a subject of debate [17].

Mineralocorticoid Receptor (MR) Antagonists

There is a growing interest in the protective role of agents that block the RAAS cascade downstream [25]. Steroidal mineralocorticoid receptor (MR) antagonists such as spironolactone and eplerenone have a limited role to play as an adjunct

therapy to ACEI/ARBs because of hyperkalemia and other adverse effects. It is thought that this is, in part, due to variations in cell-specific effects of steroidal MR antagonists and incomplete antagonism. Identification of nonsteroidal MR antagonists that have a predictable antagonistic response and more tolerable side effect profile are currently being evaluated.

In an attempt to more precisely target the mineralocorticoid receptor, potent mineralocorticoid receptor antagonists that might exhibit less potassium retention – specifically nonsteroidal compounds such has finerenone – have been developed [29]. The dihydropyridine finerenone is a selective inhibitor and less often to cause hyperkalemia [48]. The recently published Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease (FIDELIO-DKD) trail was designed to test the hypothesis that finerenone slows CKD progression and reduces cardiovascular morbidity and mortality among patients with advanced CKD and type 2 diabetes [49]. This study showed that in patients with CKD and type 2 diabetes, treatment with finerenone resulted in lower risks of CKD progression and cardiovascular events than placebo [49]. The available evidence supports a pathophysiological role for overactivation of the mineralocorticoid receptor in cardiorenal diseases, including CKD and diabetes, through inflammation and fibrosis that lead to progressive kidney and cardiovascular dysfunction. The apparent benefit with respect to CKD progression was less than that reported with canagliflozin in the recent CREDENCE trial, with one explanation for the different findings being the fact that SGLT2 inhibitors were allowed in the present trial, whereas patients treated with mineralocorticoid receptor antagonists were excluded from the CREDENCE trial [48].

Conventional therapy of DKD includes better hyperglycemic control, RAS blockers, and other managements such as lipid-lowering therapies [31]. In non-dialysis-dependent CKD, cardiovascular events are reduced by statins and ezetimibe. The KDIGO along with the Association of British Clinical Diabetologists and Renal Association (ABCD-RA) guidelines recommend statins in non-dialysis-dependent CKD [3]. In patients on dialysis, there has been a failure to demonstrate similar benefits. Since diabetic kidney disease is associated with a high cardiovascular event rate, statins are generally used in this population for their benefits as CV protective agents, despite no significant renal benefit of these medications has been seen in clinical trials [50]. KDIGO recommends a more comprehensive CKD staging that incorporates albuminuria at all stages of eGFR; this system is more closely associated with risk but is also more complex and does not translate directly to treatment decisions [17].

Endothelin Receptor Antagonists

Endothelin is a potent vasoconstrictor peptide derived from the endothelium, which can cause blood vessels to constrict. Endothelin receptor antagonists currently are used for the treatment of pulmonary hypertension. Other potential implications for

use are being explored. A recent development is the release of modestly positive results for the endothelin receptor antagonist atrasentan in the Study of Diabetic Nephropathy with AtRasentan (SONAR), in a selected group of patients designed to minimize the known side effect of fluid retention [50]. Although this study was stopped early for concern of futility, the study eventually showed a renal benefit of the same magnitude as in CREDENCE, but with no effect on major adverse cardiovascular events and a tendency toward increased heart failure, which also stopped another endothelin receptor antagonist, avosentan [51]. It is currently unknown whether this treatment approach will be developed further, and whether perhaps a combined treatment approach with SGLT2 inhibition, which promotes diuresis, may aid in potentially optimizing safety and even efficacy [50]. Beyond the currently approved glucose-lowering medications, MRAs and endothelin receptor antagonists appear to be the most likely to move into the clinic in the future for use as kidney protective therapies, although the mechanisms of hyperkalemia with MRAs and volume retention with endothelin receptor antagonists will need to be clearly understood to mitigate the risks of such side effects [30].

Lifestyle Modifications

For all patients with diabetes, lifestyle modifications, including dietary restriction of sodium and protein, adequate exercise and weight reduction, and smoking cessation, are important to lower the risk of diabetic kidney disease and cardiovascular events [14]. Salt intake, obesity, and sedentary living have been linked to morbidity and mortality in multiple epidemiological studies. Dietary sodium restriction has been demonstrated to reduce blood pressure and albuminuria and enhances the effects of RAAS inhibition [1]. For people with non-dialysis-dependent CKD, dietary protein intake should be 0.8 g/kg body weight per day (the recommended daily allowance). Compared with higher levels of dietary protein intake, this level slowed GFR decline with evidence of a greater effect over time. Higher levels of dietary protein intake (>20% of daily calories from protein or >1.3 g/kg/day) have been associated with increased albuminuria, more rapid kidney function loss, and CVD mortality and therefore should be avoided [17].

Because the development of type 2 diabetes is strongly linked to dietary habits and excess adiposity, it is reasonable to consider strategies targeting these factors in the management of DKD. Whether bariatric surgery, the most effective and sustained of the weight reduction strategies, is effective in treating DKD has recently been comprehensively reviewed [25]. In 2015, a narrative review on weight loss and DKD was published. It included 26 studies, 16 using bariatric surgery, 8 using lifestyle modifications, and 2 using pharmacologic therapies, of individuals with obesity, diabetes, and DKD. Similar to the reviews of weight loss and CKD, this review found that weight loss was associated with decreases in proteinuria and increases in GFR [52].

Conclusion

We are entering a new era in the management of patients with chronic kidney disease in the context of type 2 diabetes [29]. Having established a clear correlation between albuminuria reduction and declining eGFR in patients with type 2 diabetes receiving usual doses of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, many nephrologists and endocrinologists have spent a good part of the past 25 years hoping that other strategies that reduce albuminuria might similarly attenuate eGFR decline [2]. Previously published data showed that intensive treatment of diabetes patients with mild to moderate CKD may lead to an improvement in proteinuria but may worsen cardiovascular outcomes and even increase cardiovascular and all-cause mortality, but more recently newer agents like SGLT2 inhibitors, have been shown to improve CV and renal outcomes. (J) While elucidating such clinically important mechanistic questions, major efforts are also needed to optimize the use of therapies with proven efficacy, including SGLT2i and GLP1-receptor agonists, in patients with T2D [30].

The GLP-1RAs and the SGLT2 inhibitors appear to be promising at this stage in terms of renal outcomes, with less promise being demonstrated for the DPP-4 inhibitors [41]. SGLT2 inhibitors and GLP-1RAs should be considered for patients with type 2 diabetes and CKD who require another drug added to metformin to attain target A1c or cannot use or tolerate metformin. SGLT2 inhibitors reduce risks of CKD progression, CVD events, and hypoglycemia, while GLP-1RAs are suggested because they reduce risks of CVD events and hypoglycemia and appear to possibly slow CKD progression [17]. The contemporary management of DKD offers some reasons for optimism, including the proven efficacy of RAAS blockers, the excitement over SGLT2-inhibitors, and the possibility of novel therapies on the horizon, yet critical gaps remain in our understanding of DKD [25]. Identification of SGLT2 inhibitors as a new therapy for DKD has a huge impact, however, is not sufficient to halt the progression of DKD. There is an urgent need to better understand the pathogenesis of DKD and develop more drugs to treat these patients [31]. The ongoing FLOW study has the potential to have a major impact on improving our understanding of how to better manage people with T2D and renal disease in addition to our current management which often focuses on cardiovascular measures [36]. Insights that have been obtained from recent CVOTs and DKD trials have direct implications for the treatment of patients in general practice, and in endocrine, cardiology, and nephrology specialty clinics. SGLT2i can, based on currently available data, be used safely with other agents that influence blood pressure and renal function [30].

It is likely that combination approaches addressing a range of hemodynamic and metabolic pathways will be needed to afford superior renoprotection in diabetes. With the recent identification of major sites of interaction between glucose- and blood pressure-dependent pathways in diabetic nephropathy, it is hoped that further new targets and ultimately novel drugs will be discovered which can block multiple pathogenic pathways [50]. After years of stagnation, we are now on the brink of a new paradigm in the prevention and treatment of kidney disease in people with type 2 diabetes [2, 3].

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Chapter 18
Diabetes and Cerebrovascular Disease

Vasileios-Arsenios Lioutas and Lina Palaiodimou

Introduction

Stroke is the second leading cause of death and the leading cause of disability worldwide [1]. The last 30 years have seen a dramatic explosion of new effective therapies in acute stroke, including intravenous thrombolysis [2, 3] and mechanical thrombectomy [4]. Despite their marked effectiveness, these interventions are applicable to a relatively small fraction of all strokes even in high-income countries [5–7]. Their eligibility in low- and middle-income countries is limited which further reduces the overall impact on the burden of stroke at a population level. Besides clinically overt strokes, there is a widespread but less explored covert, subclinical, insidious cerebrovascular injury [8] which has an impact on cognitive function and contributes significantly to the burden of mild cognitive impairment and dementia; cerebral small vessel disease is at the heart of this association [9]. Thus, preventive strategies targeted at the root causes of vascular brain injury, stemming the damage at its genesis are particularly appealing: they are applicable to the entire population, they do not require extensive infrastructure, complex systems of care and rare expertise, and can be used in low-resource settings. Diabetes mellitus (DM) is one of the well-established, "conventional" modifiable stroke risk factors along with hypertension, dyslipidemia, physical inactivity, and smoking and merits special attention.

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In this chapter, we will review the epidemiologic evidence linking DM to stroke and vascular cognitive impairment, examine the effect of glycemic control on the long-term incidence of stroke and cognitive impairment, describe the specific parameters for cardiovascular risk factor control in diabetic patients, and briefly describe the pathophysiologic mechanisms of diabetic cerebral vascular injury. Lastly, we will discuss diabetes in the context of acute stroke: We will examine the effect of hypoglycemia on the outcome of acute stroke, including acute revascularization therapy outcomes and describe the current status of clinical research on diabetes and hyperglycemia management in the acute stroke setting.

Diabetes as a Risk Factor for Stroke and Cognitive Impairment, Pathophysiologic Mechanisms, and Preventive Strategies

Epidemiologic Considerations: Insulin Resistance, Diabetes, and the Risk of Stroke

Relative long-term risk of stroke in patients with DM was reported as 2- to 2.5-fold in the Honolulu Heart Study with a primarily Japanese–American population, after adjusting for confounders [10, 11]. The association remains remarkably consistent across sociodemographically diverse cohorts such as the Northern Manhattan Study [12]. The effect is cumulative with a 3% increase in the risk of non-hemorrhagic stroke per year of DM duration [12]; on a population level, 8% of all ischemic strokes are attributable to DM [13]. The prevalence of DM among stroke patients is approximately 20% in various studies. Besides overt diabetes, impaired fasting glucose is also independently associated with future stroke, even at levels below the diabetic threshold [14].

The impact of DM on stroke incidence is more pronounced in younger patients [15], among whom the relative risk is higher than in older age strata. Diabetic patients with stroke tend to be younger and with a higher prevalence of concurrent cardiovascular risk factors [16]. Although patients with type 2 DM constitute the overwhelming majority of diabetics with stroke [17], the relative risk of stroke is significantly higher for type 1 DM patients [17]. In a population-based study of young adults with type 1 DM in Sweden, the adjusted standardized incidence rate of premature stroke was 18-fold higher for men and 26-fold higher for women compared to non-diabetics of similar age [18].

Although the end result of stroke is always brain tissue destruction from deprivation of blood flow (ischemic stroke, Figure 18.1a) or local mechanical tissue disruption and toxic effect of expanding hematoma (hemorrhagic stroke, Figure 18.1b), stroke is a heterogeneous disease. A primary distinction is ischemic versus hemorrhagic stroke: Ischemic strokes are significantly more common, comprising ~85% of all strokes, while the rest 15% are hemorrhagic. DM primarily predisposes to

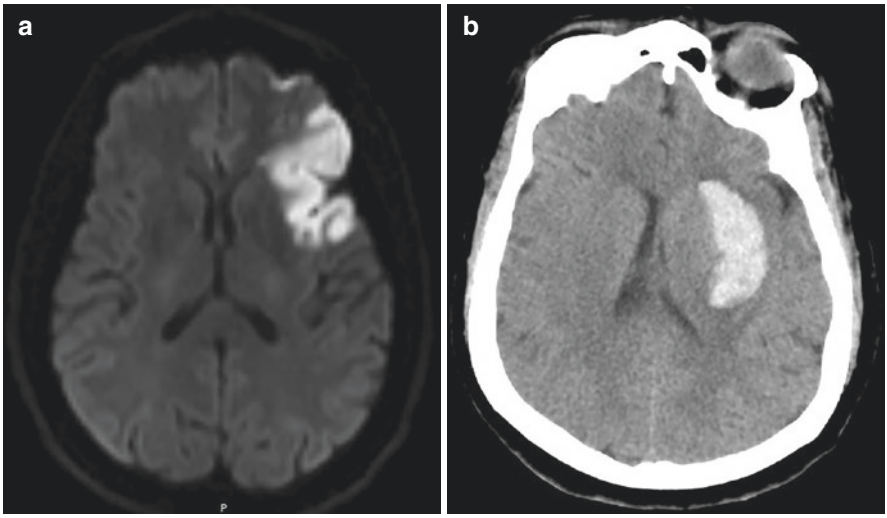

Fig. 18.1 (**a**) Brain MRI with acute left frontal ischemic stroke. (**b**) Brain CT with acute left basal ganglia intracerebral hemorrhage

ischemic stroke and does not significantly increase the risk for hemorrhagic strokes [13, 19]. Ischemic strokes are, in turn, a heterogeneous group with diverse underlying pathogenesis and pathophysiology [20], including cardioembolic infarcts, strokes from large artery atherosclerosis and small vessel, and "lacunar" strokes that are due to an intrinsic process known as lipohyalinosis. Observational and mendelian randomization studies have shown that DM primarily predisposes to the latter category of small penetrating small vessel infarcts [21–23] (Fig. 18.2), whereas other studies suggest that strokes due to large artery atherosclerosis might be equally frequent in some diabetic populations [17, 24, 25]. Thus, diabetic cerebral vascular injury can be conceptualized both as a micro- and macrovascular complication of DM.

Diabetes, Subclinical Brain Injury, and Vascular Cognitive Impairment

DM has been linked to the progression of cerebral atrophy [26–28] at a rate as high as three times that of normal aging [29]. Studies are conflicting on whether the atrophy is primarily due to gray matter loss [30, 31], white matter rarefaction and demyelination [32], or both [26]. Vascular injury in the form of cerebral small vessel disease and particularly lacunes is frequently seen in diabetics [22, 28]. Newer MRI techniques such as Diffusion tensor imaging have revealed disruptions in the white matter microstructural integrity [33, 34] even in otherwise normal-appearing white

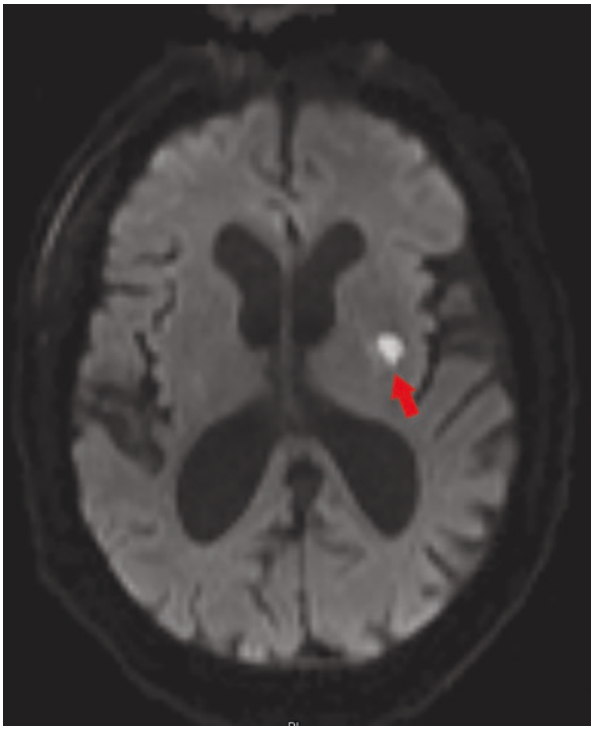

Fig. 18.2 Brain MRI with acute left basal ganglia lacunar infarct

matter, whereas functional MRI has shown impaired functional connectivity in patients with type 2 DM [35].

The functional correlates of these insidious deleterious effects of DM are cognitive impairment and dementia. Earlier epidemiologic evidence was hampered by heterogeneity in dementia definitions and incomplete information on premorbid cognitive function, suggesting, however, an increased risk of late-life dementia in diabetics [36]. A more recent study in >13,000 black and white adults in the Atherosclerosis Risk in the Communities study [37] documented a 19% greater cognitive decline over 20 years among participants with diagnosis in midlife. Both glycemic control (adjusted global *Z*-score difference, −0.16; $P = 0.071$) and longer diabetes duration (p for trend<0.001) were associated with greater cognitive decline in late life. A meta-analysis of 14 studies including >2.3 million individuals found an adjusted risk ratio of ~1.6 for dementia [38]. The risk was substantially higher for vascular dementia with a risk ratio 2.34 (95% CI 1.86–2.94) among women and 1.73 (95%CI 1.61–1.85) among men, whereas for nonvascular dementia, the risk ratio was statistically significant but lower (approximately 1.5). The pathophysiologic underpinnings of these associations are primarily vascular [39], although neurodegeneration [31] without overt vascular injury also has a significant role.

Pathophysiology of Cerebral Vascular Injury

Stroke is considered a macrovascular complication although, as we have already seen, DM has a predilection for small vessels and possibly affects the microvasculature earlier and more extensively than the large vessels. A detailed analysis of the molecular, cellular, and biochemical processes that mediate diabetic vascular injury exceeds the scope of this chapter; herein, we will highlight the main features.

Insulin resistance and chronic and repetitive hyperglycemia induce oxidative stress with reactive oxygen species production in intracellular organelles which in turn reduces nitric oxide (NO) bioavailability [40]. Additionally, both hyperglycemia and insulin resistance reduce the vasodilatory activity of NO by shifting the balance toward the production of endothelin-1 which has potent vasoconstrictive properties [41, 42] and increases the synthesis of other vasoconstrictors and prostanoids [43]. The net effect of these processes is impaired arteriolar relaxation and cerebral vasoreactivity [44] depriving the brain of its main autoregulatory mechanism and rendering it more susceptible to ischemic insults. Both hyperglycemia and insulin resistance induce a prothrombotic state [45], increasing platelet aggregation [46], promoting thrombin generation and the procoagulant activity of tissue factor, overall increasing the risk of atherothrombotic events and accelerating atherosclerotic plaque progression [47].

DM-induced structural vascular changes have primarily been examined in experimental type 1 diabetes models. These showed increased collagen deposition leading to profound thickening of the basement membrane in the cerebral microvessels [48, 49], a process reminiscent of the lipohyalinosis observed in small vessels of humans having suffered lacunar infarcts and possibly explaining the predilection for small penetrating vessel strokes in diabetic patients. Hyperglycemia and insulin resistance increase the production of molecules with proliferative properties such as endothelin [42] which also contribute to vascular remodeling with collagen deposition and increased wall thickness seen in both small and large arteries [50]. Lastly, the structural changes in the endothelium and basement membrane of capillaries increase the blood–brain barrier permeability [51, 52], impairing the clearance of molecules and proteins critical for brain homeostasis. Figure 18.3 summarizes the main pathophysiologic pathways of diabetic cerebral vascular injury.

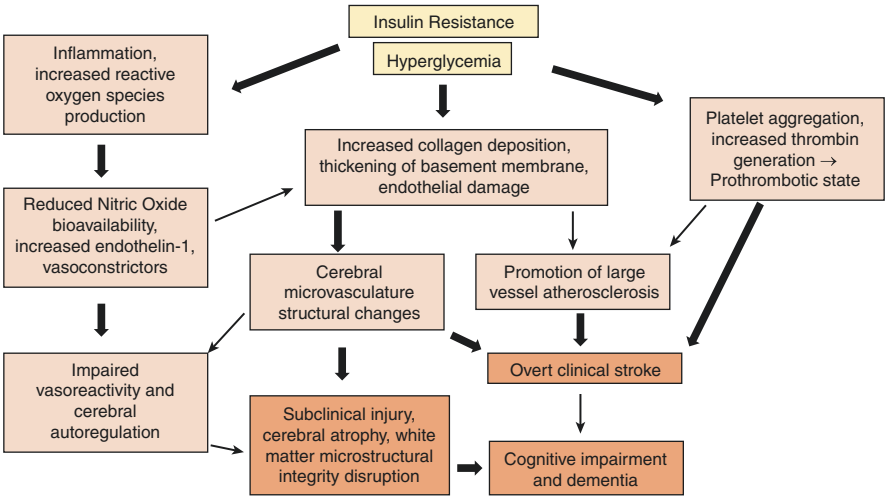

Fig. 18.3 Proposed pathophysiologic pathways of diabetic cerebral vascular injury

Glycemic Control and Mitigation of Stroke Risk: Evidence from Clinical Trials

Insulin Resistance

There has been only one randomized clinical trial specifically examining an intervention for insulin resistance or DM for the secondary prevention of stroke: The **Insulin Resistance Intervention after Stroke (IRIS)** trial randomized 3876 patients with recent ischemic stroke or transient ischemic attack and insulin resistance based on the homeostasis model assessment of insulin resistance (HOMA-IR) to receive 45 mg of pioglitazone or placebo [53]. Over 4.8 years of median follow-up, there was a significant reduction in the primary composite endpoint of stroke or myocardial infarction (HR 0.76, 95% CI (0.62–0.93). Similarly, there was a significant reduction in the progression to DM. However, there was no significance reduction in recurrent stroke, although the effect was more favorable among those with good protocol adherence [54] and the neutral study results might be in part explained by including non-compliant patients in the intention to treat primary analysis. In terms of safety, there was a significant increase in bone fractures in the pioglitazone group (6.9% vs. 4.9%, $p = 0.008$). A subsequent meta-analysis of three trials totaling >4900 patients found a significant reduction in stroke risk without difference in all-cause mortality [55].

Hyperglycemia and Intensive Glycemic Control

Given the key role of hyperglycemia in the pathophysiology of diabetic cerebral vascular injury, it had been expected that tight glycemic control would be beneficial in the reduction of stroke in diabetic patients. Although no clinical trials specifically targeted to stroke patients, several studies have attempted to address the impact of long-term intensive glycemic control on cardiovascular outcomes with conflicting results. In the following section, we will discuss the main findings of these trials pertaining specifically to stroke risk reduction; mortality and other macrovascular and microvascular outcomes have been discussed in detail elsewhere in this book.

In the **UK Prospective Diabetes Study (UKPDS) 33** [56], 3867 newly diagnosed patients with type 2 DM were randomized to an intensive regimen with a sulfonylurea or with insulin, versus conventional policy with diet. The intervention was deemed successful from a glycemic control perspective with an 11% reduction in HbA1C in the intensive group but no significant reduction in stroke in the intensive regimen; in fact, an insignificant increase was observed (5.6 vs. 5.0 per 1000 person years, $p = 0.52$). A sub-study of the UKPDS **(UKPDS 34)** randomized 753 overweight patients to an intensive blood–glucose control policy with metformin (n = 342) versus a conventional glycemic control policy ($n = 411$) [57]. A lower but statistically insignificant incidence of fatal stroke was observed in the metformin group (1.6 versus 2.1 per 1000 person-years), $p = 0.59$. Therefore, 3277 patients of the UKPDS trial entered post-trial monitoring and completed a total of approximately 17 years of follow-up, although no effort was made to maintain the assigned intervention beyond the trial completion. There was no significant difference in the cumulative incidence of stroke in the intensive therapy group (either sulfonylurea/insulin or metformin) versus the conventional group [58] (Table 18.1).

In the **Action to Control Cardiovascular Risk in Diabetes (ACCORD)** study, 10,251 patients with type 2 DM and a median HbA1c of 8.1% were randomized to either intensive therapy (goal HbA1c <6%) or standard therapy (goal HbA1c 7% to 7.9%) [59] for a median time of 3.5 years, at which point the intensive regimen was discontinued at the recommendation of the data and safety monitoring board. The interventions rapidly achieved the target HbA1C levels. There was no significant difference in the risk of stroke between the two groups (0.39% versus 0.37% per year, $p = 0.37$). A follow-up study (ACCORDION) monitored ACCORD participants for up to 7 years after the intensive glycemic intervention was stopped [60]; the results were comparable to the main study with no significant difference in the hazard of stroke (0.55% versus 0.63% per year, $p = 0.11$).

In the **Action in Diabetes and Vascular Disease (ADVANCE)** study [61], 11,140 adults with type 2 DM were randomized to either intensive glycemic control (target HbA1C 6.5%) with gliclazide or standard glycemic control, according to local guidelines; the median follow-up was 5 years and target glycemic control was achieved and maintained through the end of follow-up. There was no significant difference in the rate of major cerebrovascular events (4.3% vs. 4.4%). Including an additional post-trial follow-up period with total observation of ~10 years [62], no difference in the overall rate of stroke was observed: 8.8% vs. 8.6%, $p = 0.81$.

Table 18.1 Effect of intensive glycemic control on long-term risk of stroke in diabetic patients in randomized clinical trials

	Risk of stroke			
	Intensive	Conventional	*p*-value	Relative risk of stroke (for intensive glycemic control policy)
UKPDS 33 [56][a]	5.6/1000 PY	5.0/1000 PY	0.52	1.11 (0.81–1.51)
UKPDS 34 [57][a]	1.6/1000 PY	2.1/1000 PY	0.59	0.75 (0.19–2.93)
UKPDS long-term follow-up [58][a] (Sulfonylurea-insulin group)	6.3/1000 PY	6.9/1000 PY	0.39	0.91 (0.73–1.13)
UKPDS long-term follow-up [58][a] (Metformin group)	6.0/1000 PY	6.8/1000 PY	0.35	0.80 (0.50–1.27)
ACCORD [59][b]	0.39%/year	0.37%/year	0.74	1.06 (0.75–1.50)
ACCORD+ACCORDION 9-year follow-up [60][b]	0.55%/year	0.63%/year	0.11	0.87 (0.73–1.04)
ADVANCE [61][c]	4.3%	4.4%	NS	3% (−16% to 19%)[d]
ADVANCE-ON [62][c]	8.8%	8.6%	0.81	1.01 (0.89–1.15)
VADT [63][c]	4%	5%	0.32	0.78 (0.48–1.28)

PY person-years
[a] UKPDS: risk of stroke reported as absolute incidence rate per 1000 person-years
[b] ACCORD/ACCORDION: risk of stroke reported as %/year
[c] ADVANCE, ADVANCE-ON and VADT: risk of major cerebrovascular events reported as cumulative proportion (%) of patients with first ever event
[d] For ADVANCE, relative risk reduction rather than risk ratio is reported

The **Veterans Affairs Diabetes Trial (VADT)** [63] was an open-label trial of 1791 veterans with type 2 DM assigned to either intensive or standard glycemic control. The intensive regimen consisted of metformin plus rosiglitazone for those with body mass index (BMI) ≥ 27, whereas those with a BMI < 27 were started on glimepiride plus rosiglitazone. Over 6 years of follow-up, there was no significant difference in the rate of stroke (4% vs. 5%, $p = 0.32$).

The effectiveness of an intense glycemic strategy in reducing stroke risk is summarized in Table 18.1.

None of the large-scale clinical trials yielded the expected clinical benefit, despite a achieving improved glycemic control; this was confirmed in a subsequent meta-analysis pooling the results of all trials [64]. The reasons remain incompletely understood. Several mechanistic explanations have been proposed: First, lack of maintenance of glycemic control in the long term as patient adherence wanes over time. Second, in all trials, significantly higher risk of severe hypoglycemia was observed in the intensive therapy group (pooled risk ratio 2.34, 95% CI (1.64–3.35), $p < 0.0001$. Recurrent hypoglycemia can be as detrimental for the brain as hyperglycemia [65], thereby negating many of the positive effects of glycemic control. Third, intensive glycemic regimens are thought to cause wide and frequent fluctuations in glucose levels, resulting in increased glycemic variability [66] which compounds the detrimental effects of hyperglycemia and hypoglycemia.

Data is insufficient to compare the efficacy of specific hypoglycemic agents (e.g., metformin, sulfonylureas, and thiazolidinediones) in stroke risk reduction.

The current recommendation from the American Heart Association/American Stroke Association for glycemic control in patients with ischemic and TIA defers to the relevant guidelines from the American Diabetes Association [67] and suggests that choice of hypoglycemic agents should be individualized.

Glycemic Control and its Effect on Brain Atrophy and Cognitive Impairment

The Memory in Diabetes (MIND) study was a sub-study of the ACCORD trial. In a total of 10,251 patients, 2977 patients were included and underwent cognitive assessments at baseline, 20, and 40 months; a smaller sample of 503 patients underwent brain MRI at baseline and 40 months [68]. Intensive glycemic control significantly decelerated brain atrophy resulting in a significantly greater mean total brain volume than the standard therapy group with mean difference of 4.62 (2.0–7.3) cm^3 at 40 months. The pattern of decelerated gray matter loss was spatially heterogeneous: the benefit was primarily observed in brain areas adjacent to cortical regions that were found to be most heavily affected by diabetes at baseline [69]. Contrary to total brain volume, the intensive therapy group had significantly higher volume of abnormal white matter, driven by patients younger than 60 years. The favorable brain atrophy outcome did not translate into a cognitive benefit as there was no significant difference in any of the cognitive outcomes [68]. In long-term follow-up (80 months), neither total brain volume, abnormal white matter, or cognitive performance differed between the intensive and standard therapy groups [70]. Several possible explanations have been offered for this lack of long-term effects, similar to the ones discussed in lack of benefit in stroke risk mitigation: substantial loss to follow-up at 80 months limited the power to detect treatment effects. Treatment adherence was not sustained in the long term. As already mentioned, intensive glycemic control significantly increases the risk of severe hypoglycemia [64], which is associated with a significantly greater risk of dementia [71]. Therefore, it is possible that any benefit of strict glycemic control is nullified by the detrimental effects of severe hypoglycemic episodes that are inevitable with such a strategy.

Management of Cardiovascular Risk Factors for Stroke Prevention in Diabetic Patients

Hypertension

Hypertension often coexists with DM and its control is an important component of cardiovascular risk mitigation in DM patients. Although it had no significant impact on the composite primary endpoint, intense blood pressure reduction in the ACCORD trial led to reduction of stroke [72, 73] with a HR of 0.59. In a large meta-analysis of 13 trials including ~74,000 patients manifested that for each 5 mmHg

reduction in systolic blood pressure, there was a 13% relative risk reduction for stroke [74]. Closer inspection of the findings of the SPRINT and ACCORD BP trials in relevant subgroups suggests that the benefits of blood pressure reduction in reducing stroke risk are modified by the intensity of blood glucose control: there is a consistent benefit in among those with standard glucose control which is not seen among those with intensive blood glucose control [75].

Hypercholesterolemia

Cholesterol level reduction is of paramount importance among DM patients for both primary and secondary stroke risk reduction. In the **Collaborative Atorvastatin Diabetes Study (CARDS),** [76] 2838 patients with type 2 DM and no prior history of cardiovascular disease were randomized to receive 10 mg of atorvastatin or placebo. Significantly lower total cholesterol and LDL were achieved in the atorvastatin group. The study was terminated prematurely as a prespecified efficacy criterion if the composite primary endpoint had been met. The atorvastatin group had a significantly lower, hazard of stroke: 1.5% vs. 2.8%, HR 0.52, (95% CI 0.31–0.89). The **Heart Protection Study (HPS)** included **5963** adults with DM, randomized to 40 mg of simvastatin or placebo [77]. There was a 24% (6–39, $p = 0.01$) lower risk of stroke among diabetic patients in the simvastatin group. A subsequent meta-analysis of 14 trials including 18,686 individuals with type 1 and 2 DM confirmed the substantial stroke reduction associated with cholesterol-lowering therapy. The risk of stroke in controls was 5.4% versus 4.4% in the treatment arm, with RR 0.79, (99% CI 0.67–0.93). Statin therapy is generally recommended for all stroke or TIA patients with LDL > 100 mg/dl with further stricter target to <70 mg/dl in diabetic patients, especially if the stroke is of presumed atherosclerotic origin [67].

Diabetes and Hyperglycemia in Acute Stroke: Pathophysiology, Outcomes, Glycemic Variability, and Management of Glycemia in the Acute Phase of Stroke

Incidence and Mechanisms of Post-Stroke Hyperglycemia

Hyperglycemia after acute stroke is a common phenomenon [78, 79] reported in up to one-third of acute stroke patients [80–82]. Although it might be explained by pre-existing diabetes mellitus or other previously undiagnosed abnormalities in glucose metabolism in some patients [78, 83, 84], stress-related hyperglycemia is also encountered in non-diabetic patients likely due to release of counterregulatory hormones and cytokines in the context of acute stroke [85]: Activation of the hypothalamic–pituitary–adrenal axis and increased levels of catecholamines during acute

stroke can signal glycogenolysis, gluconeogenesis, proteolysis, and lipolysis, resulting in an excessive glucose production [86, 87].

Mechanisms of Exacerbation of Acute Ischemic Injury by Hyperglycemia

Admission hyperglycemia has been associated with pathophysiological sequalae that may lead to poor outcomes in stroke patients.

Oxidative Stress, Lactic Acidosis, and Cellular Energy Failure

Decrease of blood perfusion in ischemic stroke and subsequent anaerobic metabolism of glucose can lead to excessive accumulation of lactic acid which results in intracellular acidification, increased cell stress, and triggers neuronal apoptosis [88]. Elevated glucose increases extracellular glutamate concentration and causes intracellular calcium imbalance which triggers cell apoptosis via excessive cytochrome C leakage into the cytosol [89, 90]. There is also an increased reactive oxygen species (ROS) influx mediated by hyperglycemia through upregulation of superoxide production [91]. ROS accumulation, oxidative stress, increase of lactic acid, and cell apoptosis aggravate cytotoxic edema and reduce the volume of salvageable brain tissue [92, 93].

In acute stroke patients with a diffusion–perfusion mismatch suggesting potentially salvageable oligemic tissue, hyperglycemia was associated with increased brain lactate production [94], resulting in reduced salvage of penumbral tissue and greater final infarct size. These associations were independent of diabetic status and initial stroke severity, confirming the unique role of admission hyperglycemia in oxidative stress and neuronal damage [94]. The detrimental effects of hyperglycemic state in infarct volume change become more pronounced with longer hyperglycemia duration [95].

Reduced Collateral Blood Flow and Penumbra Salvage

Cerebral ischemia is a dynamic phenomenon that evolves over hours: The initial ischemic insult results in a core of irreversibly infarcted tissue, surrounded by an area of oligemic but potentially salvageable tissue, known as ischemic penumbra. The fate of the penumbra and the ultimate infarct size are determined by the efficiency of the collateral circulation. Hyperglycemia has a detrimental effect by downregulating the endothelial nitric oxide synthase activity which is crucial for the vasodilation and recruitment of collaterals [96–98]. Cerebral arteriolar vasomotor reactivity has also been found to be reduced in hyperglycemic patients [99, 100].

The net result is poorly regulated vasomotor response, regional cerebral blood flow imbalance, and larger final infarct size.

Blood–Brain Barrier Dysfunction

As already mentioned, DM is associated with increased blood–brain barrier permeability [51, 101]. Even in non-diabetic patients, acute transient hyperglycemia causes inflammation and endothelial injury [102, 103], destabilizing the tight junction proteins in endothelial cells [104] and disrupting the integrity of the blood–brain barrier [105, 106]. Increased blood–brain barrier permeability may worsen cerebrovascular reperfusion injury and predisposes to symptomatic intracerebral hemorrhage after acute reperfusion treatments.

Post-stroke Inflammatory Response

Acute cerebral ischemia activates microglia and astrocytes, with production of pro-inflammatory cytokines and chemokines, signaling the transmigration of inflammatory cells into brain tissue. The subsequent inflammatory cascade has detrimental effects in both blood–brain barrier integrity and tissue viability. Hyperglycemia has been shown to compound the inflammatory process by promoting leukocyte migration to the brain [107], enhancing production of pro-inflammatory cytokines [108] and overexpression of endothelial cell adhesion molecules [109, 110]. This results in cerebral edema and higher likelihood of hemorrhagic transformation and white matter degeneration following ischemic stroke [111, 112].

Hyperglycemia and Clinical Stroke Outcomes

Mortality and Functional Outcome after Ischemic and Hemorrhagic Stroke

Post-stroke hyperglycemia is associated with elevated short-term mortality [80]. Non-diabetic patients with admission glucose values of 110 to 126 mg/dL had a three-fold higher risk of in-hospital mortality or mortality within 1-month post-stroke [80]. Higher admission glucose blood value has been associated with in-hospital mortality in other cohort studies [113, 114].

Hyperglycemia has been associated with unfavorable functional recovery at 3–6 months post-stroke, regardless of diabetes status [80, 115]. Surprisingly, the association between admission hyperglycemia and long-term functional outcome has consistently been shown to be more pronounced in non-diabetic patients, whereas in diabetic patients elevated glucose levels during a stroke admission are less helpful in determining long-term outcomes [114, 116, 117]. Higher levels of

blood glucose on admission were independently associated with higher likelihood of hemorrhagic transformation of acute ischemic stroke [118].

Hyperglycemia is also detrimental in hemorrhagic stroke: Elevated fasting plasma glucose in the acute stage was shown to independently predispose to hematoma enlargement after spontaneous intracerebral hemorrhage [119, 120] resulting in increased the risk for short-term adverse events, early neurological deterioration, [121] poor functional outcome, and increased mortality [122–124].

Impact of Diabetes and Hyperglycemia on Intravenous Thrombolysis and Mechanical Thrombectomy Outcomes

Hyperglycemia inhibits fibrinolysis and may augment the synthesis of plasminogen activator inhibitor type-1 in arterial endothelial cells, resulting in impaired fibrinolytic response to IV-tPA administration [125, 126]. Higher glucose values were associated with lower recanalization rates in both diabetic and non-diabetic acute ischemic stroke patients receiving intravenous thrombolysis, translating to worse than expected long-term functional outcomes, independent of initial stroke severity [127, 128]. Admission hyperglycemia, especially for blood glucose admission values >180–200 mg/dl [129, 130], has been consistently associated with symptomatic intracranial hemorrhage after intravenous thrombolysis [127, 131–133] and reperfusion injury after mechanical thrombectomy [134].

Additional Components of Dysglycemia in Acute Stroke: Hypoglycemia and Glycemic Variability

Hypoglycemia

Blood glucose exhibits a U-shaped association with cerebral infarct volume in animal stroke models, suggesting that both hyperglycemia and hypoglycemia have detrimental effects on final infarct size [135]. In humans, a J-shape association with a nadir (lowest risk) of ~90 mg/dL has been demonstrated between glucose values and functional outcomes at 12 months [136]. Specifically, glucose values >130 mg/dL or < than 66 mg/dL are both associated with poor functional outcomes [136]. Hypoglycemia can exacerbate ischemic damage by depriving neuronal cells of their main source of energy at times of high metabolic demand, triggering a metabolic crisis [65] and is particularly consequential in patients with acute stroke.

Hypoglycemia is not an uncommon complication in acute stroke patients, affecting up 10% of the patients in a cohort study [137] and is perhaps underdiagnosed, considering the difficulty of stroke patients to report hypoglycemia-related symptoms. Dysphagia, fasting status, and attempts for aggressive management of post-stroke hyperglycemia mainly account for post-stroke hypoglycemia. Given its

detrimental effect, clinicians should be vigilant in monitoring and preventing hypoglycemic events in stroke patients.

Glycemic Variability

Glycemic variability (GV) is defined as the degree of fluctuation in glucose values over time and is considered the third component of dysglycemia, along with hyperglycemia and hypoglycemia [138]. Both upward and downward fluctuations of glucose trigger oxidative stress [139]; which is more pronounced than observed with chronic sustained hyperglycemia [66]. In the clinical setting, GV was shown to independently predict mortality and morbidity in patients with various acute illnesses [140, 141].

GV indices measured in diabetic acute stroke patients were independently associated with early neurological deterioration during hospitalization, poor functional outcome, increased risk of recurrent cardiovascular events, and post-stroke cognitive impairment [142–145], independent of HbA1c levels or hypoglycemic events during hospitalization [142]. GV is associated with stroke outcomes in non-diabetic patients as well: Higher GV values have been associated with infarct volume growth [146], progression of penumbra to irreversible ischemia [147], symptomatic intracerebral hemorrhage, and mortality [148].

Continuous glucose monitoring is a promising and feasible method for monitoring glycemic variability [149]. It has been successfully utilized in the acute inpatient setting, allowing for almost real-time information about glucose excursions [150] and could prove useful in managing glycemic monitoring in the acute phase of stroke.

Glycemic Management in the Acute Phase of Stroke

Overview

Given the abundance of observational evidence highlighting the detrimental effect of hyperglycemia on stroke outcomes, glucose normalization was anticipated to improve stroke outcomes [151, 152] and has been the target of several clinical trials employing tight glycemic control regimens. The results, however, have been perplexing: None of the relevant randomized clinical trials yielded significant clinical benefit, and increased risk of hypoglycemia has been a recurrent theme [153–165]. A recent systematic review and meta-analysis of 12 RCTs that evaluated intravenous insulin administration in hyperglycemic stroke patients versus standard of care showed no benefit in mortality or functional independence between the two arms, reconfirming a significant, fivefold higher risk of hypoglycemia in the interventional arm [166].

Several reasons have been proposed for the lack of clinical efficacy in clinical trials: There is a significant heterogeneity in the timing and glucose threshold for therapy initiation, treatment duration, optimal hypoglycemic regimen, and the target glucose range. Some have underscored the significantly higher rates of hypoglycemia and the glycemic variability introduced by tight glycemic regimens; as discussed above, both phenomena can be very detrimental in the acute phase of stroke, negating any possible benefit from hyperglycemia management. Lastly, some authors point out that hyperglycemia in the acute phase of stroke might simply be an epiphenomenon, a marker of stroke severity, explaining the lack of clinical response. In clinical practice, target glucose range, treatment initiation, and adoption of aggressive protocols differ significantly among practicing physicians, depending on their specialty [167]. In the following sections, we will describe these issues in detail.

Optimal Timing of Treatment Initiation and Treatment Duration

In the majority of clinical trials, hypoglycemic treatment in stroke patients has been initiated within 24 hours upon admission [153–155, 158, 161, 163–165]. Some suggested that this might be too delayed, missing a critical, ultra-early therapeutic window and it was postulated that an even earlier glucose control might be beneficial. In the INSULINFARCT trial, hypoglycemic treatment was initiated within 6 h after stroke onset [160]. However, neither infarct volume nor functional outcomes were improved significantly in the interventional arm of this trial [160] and the implementation of such an early treatment window was not deemed feasible. An intermediate and possibly more pragmatic time window of 12 h after stroke onset was adopted by the most recent clinical trials, including the largest and more rigorous relevant clinical trial to date, the Stroke Hyperglycemia Insulin Network Effort (SHINE) [156, 159, 162], without significant clinical benefit.

The temporal profile of post-stroke hyperglycemia consists of two main phases, as was demonstrated in a study using continuous glucose monitoring [85]. There is one early phase of hyperglycemia during the first hours of stroke onset, followed by a delayed phase, recorded at 48–88 h post-stroke in both diabetic and non-diabetic patients [85] which probably coincides with resumption of food intake. Regardless, it seems that admission hyperglycemia may be more protracted and not restricted to the early hours after admission only. Post-admission hyperglycemia was independently associated with mortality, regardless of the glucose values on admission, and even if the patients were initially normoglycemic [152]. For this reason, hypoglycemic treatment should probably aim at glucose normalization that covers the delayed phase of hyperglycemia as well, for at least 72 h post stroke. All clinical trials focused on treating patients up to 24 h after which glycemic control was left to the discretion of treating clinicians. Therefore, there is a potential missed opportunity to benefit from treatment of hyperglycemia beyond the very early phase.

Glucose Threshold for Hypoglycemic Treatment Initiation

Blood glucose values that triggered hypoglycemic treatment initiation differed among clinical trials. This variation is also found in the real-world clinical setting, according to an EFNS Task Force survey in 22 European countries, which showed that glucose thresholds that triggered intervention ranged from 133 mg/dl to 252 mg/dl [168]. Glucose values as low as 108 mg/dl have been used as a threshold for treatment initiation in the GIST-UK trial, whereas a more conservative threshold of 150 mg/dl was chosen for the THIS trial [156, 163]. Neither approach had an impact on stroke outcomes [156, 163], but in both trials, severe hypoglycemic events were observed in the active arm. In the SHINE trial, a more sophisticated, individualized approach was adopted, using different thresholds for diabetic and non-diabetic patients, considering the different pathophysiological mechanisms attributed for admission hyperglycemia and the different temporal profiles of its progression [85, 162]. A threshold of 110 mg/dl was used for patients with type 2 DM and 150 mg/dl for non-diabetic participants. However, this did not translate into a clinical benefit.

Optimal Regimen for Glucose Control

The randomized clinical studies have used a variety of regimens in different treatment protocols.

Intravenous insulin treatment has been comprehensively investigated in different clinical trials in the last two decades as the standard of care of hypoglycemic treatment [155–162, 165], without significant benefit. The highly anticipated SHINE trial, which evaluated 1151 hyperglycemic stroke patients randomized to receive either intravenous insulin with the aim of tight glycemic control or subcutaneous sliding scale insulin four times daily according to standard practice, did not improve 3-month functional outcome, whereas severe hypoglycemia defined as blood glucose less than 40 mg/dl was significantly more frequent in the intervention group [162]. Perhaps, in specific subgroups of stroke patients in need of rapid glucose correction, such as those receiving acute reperfusion therapies, intravenous insulin may still be used, but this hypothesis needs to be confirmed in the setting of a clinical trial.

Intravenous glucose-potassium-insulin regimen, also known as GKI, was thought to control hyperglycemia in a controlled way avoiding large glucose excursions and additionally preventing hypokalemia, which may complicate insulin-only treatment [169]. A regimen consisting of 16 Units of human soluble insulin and 20 mmol potassium chloride in 500 mL 10% glucose was used in the **Glucose Insulin in Stroke Trial (GIST**) explanatory trial [153], achieving significantly lower glucose values and lower 30-day mortality [153]. This was followed by a larger, Phase 3 trial, **GIST-UK**, which tested the safety and efficacy of GKI for correcting mild-to-moderate hyperglycemia in both ischemic and hemorrhagic stroke patients, regardless of the history of diabetes [163]. A small but statistically significant reduction in

plasma glucose values was demonstrated, but this treatment effect did not translate in better clinical outcomes at 3 months [163]. Rescue treatment with dextrose was necessary in 15% of GKI patients with asymptomatic hypoglycemia [163]. Similar efficacy results were observed in another small trial evaluating GKI with higher rates of asymptomatic hypoglycemia (76%) [164]. Interestingly, GKI infusion was also associated with greater infarct growth in patients with persistent arterial occlusion in an exploratory analysis [164]. Besides the lack of efficacy, GKI was deemed impractical for clinical practice by nursing staff due to increased workload from the frequent changes of specimen bags required and treating episodes of hypoglycemia [163].

Another small study evaluated insulin-potassium-saline-magnesium (IPSM) infusions for controlling hyperglycemia in diabetic and non-diabetic ischemic stroke patients [154]. The addition of magnesium in the regimen was based on its postulated anti-excitotoxic effect. The regimen resulted in a lower likelihood of neurological deterioration during hospitalization and better functional outcomes at 30 days compared with controls [154] but did not assess the occurrence of hypoglycemic in treated patients, a significant omission. It has not been further evaluated in subsequent studies.

Exenatide is a synthetic glucagon-like peptide-1 receptor agonist that has recently been tested in acute stroke patients. Based on experimental data suggesting a potential neuroprotective effect and confirming its tolerability, one pilot study investigated the safety and efficacy of subcutaneous exenatide in 11 stroke patients [170, 171]. Exenatide was well tolerated without symptomatic hypoglycemia, and glucose control under 155 mg/dl was achieved. On the other hand, the PROLOGUES trial failed to provide sufficient evidence that prehospital administration of exenatide in hyperglycemic hyperacute stroke patients led to significant glucose reduction [172]. However, this study was prematurely stopped due to slow inclusion and the results may have been confounded by the limited sample size [172]. Other clinical trials are currently evaluating exenatide as a potentially safe and effective regimen for glycemia management in the acute stroke setting and their results are awaited [173, 174].

Optimal Target Glucose Range

No consensus has been reached regarding the optimal target of glucose values in the acute stroke setting. One important limitation of achieving tight glucose control is the incidence of hypoglycemic events. The **Glucose Regulation in Acute Stroke Patients (GRASP)** trial aimed to compare three different possible target ranges: the tight one (70–110 mg/dl), the loose one (70–200 mg/dl), and the usual-care one (70–300 mg/dl) [161]. Hypoglycemia was significantly more frequent in the tight glucose range, whereas no difference was observed in the loose and the usual-care group [161]. Another important aspect disclosed in the GRASP trial was that the loose intervention was more feasible and the patients obtained longer in-target duration compared with assigned to the tight range [161]. A considerable hypoglycemic

risk was also observed in other clinical trials that used 130 mg/dl or less as the higher value of glucose target range [153, 156, 158, 159, 162–165].

Current Guidelines and Standard of Practice for Glycemic Control in Acute Stroke

Current guidelines reflect the findings of all major clinical trials and the lack of efficacy of early and aggressive induction of euglycemia on patient outcomes as well as the high risk of severe hypoglycemia with tight glycemic control regimens. Guidelines from the European Stroke Organization and the American Heart Association/American Stroke Association recommend initiation of insulin at high degrees of hyperglycemia (≥180 mg/dl) with the aim to maintain a mild hyperglycemic state (between 120 and 180 mg/dl) [175, 176], taking care to avoid wide excursions and severe hypoglycemic events. The updated European Stroke Organization guidelines explicitly caution against routine use of tight glycemic control with intravenous insulin [175] unless glucose levels exceed 180 mg/dl. Overall, no specific treatment protocol is provided, and there is a wide variability in physician care hypoglycemic regimens in acute stroke patients [167].

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Chapter 19
Diabetes and the Autonomic Nervous Systems

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The Autonomic Nervous System

The autonomic nervous system regulates body systems such as blood pressure and the rate of breathing. The autonomic nervous system has two main divisions called the sympathetic and the parasympathetic. The sympathetic system regulates the fight and flight responses, while the parasympathetic system regulates the rest and digest systems [1]. Autonomic syndromes can be grouped by the primary chemical neurotransmitters that are affected by the underlying etiology. The sympathetic nervous systems include noradrenergic, adrenergic, and cholinergic functions. These are made up of unmyelinated sympathetic neurons that comprise the thoracolumbar ganglionic chain. Parasympathetic nervous systems consist of myelinated predominately cholinergic neurons situated in the brainstem, cervical, and sacral spinal cord.

The autonomic nervous system controls pupillary function, blood pressure, heart rate, respiratory rate, body temperature, digestion, electrolyte and water balance, urination, defection, and sexual response. The two divisions coordinate to control and regulate these important functions.

Acetylcholine and norepinephrine are the neurotransmitters used to communicate within the autonomic nervous system. Nerve fibers that secrete acetylcholine are called cholinergic fibers. Acetylcholine is involved in both the sympathetic and parasympathetic systems. Fibers that secrete norepinephrine are called adrenergic fibers. Norepinephrine has sympathetic effects.

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Autonomic disorders may result from disorders that damage the brain, the spinal cord, or the more distal nerve endings. Symptomatically, autonomic disorders commonly cause orthostatic hypotension, postural tachycardia, temperature dysregulation, and dryness of the eyes or mouth. Individuals may have postprandial hypotension or gastroparesis. Individuals may have urinary incontinence or constipation.

Central Autonomic Dysfunction

Central autonomic dysfunctions can be due to primary or secondary disorders. Autonomic failure (AF) may be a major manifestation of multiple system atrophy (MSA) and idiopathic Parkinson's disease (IPD). In both MSA and IPD, AF is almost invariably associated with neuronal loss in the intermediolateral cell columns. Dysautonomia in MSA is early, severe, and progressive, including marked orthostatic hypotension and urinary incontinence, and is complicated by respiratory disturbances, such as laryngeal stridor and sleep apnea. AF in IPD is generally less severe than in MSA. Higher L-Dopa medication requirements and more side effects, abnormal urethral sphincter electromyography, and CSF markers may distinguish MSA from IPD. Secondary autonomic disorders may result from traumatic, vascular, inflammatory, demyelinating, or neoplastic lesions involving corticolimbic, hypothalamic, brainstem, or spinal autonomic networks. These disorders can cause AF or autonomic hyperactivity, such as arrhythmia, hypertension, and hyperthermia [2].

Peripheral Autonomic Nervous System

The autonomic nervous system (ANS) is composed of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). The SNS is organized at a spinal and peripheral levels such that cell bodies within the thoracolumbar segments of the spinal cord provide preganglionic efferent innervation to sympathetic neurons. The spinal cells of origin for the presynaptic input to sympathetic peripheral ganglia are located from the first thoracic to the second lumbar level of the cord, although minor variations exist. The postganglionic fibers in the SNS travel quite lengthy paths to arrive at the target organs. Interactions between the adrenal cortex and adrenal medulla constitute a critical link between the autonomic and endocrine systems [3].

The efferent nervous activity of the ANS is largely regulated by autonomic reflexes. In many of these reflexes, sensory information is transmitted to homeostatic control centers, in particular, those located in the hypothalamus and brainstem. Much of the sensory input from the thoracic and abdominal viscera is transmitted to the brainstem by afferent fibers of cranial nerve X, the vagus nerve. Many important variables in the body are monitored and regulated in the hypothalamus and the brainstem including heart rate, blood pressure, gastrointestinal peristalsis, and glandular secretion, body temperature, hunger, thirst, plasma volume, and plasma osmolarity.

An example of this type of autonomic reflex is the baroreceptor reflex. Baroreceptors located in some of the major systemic arteries are sensory receptors that monitor blood pressure. If blood pressure decreases, the number of sensory impulses transmitted from the baroreceptors to the vasomotor center in the brainstem also decreases. As a result of this change in baroreceptor stimulation and sensory input to the brainstem, ANS activity in the heart and blood vessels is adjusted to increase heart rate and vascular resistance so that blood pressure increases to its normal value.

Some autonomic reflexes may be processed at the level of the spinal cord. These include the micturition reflex and the defecation reflex. Although these reflexes are subject to influence from higher nervous centers, they may occur without input from the brain.

Each system, sympathetic or parasympathetic, is dominant under certain conditions. The sympathetic system predominates during emergency "fight-or-flight" reactions and during exercise. The parasympathetic system predominates during quiet, resting conditions. The overall effect of the parasympathetic system under these conditions is to conserve and store energy and to regulate basic body functions such as digestion and urination [1].

Sympathetic Division

The preganglionic neurons of the sympathetic system arise from the thoracic and lumbar regions of the spinal cord (segments T_1 through L_2). Most of these preganglionic axons are short and synapse with postganglionic neurons within ganglia found in the sympathetic ganglion chains. A single preganglionic neuron may synapse with several postganglionic neurons in many different ganglia. The coordinated sympathetic stimulation of many organs and tissues in the body is referred to as a mass sympathetic discharge.

Other preganglionic neurons exit the spinal cord and pass through the ganglion chain without synapsing with a postganglionic neuron. Instead, the axons of these

neurons travel more peripherally and synapse with postganglionic neurons in one of the sympathetic collateral ganglia. These ganglia are located about halfway between the CNS and the effector tissue.

Finally, the preganglionic neuron may travel to the adrenal medulla and synapse directly with this glandular tissue. The cells of the adrenal medulla have the same embryonic origin as neural tissue and, in fact, function as modified postganglionic neurons. Instead of the release of neurotransmitter directly at the synapse with an effector tissue, the secretory products of the adrenal medulla are picked up by the blood and travel throughout the body to all of the effector tissues of the sympathetic system. Most innervated blood vessels in the entire body, primarily arterioles and veins, receive only sympathetic nerve fibers. Therefore, vascular smooth muscle tone and sweating are regulated by the sympathetic system only.

Parasympathetic Division

The preganglionic neurons of the parasympathetic system arise from several nuclei of the brainstem and from the sacral region of the spinal cord (segments S_2–S_4). The axons of the preganglionic neurons are quite long compared to those of the sympathetic system and synapse with postganglionic neurons within terminal ganglia which are close to or embedded within the effector tissues. The axons of the postganglionic neurons, which are very short, then provide input to the cells of that effector tissue.

The preganglionic neurons that arise from the brainstem exit the CNS through the cranial nerves. The oculomotor nerve (III) innervates the eyes; the facial nerve (VII) innervates the lacrimal gland, the salivary glands, and the mucus membranes of the nasal cavity; the glossopharyngeal nerve (IX) innervates the parotid (salivary) gland; and the vagus nerve (X) innervates the viscera of the thorax and the abdomen (e.g., heart, lungs, stomach, pancreas, small intestine, upper half of the large intestine, and liver). The physiological significance of this nerve in terms of the influence on the parasympathetic system is clearly illustrated by its widespread distribution and the fact that 75% of all parasympathetic fibers are in the vagus nerve. The preganglionic neurons that arise from the sacral region of the spinal cord exit the CNS and join together to form the pelvic nerves. These nerves innervate the viscera of the pelvic cavity (e.g., lower half of the large intestine and organs of the renal and reproductive systems). The effects of the parasympathetic system tend to be more discrete and localized, with only specific tissues being stimulated at any given moment, compared to the sympathetic system where a more diffuse discharge is possible [3] (Fig. 19.1).

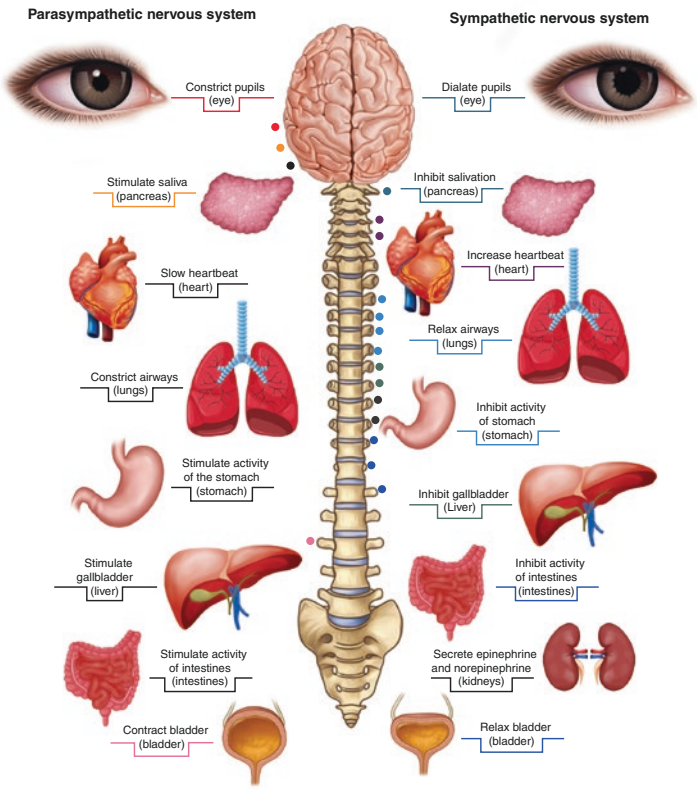

Fig. 19.1 Parasympathetic and Sympathetic Nervous system

Neurotransmitters of the Autonomic Nervous System

The two most common neurotransmitters released by neurons of the ANS are acetylcholine and norepinephrine. Neurotransmitters are synthesized in the axon varicosities and stored in vesicles for subsequent release.

Nerve fibers that release acetylcholine are referred to as cholinergic fibers. These include all preganglionic fibers of the ANS, both sympathetic and parasympathetic systems; all postganglionic fibers of the parasympathetic system; and sympathetic postganglionic fibers innervating sweat glands. Nerve fibers that release norepinephrine are referred to as adrenergic fibers. Most sympathetic postganglionic fibers release norepinephrine (Fig. 19.2).

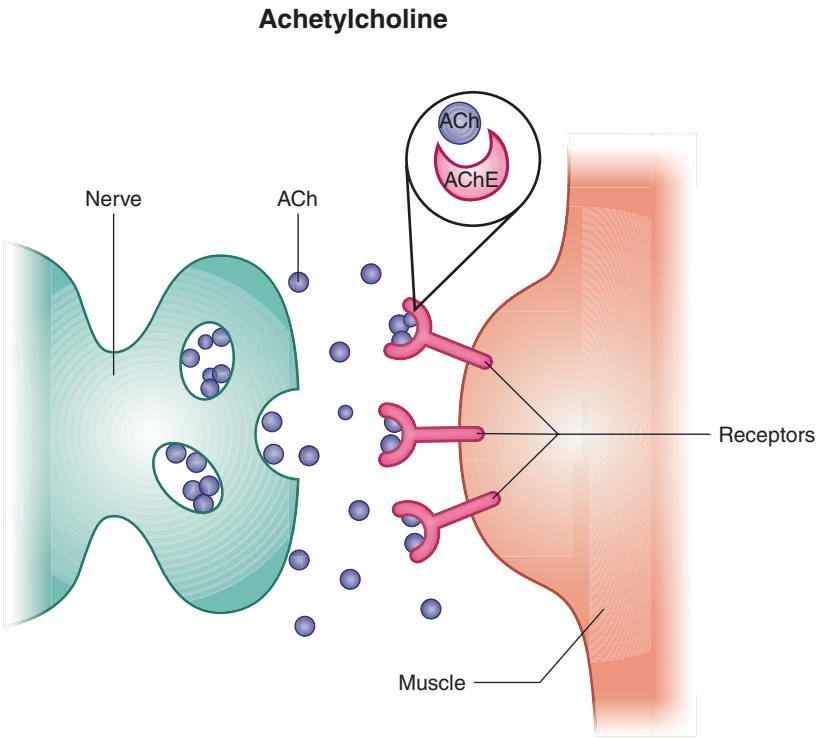

Fig. 19.2 Acetylcholine Neurotransmitter

As previously mentioned, the cells of the adrenal medulla are considered modified sympathetic postganglionic neurons. Instead of a neurotransmitter, these cells release hormones into the blood. Approximately 20% of the hormonal output of the adrenal medulla is norepinephrine. The remaining 80% is epinephrine. Unlike true postganglionic neurons in the sympathetic system, the adrenal medulla contains an enzyme that methylates norepinephrine to form epinephrine. The synthesis of epinephrine, also known as adrenaline, is enhanced under conditions of stress. These two hormones released by the adrenal medulla are collectively referred to as catecholamines.

Diabetes mellitus is the most common cause of autonomic neuropathy in the developed world. Diabetic autonomic neuropathy causes a constellation of symptoms and signs affecting cardiovascular, urogenital, gastrointestinal, pupillomotor, thermoregulatory, and sudomotor systems. Several discrete syndromes associated with diabetes cause autonomic dysfunction. The most prevalent of these are generalized diabetic autonomic neuropathy, autonomic neuropathy associated with the prediabetic state, treatment-induced painful and autonomic neuropathy, and transient hypoglycemia-associated autonomic neuropathy. These autonomic manifestations of diabetes are responsible for the most troublesome and disabling features of diabetic peripheral neuropathy and result in a significant proportion of the mortality and morbidity associated with the disease [4].

Evaluation of the Autonomic Nervous System

Clinical History

An organized clinical history can help identify the specific autonomic syndrome based on the constellation of symptoms. For example, decreased noradrenergic function can cause orthostatic hypotension, whereas increased noradrenergic function can lead to palpitations, hypertension, or postural tachycardia. Other symptoms associated with increased noradrenergic function include mydriasis, hyperhidrosis, or piloerection. Inappropriate activation of adrenergic functions can cause palpitations, tachycardia, hypertension, cold hands, and anxiety. Conversely, decreased adrenergic function will lead to fatigue. With regard to parasympathetic disorders, ask about dry mouth, dry eyes, and genitourinary symptoms including erectile dysfunction. Shortness of breath or dyspnea on exertion that may be as a result of excessive bronchial constriction [5].

Medication can affect various elements of the autonomic nervous system depending on the individual pharmacologic mechanisms of action. As such, the treating physician should explore and reconcile all prescribed medications; how the patient is taking each medicine; inquire into medication compliance and any home-holding parameters; and inquiry of any herbal supplements and dietary modifications. Finally, it is necessary to define the impact of symptoms on the patient's quality of life. A complete social history may be pertinent if the patient is no longer being able to continue employment due to the severity of disabling symptoms.

Other causes of total or generalized autonomic failure should be considered in the differential diagnosis when evaluating a patient with well-controlled diabetes mellitus, such as multiple system atrophy, as well as hypovolemia, hypopituitarism, adrenal dysfunction, or other peripheral neuropathies.

Autonomic Questionnaires

It is important to ask for a comprehensive review of systems when seeking out symptoms of dysautonomia due to the widespread and varied total body regulatory effect of the autonomic nervous system. Autonomic dysfunction impacts the quality of life for patients that suffer from chronic medical conditions such as health failure and diabetes. Often these symptoms are volunteered to their providers; however, a screening questionnaire provides systematic and thorough documentation of autonomic complaints. Screening for autonomic dysfunction in patients with diabetes assists in deciding on the battery autonomic tests used to confirm diabetic autonomic neuropathy based on the symptoms endorsed.

The Autonomic Symptom Profile (ASP) is a 169-item questionnaire that assesses 11 domains of autonomic function. ASP is a validated scale for autonomic symptoms with its results providing a composite score also known as The Composite Autonomic Symptom Scale (COMPASS) [6]. COMPASS is a survey instrument

developed at Mayo Clinic Autonomic Disorders Center, comprising 84 questions adopted from the ASP, addressing 11 autonomic domains. COMPASS-31 is a refined iteration of the original COMPASS that has condensed the survey to a set of 54 questions by removing less meaningful and redundant questions in an effort to improve ease-of-assessment and internal consistency. Notably, this revised questionnaire removed syncope and erectile dysfunction from the assessed domains and combined gastroparesis, constipation, and diarrhea into a single gastrointestinal domain [7].

A more convenient clinical questionnaire is the Survey of Autonomic Symptoms or SAS. SAS is a set of 12 questions which allows the patient to endorse 12 varied health problems and grade their severity from 1 ("not at all") to 5 ("a lot"). Symptoms listed include: lightheadedness, dry mouth/dry eyes, pale or blue feet, cold feet, decreased sweating in the feet either with exercise, hot weather, or compared to the rest of the body, increased sweating in the hands, nausea/vomiting/bloating after small meals persistent diarrhea, persistent constipation, urine leakage, erectile dysfunction if the patient is male. The complete tables SAS and ACP have shown good external consistency to each other when applied to patients with early diabetic neuropathy. When receiver operating characteristic (ROC) curves were compared, there is also no significant difference in specificity between the tests for the tested sample group [8].

Physical Exam

A physical examination of the autonomic nervous system from a neurologist's perspective is systematic and relevant to the patient's endorsed symptoms. Given the diverse constellation of symptoms related to autonomic function, there is value in developing a consistent foundation for all patients with concern for dysautonomia. In addition to the standard neurologic exam, several additional features with pertinence to patients presenting with suspicion for autonomic dysfunction are presented below.

Orthostatic vital signs should be taken in the supine, sitting, and standing positions for all autonomic patients in the clinical setting. Patients with a high suspicion for orthostatic intolerance should be instructed to record their heart rate and blood pressure at home. Data acquired at home are most useful when logged by patients consistently, and at the same time of day. One strategy is to advise patients to keep an over-the-counter BP cuff on their nightstand and measure vitals every morning upon awakening.

Pupils should be examined for anisocoria, afferent pupillary defects, and saccadic eye movements. Unilateral miosis and ptosis suggest a possible cranial nerve 3 palsy. Combined with anhidrosis, these three findings are consistent with Horner's syndrome or oculosympathetic paresis. Reduced horizontal saccades can be a sign of degenerative central nervous system diseases that cause autonomic dysfunction. Impaired nystagmus or saccades may be caused by optic nerve pathology. The

presence of these features do not exclude the presence of cerebral or brainstem pathology. Damage to the optic tract and cortex may also cause impaired nystagmus, smooth pursuit, or saccades. The presence of a visual field deficit or oculomotor apraxia would not be expected from solely a peripheral nerve pathology and a central cause should be considered.

Pinprick testing is helpful in assessing patients with diabetic neuropathy as length-dependent loss of large fibers can cause loss of pain sensation. Patients with small fiber neuropathy, either secondary to diabetes or otherwise, will be unable to confidently and consistently distinguish between light touch and pain. Light touch and vibration are innervated separately from pain and proprioception.

Patients will typically have shoes and socks removed for diabetic foot exams. This is a good opportunity to test length-dependent pain sensation. Using a safety pin, gently prick the skin without drawing blood at the sternum with a bare chest, where pain perception is expected to be preserved. In order to confirm full pain perception, pin prick at the chest is compared with the patient's forehead and nape of neck. Using the location with most pain as the control group, the skin is pricked starting from the sole of the foot and moved proximally up the leg. The patient states when full pain is perceived, where the sensation of pain from the pin is equal to that of the chest, forehead, or nape of the neck. This examination is repeated for the upper extremities starting from the dorsal surface of the hand and continuing up the arm. If the full length of the extremity is reached without eliciting a full pain response, trial pinpricks on the torso and lower back should be performed. An alternate approach is to have the patient label their pain sensation on the extremities as a percentage of full pain response compared to the control.

Patients with length-dependent diabetic neuropathy will elicit a length-dependent loss of pain sensation, also known as the "stocking-glove" pattern. Typically, if the loss of pain sensation rises superior to the knees, the upper extremities will also be involved starting with the fingertips. While this is the most identifiable pattern of sensory loss, there are other patterns of sensory loss. Incongruous patches of sensory loss may be found in small fiber neuropathy and large fiber plexopathy, both of which may be secondary to diabetic neuropathy. Documenting the extent of involvement is a useful data point in tracking improvement or worsening of denervation.

Patients with large fiber diabetic neuropathy may also have hyporeflexia given involvement of peripheral neuropathy. Achilles and patellar reflexes may be decreased or absent when compared to age-equivalent counterparts. There is frequently physiologic hyporeflexia in the elderly and hyperreflexia in children. The patellar reflex is frequently absent in patients who have undergone total knee arthroplasty. Diffusely brisk hyperreflexia in the elderly patient should be concerning for upper motor neuron pathology and would not be expected solely from peripheral nerve disease resulting from diabetes or other pathologies.

A skin exam may compliment the autonomic nervous system exam. Damage to sympathetic fibers may cause inappropriate skin flushing (hyperemia) and sudomotor dysfunction (anhidrosis). This may also be noted in a length-dependent fashion on the extremities.

Table 19.1 The composite autonomic scoring scale for laboratory quantification of generalized autonomic failure

Sudomotor index
1. Single site abnormal on quantitative sudomotor axon reflex test *or*
Length-dependent pattern (distal sweat volume) *or*
Persistent sweat activity at foot
[On thermoregulatory sweat test, anhidrosis present but <25%][a]
2. Single site <50% of lower limit on quantitative sudomotor axon reflex test
[On thermoregulatory sweat test, anhidrosis 25–50%][a]
3. Two or more sites <50% of lower limit on quantitative sudomotor axon reflex test
[On thermoregulatory sweat test, anhidrosis >50%][a]
Adrenergic index[b]
1. Phase II_c decrease of <40 but >20 mmHg mean BP *or* Phase II_I does not return to baseline *or* Decrease in pulse pressure to ≤50% of baseline
2. Phase II_c decrease of <40 but >20 mmHg mean BP + phase II_I or IV absent
3. Phase II_c decrease of >40 mmHg + absent phases II_I and IV
4. Criteria for 3 + orthostatic hypotension (systolic BP decrease of ≥30 mmHg; mean BP decrease of ≥20 mmHg)
Cardiovascular heart rate index
1. $HR_{\&\$\$;}$ or VR mildly decreased (above 50% of minimum)
2. $HR_{\&\$\$;}$ or VR decreased to <50% of minimum
3. Both $HR_{\&\$\$;}$ and VR decreased to <50% of minimum

BP blood pressure, $HR_{\&\$\$}$ heart rate response to deep breathing, *VR* Valsalva ratio
[a]Could be substituted for results of quantitative sudomotor axon reflex test
[b]Phases refer to components of the Valsalva maneuver: II_c and II_I = early and late portions, respective, of phase II

While no single test confirms autonomic neuropathy, the diagnosis of autonomic dysfunction should be supported with an arsenal of laboratory testing including orthostatic, cardiovascular, and skin histologic testing. The Composite Autonomic Scoring Scale (CASS) had been developed as a 10-point reflex screen involving sudomotor, adrenergic, and cardiovagal testing. A score of 5 or more would be consistent on CASS to have autonomic failure [9]. CASS scores have been studied in patients with mild diabetic neuropathy and demonstrated weak specificity, implying a need to evaluate symptoms beyond the scale [10] (Table 19.1).

Autonomic Testing

Tilt Table Testing

Patients demonstrating impaired vasovagal reflex are prone to vasovagal and vasodepressor syncope. In vasovagal syncope, a positional change results in a sudden fall in BP and HR resulting in loss of consciousness, whereas in vasodepressor

syncope, only the BP falls. Syncopal events captured with tile table testing can assist with identifying the exact orthostatic intolerance [11].

Cardiovagal Testing

Cardiovagal function can be evaluated by testing heart rate response to both deep breathing and the valsalva maneuver. In a physiologic Valsalva maneuver, the temporary rise in thoracic and abdominal pressure will both decrease and narrow the gap between systolic and diastolic blood pressures. This is correlated to a simultaneous rise in heart rate followed by a rapid return to normotension and normocardia [11].

Adrenergic failure due to autonomic dysfunction will result in delayed blood pressure recovery after an attempt at Valsalva. An impaired baroreflex prevents any physiologic rise in heart rate during this maneuver. Formal laboratory testing in autonomic function typically involves the patient sustaining a 15-s expiration against a fixed pressure resistance through a mouthpiece, while heart rate and breath-by-breath blood pressures are recorded.

Deep breathing is another form of cardiovagal testing wherein patients are instructed to breathe through a pCO2 mouthpiece at precisely 6 breaths per minute, or 5-s inhalation followed by a 5-s exhalation. Patients are instructed not to breath-hold or hyperventilate, which is verified by pCO2 monitoring. The difference in heart rate between end-of-expiration and end-of-inspiration is known as respiratory sinus arrhythmia (RSA). RSA values are usually averaged over 6 breath cycles after 1 min of unrecorded deep breathing. The patient's RSA is compared to a table of normative values stratified by age [12]. Impairment in the cardiac parasympathetic system causes a loss of physiologic heart rate change due to deep breathing.

Sudomotor Function Testing

Sweat gland or sudomotor dysfunction may be indicative of loss of thermoregulation, found in synucleinopathies such as MSA, Parkinson's disease, or other causes of autonomic dysfunction. Signs of peripheral thermoregulation are predominately two mechanisms regulated by the hypothalamus: vasodilation, vasoconstriction, and sweat production. Unmyelinated postganglionic sympathetic C-fibers innervate eccrine sweat glands and are activated by acetylcholine [13].

Quantitative sudomotor axon reflex testing (QSART) is the most widely used test for sudomotor function over a limited surface area, typically the forearm, leg, or dorsal foot. The technique is able to characterize the topographic pattern for sweat across the sweat surface. Acetylcholine (10%) is administered transdermally via iontophoresis to the prepared skin, which activates the nicotinic and muscarinic receptors in the terminal nerve endings of the dermis, causing a local direct sweat

response. Sweat from glands in adjacent skin are evoked by collateral innervation of adjacent sudomotor nerve fibers and characterized as an indirect sweat response. Both sweat responses are quantified as a change in relative humidity over time and then compared to corresponding sweat output control values, usually grouped by sex and body location.

In presumed diabetic autonomic dysfunction, QSART sudomotor testing demonstrates the loss of acetylcholinergic function with distal C-fibers, and the resulting postganglionic sudomotor dysfunction.

The Thermoregulatory Sweat Test (TST) is the gold standard for comprehensive sudomotor evaluation. Quinizarin color indicator dye is applied to the ventral skin surface of a patient in both humidity- and temperature-controlled rooms. The patient is then heated to 1 °C above baseline-measured temperature to a maximum of 38 Celsius for up to 70 min. The percentage of anhidrotic skin and distribution of sweating patterns is digitally logged for analysis. The sweat pattern in diabetic neuropathy typically shows length-dependent stocking distribution of impaired sweat gland function.

Skin Biopsy for Immunohistochemistry

Intraepidermal nerve fiber density is correlated with the progressive loss of nerve fibers in diabetic autonomic neuropathy. The overall reduction in the sympathetic adrenergic innervation within the pilomotor muscles is correlated with the severity of diabetic neuropathy.

Skin biopsies 3 mm in diameter and 3–5 mm in depth are sampled from the distal leg or thigh, and the frozen sections are processed. Typical immunolabeling is applied with various stains to identify sweat gland basement membrane, epithelium, muscle errector pilorum, noradrenergic fibers, and sudomotor cholinergic fibers. Subsequent washes and antibody staining can be applied to the pathology in order to identify endothelium, sweat gland tubules, and other clinically relevant structures [14, 15].

Practically speaking, it is not necessary to send for pathology to confirm autonomic neuropathy in diabetes. However, a skin biopsy sent to evaluate for small fiber neuropathy in patients can be useful in quantifying the severity of autonomic and peripheral nerve system disease. A decline in sudomotor and pilomotor nerve fiber density is also expected in prolonged nerve damage from diabetes and is associated with high Hgb A1c levels [16]. A reduction in sympathetic adrenergic innervation within pilomotor muscles correlates with the severity of diabetic neuropathy.

Other Clinically Appropriate Testing

Erectile dysfunction or urodynamic insufficiency should be evaluated by Urology or Gynecology. If there is a clinical suspicion of autonomic dysfunction involving the gut, motility studies, including antroduodenal manometry [17], should be performed,.

Clinical Manifestations of Autonomic Dysfunction in DM

Early onset of type 1 diabetes results in a longer burden of disease with a resulting increase in diabetic-related complications seen in the aging population along with a lower quality of life in those adults with worse glycemic control [18]. Individuals with type 2 diabetes tend to develop diabetes-related complications at or early after diagnosis including neuropathy and nephropathy along with other micro- and macrovascular impairments. This may be related to the delay in treatment of type 2 diabetes as a result of individuals going undiagnosed for many years.

Peripheral Neuropathy

The most common neurological manifestation of DM is peripheral neuropathy. The most common causes of peripheral neuropathy in the United States and Europe are pre-diabetes and type 2 diabetes (T2D). At least half of all diabetic patients, including patients with type 1 diabetes (T1D), develop some form of neuropathy during their lifetime. Over 20 million Americans currently have neuropathy secondary to either pre-diabetes, T1D, or T2D, and this number will double as more Americans develop pre-diabetes and T2D.

Diabetes can produce several types of peripheral nervous system (PNS) damage. The most common type of nerve damage is bilateral and symmetrical damage to nerves of the feet, with a distal-to-proximal gradient of severity, known as a stocking-glove neuropathy. Because this pattern of nerve injury is so common, this neuropathy is synonymously called diabetic neuropathy (DN) [19]. A similar pattern of injury occurs with pre-diabetes, supporting the idea that nerve injury secondary to diabetes is a continuum from normal glycemia to varying levels of hyperglycemia.

DN is primarily a disorder of sensory nerves, and, early in the course of DN, patients commonly experience positive sensory symptoms in the feet such as pain, tingling, and paresthesias as well as negative symptoms such as numbness; disordered sensory processing may evoke pain when the feet are touched (allodynia) and increase sensitivity to noxious stimuli (hyperalgesia). Only much later in the course of the disease is there evidence of motor nerve dysfunction with distal weakness of the toes, or in extreme cases, the ankles and calves. The progressive loss of lower extremity sensation and subsequent motor weakness, results in loss of balance, falls, and a numb, insensate foot [20, 21].

Orthostatic Hypotension

Orthostatic intolerance is common in patients with dysautonomia. Patients typically endorse symptoms provoked with change in posture from lying down, to sitting, and standing. Specifically, OH is defined as a drop in systolic blood pressure (SBP) of

at least 20 mmHg or a drop in diastolic blood pressure (DBP) of at least 10 mmHg within 3 min of standing. Symptoms range from lightheadedness, vision blurring, cognitive difficulty colloquially called "brain fog," fatigue, or abnormal movements such as tremors. Patients may also complain of palpitations, dyspnea, or loss of consciousness as a result of impaired baroreflexes. Formal tilt table testing can assist in the formal diagnosis of Orthostatic Hypotension (OH) or Postural orthostatic tachycardia syndrome (POTS).

A meta-analysis determined that the prevalence of orthostatic hypotension (OH) in DM was 24% (95% CI: 19%–28%). In this meta-analysis, HbA1c, hypertension, and diabetic nephropathy were significantly associated with the increased risk of OH in DM. Therefore, it is vital to control the blood glucose and BP and delay the progression of diabetic nephropathy in diabetic patients. The risk factors associated with OH include older age; and comorbidities such as hypertension, congestive heart failure, and kidney disease [20, 21] along with the use of antihypertensive drugs (including diuretics, vasodilators), tricyclic antidepressants, and insulin.

The prognosis of OH in DM is associated with a higher risk of total mortality and cardiovascular events. An orthostatic evaluation should also be a part of the diagnostic workup in diabetic patients.

POTS is defined as an increase in HR > 30 bpm within 5 min of positional change without OH. A diagnosis of POTS almost always requires tilt table testing to rule out the presence of OH.

Gastroparesis

Gastroparesis is a delayed gastric emptying in the absence of obstruction, a complication seen in patients with both T1D and T2D. It occurs with a prevalence of up to 40% of gastroparesis in patients with T1D and 10–30% in patients with T2D. Over a 10-year period, approximately 5.2% of patients with T1DM developed gastroparesis, whereas 5 times fewer (1%) patients with T2DM developed gastroparesis over that same period [22].

Symptoms associated with gastroparesis, such as early satiety, prolonged fullness, nausea, vomiting of undigested food, and bloating are some of the most common complaints and affect the patient's quality of life. Nausea is the most common symptom; however, other causes of nausea such as gastroesophageal reflux disease (GERD) or constipation or gallbladder disease, common disorders in patients with diabetes, must be considered. Gastroparesis also affects nutrient absorption, and, as a result it may affect medication absorption, which, in turn, affects glucose control. Nausea typically worsens postprandially. Symptoms are similar in patients with T1DM and T2DM, although patients with T2DM tend to have more fullness and bloating [23]. Tests to evaluate for the presence of gastroparesis and gastric dysrhythmias include nuclear medicine scintigraphy, wireless capsule endoscopy, and electrogastrography (EGG).

Temperature and Sweat Changes

Patients with T1D and T2D may have abnormal core body temperature control and encounter difficulties with body heat dissipation. This occurs primarily through decreased capacity of eliminating heat through sweat production in warmer temperatures or due an attenuated capacity to increase metabolic heat production and decrease skin blood flow during cold exposure. More specifically, the patients have lower skin blood flow and sweating responses during heat exposure which can have important consequences on cardiovascular regulation and glycemic control.

This presence and severity of the temperature and sweat dysregulation in patients with T1D is directly correlated with longer disease duration [24].

Pathophysiology of Diabetic Autonomic Neuropathic Changes

The pathophysiology of DN is multifactorial with structural and metabolic alterations within axons, Schwann cells, and microvascular elements within the endoneurium and extracellular matrices [25]. Newer findings suggest that changes in the endoneurial capillary morphology and vascular reactivity are present before the development of diabetic neuropathy [26]. In addition, there is an association between the level of endoneurial hypoxia and reductions in nerve conduction velocity [27]. There are a number of pathways felt to be at play in diabetic neuropathy (Fig. 19.3).

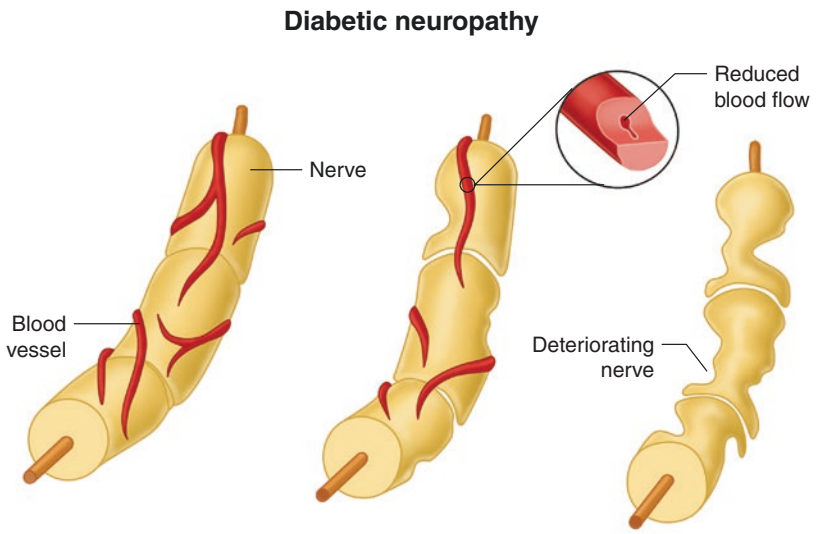

Fig. 19.3 Diabetic neuropathy

Increased glycolysis in response to excess glucose disrupts several metabolic pathways that can promote neuronal injury. The glycolysis intermediate fructose-6-phosphate enters the hexosamine pathway and undergoes a series of reactions to form uridine 5-diphosphate-N-acetylglucosamine (GlcNac). GlcNac is one of the sugar moieties that bind serine/threonine residues on common transcription factors, such as Sp-1, promoting lipid dyshomeostasis, inflammation, and injury of complication-prone tissues, including peripheral nerves [28]. Increased glycolysis leads to the accumulation of another intermediate, dihydroxy-acetone phosphate, which is converted to diacylglycerol (DAG). Increases in DAG are also well documented in complication-prone tissues, especially nerves, where DAG activates neuronal Protein Kinase C (PKC) [29]. Activated PKC leads to multiple metabolic impairments that range from insulin resistance to disrupting the function of Na/K ATPase to altering gene expression of vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β), leading to vasoconstriction, hypoxia, and neuronal damage [30].

In the polyol pathway, intracellular glucose is converted to sorbitol by the rate-limiting enzyme aldose reductase [31]. The activation of this pathway may result in osmotic damage and diminution of Na+/K+-ATPase activity [32]. These processes lead to increased intracellular oxidative stress [33].

Oxidative Stress

Oxidative stress is considered a central facet in the development of diabetes and associated micro- and macrovascular complications.

Tumor Necrosis Factor-Alpha

Cytokines are produced by cells from the immune system including mast cells, Schwann cells, fibroblasts, and sensory neurons. Tumor necrosis factor-alpha (TNF-α) is a potent systemic pro-inflammatory cytokine and is a central component of the inflammatory response, in various immune-mediated inflammatory diseases. It is a pathophysiological feature of such disorders, such as rheumatoid arthritis, which are characterized by chronic inflammation. TNF-α is produced in Schwann cells and has a role in peripheral nerve regeneration and regulation of apoptosis. T2DM patients with neuropathy have higher levels of TNF-α compared to patients without neuropathy and healthy controls [34]. In addition, treatment with anti-TNF-α monoclonal antibody in an animal model of painful diabetic neuropathy exhibited a neuroprotective effect [35].

Mitochondrial Dysfunction and Diabetes

Accumulating data suggest that Schwann cells (SCs) are much more than passive insulators for axons. SCs may be critical sensors of axonal activity and provide the needed energy for axonal function. Thus, an emerging idea is that disruption of the

normal "bioenergetic cross-talk" between SCs and axons during T2D underlies DN. It is possible that this reprogramming leads to SC insulin resistance and axonal starving. Alternatively, substrate overload from the SCs to the myelinated axon results in pH changes and/or axonal mitochondrial injury, characterized by mitophagy and/or the transfer of toxic lipid species from affected SCs to the axonal compartment, leading to mitochondrial injury. Collectively, these insults to mitochondria in experimental diabetes result in loss of axonal energy stores and axonal injury, promoting DN [36].

Axons are highly vulnerable to diabetes-mediated injury due to their abundant expression of ion channels. Axons are known to express a number of distinct voltage-gated sodium channels (VGSCs) as well as the Na^+Ca^{2+} exchanger isoform 2 (NCX2) in their termini [37]. Na/K ATPase is required to export intra-axonal Na^+ that accumulates following action potential propagation; however, this function fails when ATP levels are below normal. This, in turn, leads to increased intra-axonal Na^+, reversal of the NCX2, increased intracellular Ca^{2+}, and axonal degeneration.

Interventions for Autonomic Dysfunction

Conservative Therapies for Orthostatic Hypotension

Drinking water and increasing salt intake increase plasma volume, which help to maintain blood pressure upon standing. The recommended daily intake of water is 2.0 L/day and of sodium chloride is 3–6 g [38]. Increasing fluid intake has been shown to have a positive effect comparable to that of orthostatic hypotension medications [39, 40] and has only mild adverse outcomes. The salt may be incorporated into meals or can be supplemented in tablets. Patients may find electrolyte tablets or liquids easier to tolerate than salt tablets. Fluids and electrolytes should be concentrated in the morning and afternoon and minimized after dinner to reduce nocturia [41]. High-salt intake must be monitored carefully because it may lead to cardiovascular complications [42].

Compression stockings reduce venous pooling in the lower extremities and promote venous return and cardiac output. The categories of compression stockings include knee-length, thigh-length, full-length, and abdominal compression. The current literature reports modest efficacy and inconsistencies in the degree and location of compression. Although compression stockings provide orthostatic relief, there are challenges with compliance. Leg compression sleeves at 20mmHG which do not cover the feet are the easiest to put on and are associated with the best compliance. This is an important component of the therapeutic plan. The stockings should be put on in the morning and worn when the patient is out of bed. Compression stocking use is advised during exercise, with larger stockings producing better results. Ideally, if tolerated, a patient should wear full-length lower extremity stockings producing a pressure of 30–40 mmHg. Abdominal compression can additionally reduce pooling in the splanchnic circulation [43, 44].

Pharmacological Management for Orthostatic Hypotension

In most cases, pharmacological treatments are administered with non-pharmacological treatments. These medications have different mechanisms of action and are often used in combination.

Fludrocortisone

Fludrocortisone, primarily used for adrenocortical insufficiency in both the USA and Europe, is a first-line monotherapy agent to manage orthostatic hypotension. The recommended dose is 0.1–0.2 mg/day, and it can take up to 5 days to see the full effects. Fludrocortisone acts as a systemic corticosteroid increasing sensitivity to circulating catecholamine [45]. Fludrocortisone is often the first medication added to help in the management of orthostatic hypotension as it increases plasma volume. Because fludrocortisone acts by intravascular volume expansion, its pressor effect is gradual. Fludrocortisone elevates both standing systolic and diastolic blood pressure [46, 47] and decreases orthostatic symptoms [48]. Patients should be monitored for hypokalemia and hypomagnesemia. It can also cause supine hypertension, and therefore is not recommended for patients with congestive heart failure or chronic renal failure.

Midodrine

Midodrine, a peripheral α-1 adrenoceptor agonist, exerts a pressor effect on both venous and arterial constriction, and is effective 1 h after ingestion [49]. The recommended dose (typically given in the morning, noon, and afternoon to avoid supine hypertension in the evening) is up to 10 mg three times daily; each dose typically lasts for 4 h, consistent with blood levels of the active metabolite desglymidodrine. In double-blind studies, midodrine gave a dose-dependent increase in mean standing systolic blood pressure and resulted in significantly higher mean global improvement of orthostatic symptoms scores compared with placebo [50, 51]. Other potential side effects include piloerection, itchiness, and urinary retention [52]. Midodrine is often used in combination with fludrocortisone and/or droxidopa although clinical trials evaluating these combinations have not been conducted.

Droxidopa

Droxidopa (L-threo-dihydroxyphenylserine) is a synthetic prodrug that is converted into norepinephrine by the ubiquitous enzyme dopa-decarboxylase. In numerous trials, droxidopa decreased postural drop in patients with orthostatic hypotension [53]. In a recent phase III clinical trial, droxidopa improved Orthostatic Hypotension

Symptom Assessment and Orthostatic Hypotension Daily Activity Scale in patients with neurogenic orthostatic hypotension [54]. Droxidopa treatment increased standing systolic blood pressure, reduced dizziness upon standing, and reduced the number of falls. Droxidopa is safe and well tolerated by patients with symptomatic neurogenic orthostatic hypotension [55]. Furthermore, it did not significantly increase supine blood pressure in the evening, thereby minimizing the risk for supine hypertension overnight [56]. The US FDA-recommended dose is 300–600 mg three times daily.

Pyridostigmine (Mestinon)

Pyridostigmine is a cholinesterase inhibitor that improves neurotransmission at acetylcholine-mediated neurons of the autonomic nervous system. In a double-blind crossover study, patients were randomized to groups receiving 60 mg of pyridostigmine; 60 mg of pyridostigmine with 2.5 mg of midodrine; 60 mg of pyridostigmine with 5 mg of midodrine; or placebo. Compared with the placebo group, treatment groups demonstrated a decreased drop in standing diastolic blood pressure without worsening supine hypertension. Adverse effects include loose stools, diaphoresis, hypersalivation, and fasciculations [57].

Treatment of Neuropathic Pain

Tricyclic Antidepressants

TCAs are one of the most studied antidepressants for the treatment of neuropathic pain. They have been shown to be effective in the treatment of peripheral neuropathy, post-herpetic neuralgia, and neuropathic pain, post-spinal cord injury and of limited effect in radiculopathy, HIV, and chemotherapy-induced peripheral neuropathy [58].

TCAs have multiple modes of action, with the most important pain-relieving effect likely being via inhibition of serotonin and noradrenaline re-uptake [59]. However, they also block histamine, adrenaline, acetylcholine, and sodium channels, accounting for their broad side effect profile [60]. Caution is required in the use of TCAs in the elderly and frail to avoid potential adverse effects such as falls, cardiac arrhythmias, orthostasis, urinary retention, and dry mouth.

Serotonin and Norepinephrine Reuptake Inhibitors

Serotonin and norepinephrine reuptake inhibitors (SNRIs) are considered first-line treatment in multiple international guidelines [61]. The most commonly studied are duloxetine and venlafaxine. They facilitate descending inhibition by

blocking serotonin and noradrenaline reuptake [62]. They have been shown to be effective in peripheral diabetic neuropathy and painful peripheral neuropathy [63].

Gabapentinoids Include Gabapentin and Pregabalin

They are a group of anticonvulsant medications that act by blocking presynaptic alpha-2-delta calcium channels in the dorsal horn, inhibiting neurotransmitter release [64]. They are considered first-line agents in the treatment of neuropathic pain by multiple international societies [65]. Gabapentin and pregabalin both have been shown to be effective in post-herpetic neuralgia and diabetic peripheral neuropathy [66].

Gabapentinoids should be trialed for a 4- to 6-week period with 2 weeks at the maximum tolerated dose [67]. Poorly tolerated side effects or inadequate pain relief should prompt dosage adjustment, cessation of the medication, progression to other first line agents, or a trial of combination therapy. The most common adverse effects include somnolence, fatigue, dizziness, and lower extremity edema [68].

Combination Therapy

Combination therapy may increase efficacy and, as a result of the smaller doses of the individual drugs, have less side effects. No one drug is effective for all patients, and, as seen above, pain relief is usually partial and side effects limit tolerability. Not surprisingly, 45% of those with neuropathic pain utilize two or more medications for their pain [69]. Ninety percent of patients with painful DN require multiple medications for their pain [70].

In diabetic peripheral neuropathy, nortriptyline plus pregabalin was shown to be more effective at decreasing pain than monotherapy [71]. Similarly, the combination of the TCA, imipramine, and pregabalin saw improved pain scores, with an average two-point (31%) decrease on the Numeric Pain Rating Scale (NPRS) scale, significantly greater than pregabalin or imipramine alone; however, side effects were higher [72]. Combination therapy should be trialed for the trial duration of the second medication and ceased if ineffective or if there are significant side effects.

Future Research: GLP1

Like insulin, glucagon-like peptide 1 (GLP-1) may have direct trophic actions on the nervous system, but its potential role in supporting diabetic sensory neurons is uncertain. There are significant GLP-1 receptors on dorsal root ganglia sensory neurons of diabetic and nondiabetic mice. Exendin-4, a GLP-1 agonist, increased neurite outgrowth of adult sensory neurons in vitro.

High-dose insulin alone reversed hyperglycemia in type 1 diabetic mice, partly reversed thermal sensory loss, improved epidermal innervation but failed to reverse electrophysiological abnormalities. Exendin-4 improved both sensory electrophysiology and behavioral sensory loss. Low-dose insulin was ineffective.

In type 2 diabetes, hyperglycemia was uncorrected, and neither insulin nor exendin-4 reversed sensory electrophysiology, sensory behavior, or loss of epidermal axons. However, exendin-4 alone improved motor electrophysiology. These results suggest that although GLP-1 agonists and insulin alone are insufficient to reverse all features of diabetic neuropathy, in combination, they might benefit some aspects of established diabetic neuropathy [73].

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Chapter 20
Diabetes and Erectile Dysfunction

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Introduction

Metabolic syndrome describes a constellation of cardiovascular risk factors that include elevated blood pressure, insulin resistance, hyperlipidemia, and central obesity. The global prevalence is increasing and is a significant cause of morbidity and mortality [1]. The implications of metabolic syndrome include the development of type 2 diabetes mellitus (T2DM), coronary artery disease (CAD), hypertension (HTN), and other ailments.

Persistent hyperglycemia, such as in T2DM, can lead to heart disease and stroke. Further health issues such as peripheral neuropathy, nephropathy, retinopathy, and sexual dysfunction are known complications of T2DM. Similarly, HTN can lead to heart failure, coronary artery, stroke, nephropathy, peripheral vascular disease, and vascular dementia. In both disorders, sexual dysfunction is an unfortunate complication. Although not life threatening, it can have significant psychological effects, such as depression, anxiety, self-esteem issues, and overall reduced quality of life [2]. Erectile dysfunction (ED) is characterized by the inability to obtain and or maintain an erection sufficient for sexual activity [3]. Before understanding the diagnosis and management options, it is crucial to understand the interplay between ED and these chronic diseases as they are intimately connected.

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Epidemiology

The global prevalence of T2DM in 2019 was estimated to be 463 million people (9.3%) and projected to rise to 578 million (10.2%) by 2030 and 700 million (10.9%) by 2045 [4]. Likewise, in 2010, there was an estimated 1.38 billion people, or 31.1% of the global population, meeting HTN criteria [5]. This number is projected to increase to 1.56 billion people worldwide by year 2025 [6].

As these numbers rise, medical professionals are facing further challenges in treating such complex patients. Among many cardiovascular complications, erectile dysfunction (ED) is a common consequence of chronic illnesses such as T2DM, HTN, and CAD. Andrologists have seen an increase in patients presenting with ED, including those belonging to a younger age group. Classically, ED has been considered an age-dependent disease and more common in men older than 40 years. However, recent data suggests that the prevalence of ED even in men less than age 40 has been increasing [7, 8]. Although psychogenic ED is commonly identified in younger individuals, metabolic disorders are more frequently diagnosed as the culprits in this age group. Other known risk factors for developing ED include tobacco use, obesity, and lack of physical activity [9, 10].

ED's global prevalence has been documented to be as high as 76% across several studies that include men aged 18 to 80 years [11]. The significance is that ED may be a sole presenting sign of an underlying undiagnosed cardiometabolic disease and should be evaluated as such. Montorsi et al. found that in 147 patients with ED and CAD, approximately 67% of patients presented with ED symptoms before CAD [12]. Understanding the relationship between ED and common cardiometabolic disorders is essential for providers to maintain a global view and to have a higher index of suspicion to identify these severe illnesses at earlier stages.

Physiology of Erectile Function

Basic Anatomy

Smooth muscle dilation, adequate systolic arterial inflow, occlusion of venous outflow, and an intact neurologic system are necessary to achieve an erection. The penile anatomy is comprised of two dorsally located corpora cavernosa and one ventral corpus spongiosum. The corpora cavernosa are filled with sinusoids and smooth muscle cells that are ultimately responsible for erections, and the corpus spongiosum encases the urethra. The tunica albuginea is an elastin-rich multilayer covering of the corpus cavernosum that houses emissary veins between the layers. Compression of these emissary veins allows for more efficient blood trapping during erections.

The internal iliac artery gives off the internal pudendal artery, which becomes the common penile artery, dividing into three branches (dorsal artery, bulbourethral artery, and cavernous artery) that will supply the penis. The cavernous artery runs in the center of the corpora cavernosa, gives off helicine arteries, and is responsible for adequate blood flow during erections.

From a neurologic standpoint, the cavernous nerves contain sympathetic and parasympathetic nerve fibers that relay fibers to the dorsal nerves, which travel atop the penile shaft. Sacral parasympathetic input and thoracolumbar sympathetic input will join the pelvic nerve plexus where the cavernous nerve emerges. Parasympathetic input stimulates erections while sympathetic input initiates detumescence.

Mechanism of Erectile Function

Understanding the multiple pathways that mediate erections can clarify the rationale behind the various treatment options for erectile dysfunction. In the flaccid state, the smooth muscle of the corpus cavernosum is tonically contracted. Smooth muscle contraction is mediated by two primary pathways: an increase in cytosolic calcium that signals smooth muscle contraction and the Rho kinase pathway that serves as a calcium sensitizing mechanism.

Several pro-erectile factors include nitric oxide (NO), prostaglandin E1 (PGE1), papaverine, and phentolamine. NO released from nonadrenergic/noncholinergic (NANC) nervous endings is the primary neurotransmitter that facilitates erections via a decrease in cytosolic calcium concentration. NO synthase (NOS) controls the production of NO either in the cavernous nerve terminals by nNOS or in the endothelium by eNOS. NO diffuses into smooth muscle cells and activates the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) via guanylyl cyclase [13]. Phosphodiesterase 5 (PDE5) is responsible for the breakdown of cGMP, and thus PDE5 inhibitors are used as a standard treatment option for erectile dysfunction by preventing the degradation of cGMP [14].

Similarly, alprostadil, a PGE1 analog, activates adenosine triphosphate conversion (ATP) to cyclic adenosine monophosphate (cAMP). Papaverine works to prevent the degradation of cAMP by inhibiting PDE 2, 3, and 4. Overall, increased cGMP levels and cAMP lead to decreased intracellular calcium and thus allow for erections [15]. Phentolamine, a nonselective alpha-receptor blocker, acts via a slightly different mechanism than the other molecules. Typically, activation of the alpha-1 receptor on the smooth muscle cell will raise inositol triphosphate and subsequently initiate a release of calcium from the sarcoplasmic reticulum. The increased cytosolic calcium levels lead to smooth contraction. In contrast, blockade of this mechanism with the use of phentolamine promotes smooth muscle dilation and erections.

Clinical Evaluation of Erectile Function

There are several validated instruments used as an initial evaluation tool to determine the presence of ED. Two commonly used questionnaires include the International Index of Erectile Function (IIEF) and the Sexual Health Inventory for Men (SHIM). IIEF is a 15-item multidimensional self-administered questionnaire categorized into five different domains: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction [16]. The SHIM is an abbreviated 5-item form of the IIEF intended to be a more simplified tool for patients and physicians [17].

Erectile Dysfunction and Diabetes Mellitus

Diabetes mellitus is an endocrine disorder that is commonly divided into two subtypes. Type I diabetes mellitus (T1DM) is characterized by young age at presentation and autoantibodies against pancreatic islet β-cell antigens [18]. Type II diabetes mellitus (T2DM) is more prevalent and an acquired chronic illness among older age groups. Diabetes is accompanied by elevated blood glucose levels secondary to inadequate insulin or ineffective response to the insulin.

Molecular Mechanisms of ED in T2DM

There are several mechanisms by which T2DM can lead to erectile dysfunction, although not all details are well understood (Fig. 20.2). Inadequate vasodilation may occur as a result of somatic or autonomic nerve dysfunction [19]. It is proposed that T2DM can lead to decreased nNOS activity or endothelial dysfunction and, thus, overall decreased NO expression. Recall that nNOS is critical in the synthesis of NO at cavernous nerve terminal endings. An additional proposed mechanism is that the subsequent hemodynamic changes and shear stress that occur during early erections then lead to activation of eNOS to further release NO [20]. Endothelial dysfunction, in addition to decreased expression of vascular endothelial growth factor (VEGF), may lead to impaired or decreased eNOS activity. In ordinary circumstances, VEGF promotes endothelial cell proliferation and angiogenesis and increases eNOS phosphorylation and activity. Another likely contributing factor is oxidative stress. T2DM can lead to a persistent inflammatory state secondary to chronic hyperglycemia. In turn, the reactive oxygen species (ROS) that form can result in endothelial dysfunction and other forms of cellular damage [21]. An additional hypothesis that has been studied in mouse models is that insulin resistance and hyperinsulinemia can lead to upregulated sympathetic activity and, ultimately smooth muscle contraction [19].

Erectile Dysfunction and Hypertension

An interplay exists between hypertension and erectile dysfunction, stemming from their shared origin in vascular abnormality and endothelial dysfunction. Hypertension is the leading modifiable risk factor for cardiovascular disease and all-cause mortality worldwide. In 2017, the American College of Cardiology and American Heart Association Task Force on clinical practice guidelines redefined HTN in adults as systolic BP $\geq$ 130 mmHg and/or diastolic BP $\geq$ 80 mmHg. This change was based on multiple studies indicating a significant increase in the risk of developing cardiovascular disease with increased blood pressure above systolic BP 115 mmHg, as well as data showing that intensive blood pressure lowering reduces cardiovascular disease and all-cause mortality to a greater extent than does standard blood pressure lowering to a target of systolic BP $\leq$ 140 mmHg [22].

Structural Changes Caused by HTN Leading to ED

Certain pathophysiologic modifications that may result from endothelial dysfunction and tissue remodeling have been reported within the penis that mirror changes found hypertensive patients' blood vessels, including increased medial wall thickness and reduced vessel lumen diameter. These alterations seen in the systemic vasculature of hypertensive patients may similarly affect the small penile vessels in addition to the pudendal arteries, leading to a global decrease in blood supply to the penis necessary for firm erection achievement [23]. Experimental animal models have shown that ultrastructural pathologic changes occur in hypertensive rats, including decreased elastin and collagen content within the penile cavernosal sinus, thinned out tunica albuginea, and increased vascular resistance within penile vessels [24]. There is also an association between the degree of arterial hypertension and the degree of vascular smooth muscle proliferation and fibrosis within penile arteries. These changes limit arterial inflow to the cavernosal sinus leading to decreased erection rigidity [25]. Finally, there is a similar association between arterial hypertension and degree of smooth muscle proliferation within the cavernosal tissue itself, limiting the capacity of the smooth muscle relaxation mechanism necessary for penile engorgement [25]. Additional proposed structural relationships between hypertension and erectile dysfunction related to inflammatory response, neural changes, Schwann cell degeneration, and genetic factors continue to be investigated [26].

Erectile Dysfunction as a Marker of Cardiovascular Disease

Erectile function is dependent mainly on vascular and nervous system health. So, it is not surprising that ED is an unfortunate sequela of both HTN and DM in both early and late stages of the diseases. Furthermore, macro- and microvascular

complications are well-known consequences of these chronic illnesses, which overall lead to a higher risk of cardiovascular disease. Nonetheless, in the absence of HTN or DM, vasculogenic ED is a manifestation of the same disease process that leads to clinical cardiovascular disease.

Several animal and human studies have now documented the relationship between ED and T2DM. It is estimated that the incidence of ED is 2–3 times higher in men with T2DM [27, 28]. ED often presents within 10 years of the DM diagnosis. In men who present with ED alone, up to 12% will have underlying undiagnosed DM. For this reason, ED should be considered a marker of disease and should trigger screening for T2DM [29].

Similarly, approximately 30% of hypertensive male patients concomitantly endorse ED. The prevalence of ED is often higher, and the extent of ED is often more severe in patients with hypertension when compared to an age-matched general population [30, 31]. In men who report symptoms of ED alone, approximately 8–10% will have undiagnosed HTN at the time of presentation [32]. A large survey of 7689 men demonstrated that ED was present in 67% of men who had hypertension alone, in 71% with diabetes only, and 77% of men who had both [33].

Besides HTN and DM, additional risk factors include smoking, obesity, age, metabolic syndrome, and hyperlipidemia. ED may be the first presenting symptom of a more serious systemic illness, and it may manifest up to 5 years before a cardiovascular event [34]. A meta-analysis of 36,744 patients revealed that compared to men without ED, men with ED may experience an increased risk to 48% for cardiovascular disease, 46% for coronary artery disease, 35% for stroke, and 19% for all-cause mortality [35]. The 2018 American Urological Association Guidelines on ED underscores the importance of considering ED as a marker for cardiovascular disease [36]. With mounting evidence in this field, other major societies are likely to adopt these recommendations into their guidelines as well.

Treatment of Hypertension

Multiple non-pharmacologic interventions aimed to lower blood pressure include dietary modification (low salt, high potassium), moderation of alcohol consumption, weight loss, and increased aerobic physical activity [37]. Beyond lifestyle modifications, pharmacologic agents are the next step. Certain antihypertensive agents are also known to cause erectile dysfunction, with higher reported cases of erectile dysfunction seen in patients receiving combination therapy [38]. This begs the question of whether some hypertensive patients develop erectile dysfunction as part of their shared pathophysiology or as a sequela of their hypertensive treatment. Furthermore, the medication side effect of erectile dysfunction has also been cited as a reason for antihypertensive medication noncompliance [39]. Numerous agents are on the market to assist in blood pressure control including thiazide diuretics, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs), and calcium channel blockers.

Thiazide Diuretics

Thiazide diuretics have historically been among the most implicated class of antihypertensives to cause erectile dysfunction. They work to reduce blood pressure by decreasing sodium reabsorption by inhibiting the NA+/Cl- co-transporter within the distal convoluted tubule. As sodium and water are excreted, plasma volume is decreased and cardiac output decreases, driving a systemic response toward vasodilation. Thiazides have also been found to have some adverse metabolic side effects such as increased serum blood sugar, increased serum triglycerides, increase total cholesterol, and increased serum uric acid [40]. The mechanism for thiazide-induced erectile dysfunction has been hypothesized to be due to alterations in these metabolite levels or possibly related to volume depletion [41]. Multiple clinical trials have supported the finding that thiazides exert a negative effect on sexual function, whether used as monotherapy or adjunct therapy [42]. The Treatment of Mild Hypertension Study (TOMHS) reported that patents randomized to thiazide diuretic use experienced a significantly higher incidence of erectile dysfunction at 2 years than participants randomized to placebo, although this difference was not statistically significant at 4 years [43]. The Trial of Antihypertensive Interventions and Management (TAIM) study additionally suggested that men receiving thiazide diuretics experienced worsening of their erectile function when compared to those taking a beta-blocker and those receiving a placebo [44]. Consideration of these possible effects on erectile dysfunction should be taken when counseling hypertensive patients on their pharmacotherapy treatment options.

Beta-Blockers

Beta-blockers are a commonly used antihypertensive medication that has also been widely associated with sexual dysfunction. A large variety of beta-blocker formulations exist that vary in their β1/β2 selectivity, sympathomimetic activity, and vasodilatory capacity [30]. They function to lower blood pressure by reducing cardiac output, suppressing the renin-angiotensin-aldosterone (RAAS) system, and decreasing adrenergic outflow from the central nervous system to promote vasodilation [45]. There are multiple proposed etiologies for how beta-blockers contribute to erectile dysfunction including peripheral vasoconstriction, interaction with the adrenergic processes involved with erection and ejaculation, and reduction of testosterone levels [26].

The long-standing association between beta blockade and sexual function is supported by a number of studies. In particular, atenolol and carvedilol have both been shown to significantly reduce the number of sexual intercourse events that hypertensive patients engage in per month [46–48]. While older generations of beta-blockers have been historically implicated in worsening sexual function, newer formulations may have promising neutral and even beneficial effects on erectile function. A study

by Brixius et al. demonstrated that the substitution of metoprolol for nebivolol significantly improved IIEF scores in hypertensive patients, while both medications performed equivalently in lowering blood pressure [49]. This spectrum of effects on sexual function should be considered carefully when determining which beta-blocker therapy may be most appropriate for the individual hypertensive patient.

Calcium Channel Blockers

Calcium channel blockers exert their effect by blocking the transmembrane flow of calcium ions through voltage-gated L-type (slowly inactivating) channels leading to smooth muscle relaxation and vasodilation. This calcium signal blockage within the adrenal cortex decreases aldosterone production leading to additional antihypertensive effect, and nondihydropyridine-type calcium channel blockers specifically decrease myocardial contractility [50, 51].

In the literature, calcium channel blockers have been shown to exhibit a largely neutral effect on sexual function. In animal studies, amlodipine did not provide any protective role against structural changes in the vessels and cavernous spaces of erectile tissue caused by systemic hypertension [52]. In clinical study, nifedipine has been linked to decreased sexual desire, erectile dysfunction, and difficulty with ejaculation upon initiation of daily treatment; however, these symptoms were no longer reported at long-term follow-up [46]. In contrast, nifedipine and diltiazem may have an overall beneficial effect upon sexual function and satisfaction, although these effects were not statistically significant [53]. Overall, calcium channel blockers are effective antihypertensive agents and appear less detrimental to sexual function than other common medications currently available.

ACE Inhibitors

ACE inhibitors are effective antihypertensive agents due to their direct effect on the renin-angiotensin-aldosterone system (RAAS). They act to reduce angiotensin-II production and attenuate the degradation of bradykinin, a potent vasodilator and stimulator of nitric oxide release [26]. The RAAS system has been shown to have direct expression within the cavernosal tissue where it acts to modulate smooth muscle contraction and tone. In fact, higher levels of angiotensin II are found in the corpus cavernosum when compared to the systemic circulation [54]. Animal studies have indicated that hypertensive rats treated with captopril had improved erectile response while simultaneously returning blood pressure to control levels [55].

This potentially beneficial effect of ACE-i upon erectile function has not been reliably demonstrated in human studies. One study found that untreated

antihypertensive patients initiated on lisinopril endorsed a significant decrease in sexual activity during the first month of therapy, but recovered to baseline sexual activity at 3 month follow up [47]. A similar effect on sexual function has been seen with enalapril and captopril [46, 56]. This apparent discrepancy between expected benefit in erectile function with ACE inhibition may be explained by a feedback mechanism which allows for angiotensin breakthrough. Monotherapy with an ACE-i increases the concentration of circulating angiotensin I due to a loss of feedback inhibition of angiotensin II on renin secretion. Increased angiotensin I in turn may partially mitigate the ACE inhibiting effect of the antihypertensive medicine, restoring concentrations of active angiotensin II back toward pretreatment levels [57]. Overall, the present data suggests that ACE-i medications confer a neutral effect on sexual dysfunction but may have the potential for therapeutic benefit as our understanding of the complex RAAS pathway evolves.

ARBs

Of all the available antihypertensive agents, ARBs have been shown to have the largest overall positive effect on erectile function. ARBs are a newer class of antihypertensive agents that influence the RAAS pathway by providing competitive antagonism at angiotensin II receptors. This mechanism of action allows this medication class to overcome the issue of angiotensin breakthrough seen with ACE-i as described above [58]. Animal studies have shown that intracavernosal injection of angiotensin II caused contraction of cavernosal smooth muscle and terminated spontaneous erection, whereas administration of losartan increased intracavernosal pressure in a dose-dependent manner and caused erection [59]. This effect was similarly observed in a study of human cavernosal tissue in which losartan increased cavernosal relaxation and inhibited cavernosal smooth muscle contraction induced by angiotensin II [60].

Multiple clinical studies have shown similar positive effects on erectile function when treating hypertension with these agents. Losartan and valsartan have both been shown to improve self-reported sexual satisfaction and frequency of sexual activity in hypertensive patients [47, 48, 61]. Two large prospective studies looked at the effect of valsartan specifically on sexual function. In a study by Düsing et al., 75.4% of 3502 study patients were diagnosed with ED by IIEF score prior to valsartan therapy, and 6 months after medication initiation, the number decreased to 53% [62]. Similarly, an additional study reported a 60% increase in rate of sexual intercourse events in patients taking valsartan compared to an overall decrease in events in those taking beta-blockers, ACE-i, calcium channel blockers, and diuretics [63]. These initial outcomes provide a promising groundwork for the use of ARBs not only for blood pressure control but also for preserving erectile function, promoting overall patient satisfaction, and improving medication compliance in the hypertensive patient.

Treatment of Diabetes Mellitus

Given the progressive nature of DM, both type I and II, it is important to begin treatment in its early stages. Recall that T1DM is the result of insulin deficiency due to a T-cell autoimmune mediated destruction of pancreatic β cells [18]. T2DM occurs secondary to insulin resistance or insulin deficiency typically in the setting of obesity, poor diet, and sedentary lifestyle. DM can often be a difficult disease to control, and over the years various pharmacological agents have emerged. Primary classes of medication include insulin, biguanides, sulfonylureas, thiazolidinediones, glucagon-like peptide 1 (GLP-1) analogues, dipeptidyl peptidase-4 (DPP4) inhibitors, and sodium-glucose co-transporter-2 (SGLT2) inhibitors. The majority of oral glycemic agents are only approved for use in T2DM. One commonly used clinical measure is a serum hemoglobin A1c (HbA1c), which is an indirect measure capturing a patient's glycemic control over the span of several months. HbA1c below 5.7% is considered normal, 5.7–6.4% is in the prediabetic range, and 6.5% or higher marks a diagnosis of diabetes [64]. This value is used as a marker to track severity of disease in addition to response and adherence to treatment. When tracking a patient's progress, the target HbA1c is 7.0% or lower. Fasting plasma glucose as well as 2-h plasma glucose are alternative diagnostic tests.

Insulin

Subcutaneous insulin injections are the primary mode of treatment for Type 1 DM and may be used for Type 2 diabetics in whom glucose control is poor with oral hypoglycemics alone. Traditionally there have been two distinct types of insulin, regular insulin and intermediate-acting insulin (Neutral Protamine Hagedorn—NPH). Over the past two decades, additional insulin treatments have emerged, now including short-acting and long-acting insulins. The longer acting insulins (glargine, detemir, ultralente) can serve as a basal treatment, allowing for more stable concentrations throughout the day. More rapid-acting insulins (aspart, lispro) tend to have a quick onset and a shorter duration of action compared to regular insulin. These short acting formulations are particularly useful for control and improvement of prandial glucose levels. Among these rapid-acting agents, there is also an inhaled insulin which may be considered. Inhaled insulin is contraindicated in patients with chronic lung disease [65]. For those with needle phobias, insulin pumps are also an option, where there is continuous subcutaneous delivery of insulin [66]. For type 1 diabetics who primarily rely on insulin, a typical treatment regimen may include a long acting insulin administered at night in addition to shorter acting insulins administered during the day with meals.

Biguanides

One of the most common and widely prescribed medications is metformin. The overall function is to increase insulin sensitivity and to inhibit hepatic gluconeogenesis [67]. Activation of AMP-activated protein kinase will decrease hepatic glucagon production. Metformin also promotes insulin sensitivity as well as peripheral glucose uptake via phosphorylation of glucose transporter type 4 (GLUT4) which is responsible for diffusion of glucose into muscle and fat cells. The medication may also result in reduced gastrointestinal absorption of glucose. The net effect is lowering of blood glucose.

According to the American Diabetes Association (ADA), it is the preferred initial oral medication for the treatment of T2DM [66]. Secondary oral agents or insulin may be ultimately added to metformin for combination therapy. However, it is important to concomitantly employ weight loss with the use of metformin, otherwise the positive effect will be seen to a certain degree before glucose levels begin to rise again. The risk of hypoglycemia is generally low, but other common side effects include diarrhea, abdominal pain, and nausea. While metformin is considered a safe treatment option, it has been associated with vitamin B12 deficiency and subsequent exacerbation of neuropathies [68]. If the target HbA1c is not met after 3 months of metformin monotherapy, then secondary agents should be introduced into the regimen. There are no formal recommendations or algorithm with regards to combination therapy and which additional drugs should be initiated. Medication choices are generally determined by side effect profile, patient factors, and cost [69].

Sulfonylureas

Sulfonylureas emerged and became available as a first line therapy along with metformin. The specific mechanism is the stimulation the release of insulin from existing pancreatic β-cells [67]. Therefore, its efficacy is contingent upon the presence of endogenous β-cells. Commonly used medications include glimepiride, glipizide, and glyburide. Frequently seen side effects include hypoglycemia and weight gain. There are also some controversies around cardiovascular safety as an initial study demonstrated an increase in cardiac deaths, but newer agents of the same class have shown mixed results [70]. This has overall led to an increased utility of other agents that may afford some cardiovascular benefits. Nonetheless, sulfonylureas may still be utilized as a first line monotherapy, particularly for those patients who cannot tolerate metformin or who have specific contraindications [71]. It is also less expensive compared to newer agents, so cost continues to play a significant role in selection of treatment options. Otherwise, it may also be used as a second line adjunct therapy to metformin, similar to other glucose lowering therapies.

Thiazolidinediones

Thiazolidinediones (TZDs) are peroxisome proliferator activated receptor (PPAR) activators. These medications function by improving insulin sensitivity as well as maintain β-cell function by preserving insulin secretion [67]. Weight gain is a common side effect. The two FDA approved agents include rosiglitazone and pioglitazone, which can be considered as second line agents. Earlier agents of the same class were found to cause significant hepatotoxicity, but these two newer agents were found to be safe on the hepatic system. Rosiglitazone was the first target of criticism and was previously banned due to concern of associated cardiovascular events, but this has since been rescinded [72]. It should still be used with caution in elderly patients with cardiac failure. Pioglitazone then gained attention with regards to a possible increased in bladder cancer risk [73]. While the data is not robust, it is recommended to avoid its use in patients with a history of or active bladder cancer. Aside from glucose lowering benefits, the advantages of TZDs include lower cost, preservation of β-cell function, an increase in HDL cholesterol levels, and a delay in progression of diabetic nephropathy [74, 75].

Dipeptidyl Peptidase-4 Inhibitors (Gliptins)

Dipeptidyl peptidase-4 (DPP4) inhibitors are among the more recent classes of antidiabetic agents. They function to block an enzyme called dipeptidyl peptidase 4 [67]. DPP4 inhibition overall leads to increased activity of gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). GIP and GLP-1 work to stimulate insulin synthesis from β-cells. GLP-1 also works to reduce glucagon secretion. Common agents include alogliptin, saxagliptin, linagliptin, and sitagliptin. Side effects do not include hypoglycemia or weight gain, and are in fact generally minimal compared to other class of agents. DPP4 inhibitors may also play a protective role in atherosclerosis and coronary artery disease [76]. This class of agents is overall becoming increasingly utilized due to its glucose-lowering efficacy as well as favorable safety profile.

Glucagon-Like Peptide 1 Receptor Agonists

GLP-1 receptor agonists are considered incretin-based therapies that act to increase insulin secretion and reduce glucagon secretion. As previously discussed, the activation of GLP-1 receptors on pancreatic β-cells will stimulate insulin synthesis and secretion. It is considered a standard second line treatment, and it is the preferred injectable therapy compared to insulin when modifying a treatment regimen, particularly when other oral agents are ineffective [66]. While side effects are primarily

gastrointestinal, the risk of hypoglycemia and weight gain is lower compared to other agents. Common medications include exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, and semaglutide. These are primarily injectable medications, but semaglutide is an available oral formulation.

Sodium-Glucose Co-Transporter-2 Inhibitors (Gliflozins)

Sodium-glucose co-transporter-2 (SGLT2) inhibitors are another newer class of medications that overall lead to increased urinary glucose excretion. SGLT2 is a protein that typically functions by reabsorbing almost 90% of glucose in the proximal renal tubules [67]. Per ADA guidelines, when considering additive therapy to metformin, SGLT2 inhibitors may be specifically considered in patients with cardiovascular disease, renal disease, or heart failure [66]. Common medications in this class include ertugliflozin, canagliflozin, dapagliflozin, and empagliflozin. Side effects include urinary tract infections, genital mycotic infections, and volume depletion. While the cardiovascular and renal benefits have been proposed, longer term studies will provide more insight.

Treatment of Erectile Dysfunction

The overall treatment options for ED have evolved over the past 30 years. First and foremost, the underlying disease process as well as lifestyle should be optimized. Patient education about associated risk factors as well as the relationship with these chronic diseases is an integral first step.

Oral Agents

Beyond lifestyle modifications, oral medications are a first line therapy for ED. Phosphodiesterase (PDE) inhibitors, specifically PDE5 inhibitors, prevent the breakdown of cGMP, a molecule that allows for smooth muscle dilation and thus erections (Fig. 20.1). Commonly used FDA-approved PDE5 inhibitors include sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra ®), and avanafil (Stendra®). The underlying mechanism is similar across these medications; however, duration of onset, half-lives, and conditions of absorption can vary slightly (Table 20.1). The success of these medications depends on intact neural pathways as well as appropriate sexual stimulation to initiate the cascade leading to the initial production of the cGMP. It is important to note that PDE5 inhibitors act to potentiate erections, not to induce them.

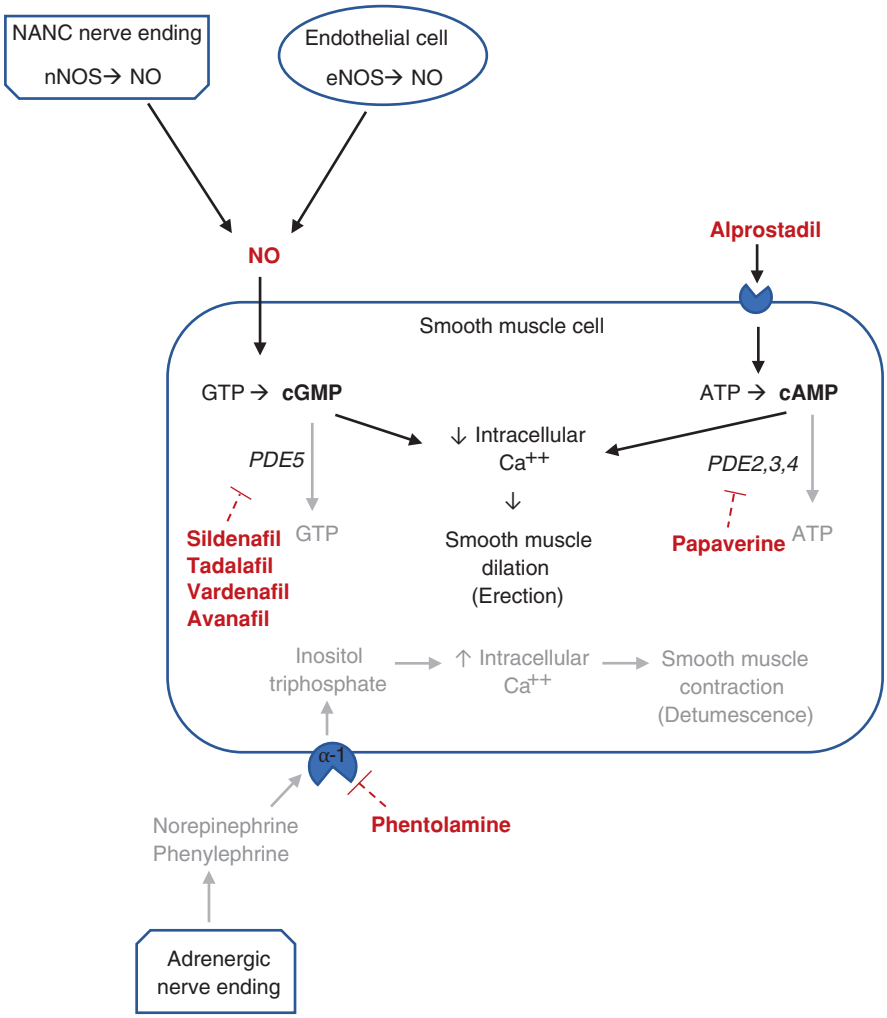

Fig. 20.1 Erectile physiology

Table 20.1 PDE5i

	Sildenafil	Tadalafil	Vardenafil	Avanafil
Dosage	20–100 mg PRN	5–20 mg PRN 2.5–5 mg daily	5-20 mg PRN	50–200 mg PRN
Administration time prior to activity	1 h	0.5 h	1 h	0.5 h
Half-life	3–5	17.5 h	4–5 h	3–5 h
Duration of action	4–8 h	36 h	4–8 h	4–8 h
Absorption with fatty food	Decreased	Unaffected	Decreased	Decreased

All medications are recommended to be taken on an as needed basis for sexual activity. Tadalafil is the only medication that is approved to be taken as a daily low dose in addition to the traditional on demand dosing [77]. The daily dose may have an added benefit in alleviating some lower urinary tract symptoms in men with benign prostatic hyperplasia [78]. For men with severe ED, a combination therapy with a daily tadalafil and on demand sildenafil can be considered [79].

Common side effects include headaches, facial flushing, nasal congestion, and heartburn. Visual disturbances secondary to cross reactivity with PDE6 can result in diplopia, blurred vision, and loss color vision. All are reversible with medication cessation. There are several contraindications and cautions when it comes to the use of PDE5 inhibitors. These medications should not be used within 6 months of a myocardial infarction. Additionally, concomitant use of nitrates is contraindicated as PDE5 inhibitors will potentiate vasodilatory and subsequently hypotensive effects. It is recommended that sildenafil, avanafil, and vardenafil not be administered within 24 hours of taking a nitrate. Similarly, there should be a 48 h separation between tadalafil and nitrates [80]. Additional medication interactions can occur with antifungals, antiretrovirals, and alpha blockers.

It is also important to note that certain classes of medications can lead to erectile dysfunction. Responsible medications may include antihypertensives (thiazides, nonselective beta-blockers), antidepressants (tricyclics, selective serotonin reuptake inhibitors), antipsychotics (phenothiazines), antiandrogens, antiulcer drugs (H2 receptor blockers), cytotoxic drugs (cyclophosphamide, methotrexate), and opiates (morphine) [81]. While it is not always possible to discontinue or switch the medication, it is valuable to understand possible underlying etiologies (Fig. 20.2).

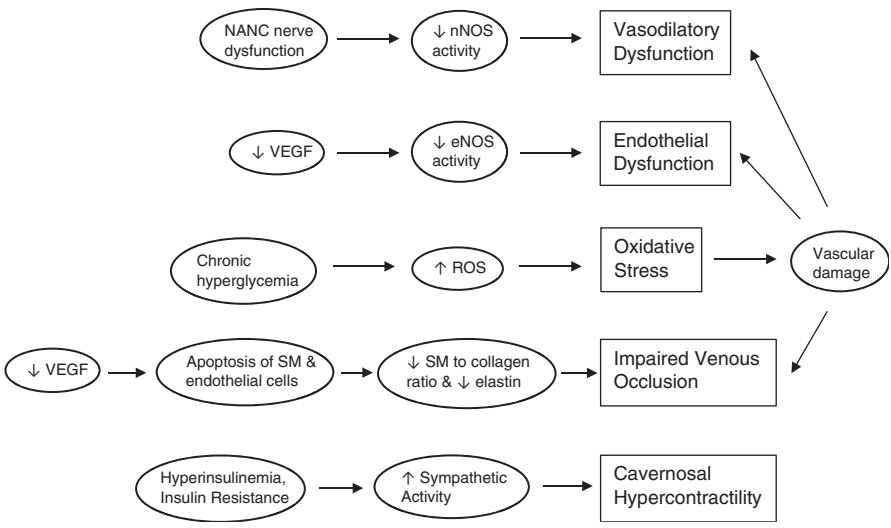

Fig. 20.2 ED and diabetes

Early studies demonstrated that PDE5 inhibitors led to more than 70% of successful intercourse attempts [80]. Diabetics are less likely to successfully respond to PDE5i, with rates between 50% and 60% [82]. A patient's response to PDE5 inhibitors relies on the quality of the pudendal nerves and their ability to release NO, degree of endothelial cell function, and the level of pudendal blood flow. For this reason, diabetic men may not have such a robust response compared to nondiabetic men [83]. Moreover, for those with an adequate initial response, the efficacy may diminish over time. Nonetheless, PDE5 inhibitors remain a first-line treatment option for diabetics, but medical professionals should be prepared to transition to additional therapies should the medication be unsuccessful.

Intracavernosal Injections

In patients for whom oral agents are ineffective or contraindicated, an appropriate next step is to discuss localized vasoactive agents such as intracavernosal injections (ICI) or intraurethral suppositories. Multiple vasodilatory agents can be incorporated into a mixture for ICI, commonly consisting of alprostadil, papaverine, and phentolamine. Alprostadil activates the conversion of ATP to cAMP, and papaverine prevents the degradation of cAMP. Through alpha blockade, phentolamine inhibits the cascade initiated by inositol triphosphate. Collectively, all allow for smooth muscle dilation (Fig. 20.1).

The medication is injected directly into the lateral base of the penile corpora, avoiding the urethra ventrally and the neurovascular bundle dorsally (Fig. 20.3).

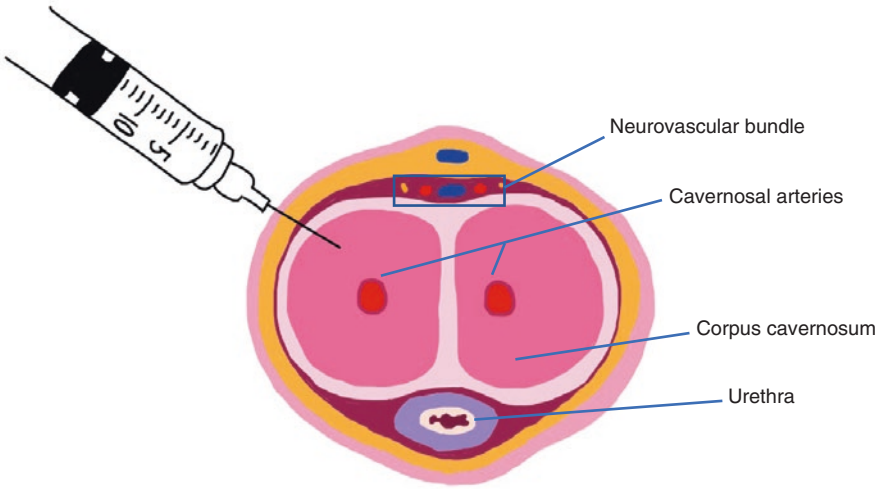

Fig. 20.3 Depiction of intracavernosal injection into the corpus cavernosum. The neurovascular bundle and urethra should be avoided

Unlike oral medications, ICI can induce erections without significant sexual stimulation and do not necessitate intact neural pathways. Therefore, for patients with diabetes or nerve damage in general, ICI may be a more effective alternative. Possible mixtures include alprostadil alone, papaverine and phentolamine (Bimix), or all three (Trimix). Mixtures can be tailored according to patient needs, initial responses, and side effects. An in-office trial of the injection should be performed to ensure that the patient can safely inject themselves without systemic side effects and to ensure that priapism does not occur. Priapism, a prolonged erection lasting longer than 4 h, is a possible side effect of any pharmacologic erectogenic treatment.

Patient satisfaction rates with the use of ICI are between 70% and 90%, and 80–90% would recommend the treatments to friends [84, 85]. For patients who respond to both ICI and sildenafil, it is unclear if ICI is truly more efficacious, but it has been shown that ICI delivers higher satisfaction rates [86]. Multiple studies confirmed these results revealing that despite response to both PDE5 inhibitors and ICI, 50% of long-term ICI users would prefer ICI to oral medications, citing improved rigidity and reduced side effects [85]. While side effects are rare, they can include pain at the injection site, bruising, development of scar tissue and possibly mild penile curvature, and priapism. While a seemingly effective treatment among long-term users, ICI can induce some hesitation among newer users due to anxiety around penile injections. When studying patient anxiety, Mulhall et al. found that 65% of new users reported high injection anxiety, and 42% of patients continued to have injection anxiety at 4 months [87]. Therefore, proper education and counseling are the principal components of effective injection use and acceptance.

Intraurethral Suppositories and Gels

For patients who are hesitant to proceed with penile injections, intraurethral alprostadil can either be instilled into the urethra by means of a suppository or a gel. Early studies have demonstrated variable results with regards to intraurethral alprostadil (MUSE®), with success rates as high as 64% and as low as 30% [88, 89]. Furthermore, a retrospective study by Jaffe et al. suggests that 30% of patients who specifically fail sildenafil will respond to intraurethral alprostadil [90].

The systemic side effects seen with oral agents are minimized with the intraurethral formulations. The most common side effect reported is mild penile or urethral pain, which may be experienced in approximately 10% of patients [88]. Hypotension and dizziness, indicating absorption of the medication into the systemic circulation, are rare but may be seen in up to 3% of patients. It is advised that patients use condoms with use of the intraurethral medications, particularly those containing prostaglandin, when engaging in sexual activity with pregnant partners due to risk of exposure [91].

With the use of compounding pharmacies, multiple agents can be incorporated into these suppositories or gels and specially formulated for patients. Similar to intracavernosal injections, alprostadil, papaverine, and phentolamine can be used in

combination at varying amounts. It is important to keep in mind that for those experiencing penile pain with intraurethral or intracavernosal treatments, it is likely the alprostadil that is responsible. In these cases, adjustments to the composition and dosage should be made accordingly.

Mechanical Devices

Noninvasive drug-free alternatives exist in the form of vacuum erection devices (VED). This is cylindrical device that is placed over the penis and draws the penis into the device via a vacuum mechanism that is controlled by the patient with the use of a pump. This allows for increased blood flow into the penile corpora and therefore rigidity. In order for the blood to remain trapped, a constriction band must be placed at the base of the penis. Once the band is removed, blood flow and subsequently the erection dissipates.

An early study in 1991 demonstrated that 75% of diabetic men were able to engage in satisfactory intercourse with the use of a VED [92]. However, reasons for discontinuation include bruising of the penis, pain related to use of the constriction band, or changes in sensation of the penis such as coldness or numbness [93]. A more recent study with a small sample size of veterans demonstrated that after comprehensive teaching sessions, 96% of patients were able to maintain an erection with the device, and 100% would recommend it to a friend [94]. Additional conclusions were that patients with poor dexterity or those more advanced in age may not have success with VEDs. In men for whom PDE5 inhibitors are contraindicated or ineffective, are unable to tolerate intraurethral or intracavernosal treatments, and are not interested in pursuing surgery, VEDs are good alternatives. It is also important to note that VEDs can be used concurrently with any of the pharmacologic treatment options, and may indeed enhance erectile response [95].

Penile Prosthesis Surgery

In medical refractory cases, surgical treatment with a penile prosthesis is an effective long-term treatment option. Surgery entails placement of artificial cylinders into the penile corpora. The cylinders may be composed of a material that remains in a rigid state at all times but can be easily manipulated such that the device is readily hidden under clothing or can be positioned for use during intercourse. These are termed semirigid, or malleable, devices. Alternatively, the corporal cylinders can be inflatable whereby a pump, typically in the scrotum, is used to propel sterile saline from an abdominally placed reservoir into the cylinders until the desired rigidity is achieved. The malleable devices are more appropriate for patients who have poor

manual dexterity or who desire a simpler prosthesis. Otherwise, the inflatable devices are considered more natural and physiologic as they can be inflated to a rigid state and deflated back to a flaccid state when desired.

Overall, satisfaction rates with penile prostheses for both patients and their partners are upward of 85% [96]. Despite high success and satisfaction rates, some patients may be unhappy with the results. The most commonly cited complaints are a decrease in postoperative penile length and difficulty with pump manipulation. Therefore, preoperative counseling and patient selection are imperative.

Conclusion

The relationship between ED and major chronic diseases such as HTN and DM is unequivocal. The underlying pathologic processes are in fact quite similar and explain why ED is increasingly considered an independent risk factor of cardiovascular disease. Early diagnosis with appropriate screening and attempts to minimize disease progression via lifestyle changes and pharmacotherapy are crucial steps. Although this cannot necessarily reverse the molecular damage that has already been done, it is important for patients to know that there are individualized treatment options for ED as well. Continuing to maintain a global view of the patient's health will allow medical professionals to prevent serious events that may lead to significant morbidity and mortality and to effectively treat all associated aspects of the disease.

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Part IV
Peripheral Vascular Disease

Chapter 21
Peripheral Vascular Disease in Patients with Diabetes Mellitus

Scott G. Prushik and Erin Mcintosh

Introduction

Vascular disease is the most significant cause of morbidity and mortality in patients with diabetes mellitus (DM), who suffer from complications due to coronary artery disease, cerebrovascular disease, and peripheral vascular disease (PVD). Patients with DM and PVD often present with a unique disease burden that can be challenging to evaluate and manage, compared to patients without diabetes. For the estimated ten million people with diabetes in the United States, one of the most common reasons for hospitalization is diabetic foot ulceration with or without superimposed infection. It is estimated that the lifetime incidence of foot ulcers among diabetic patients may be as high as 25% with an increased risk of amputation as high as 15–30 times that in non-diabetics [1–3]. Diabetes significantly increases the incidence and severity of limb ischemia and the distribution of peripheral arterial disease (PAD) in diabetics differs from those without it, tending to involve the more distal, infrapopliteal arteries. While diabetic patients are prone to microvascular complications of nephropathy, retinopathy, and neuropathy, it is an important principle to remember when assessing diabetic patients with PVD that the ischemia that results in non-healing ulcers and infections is due to macrovascular occlusive disease secondary to atherosclerosis [4–6]. The complex interplay between the micro- and macrovascular pathologic mechanisms that lead to peripheral neuropathy and ischemia, create an ideal setting for the development of pressure necrosis, non-healing ulcers, and polymicrobial infection, which can ultimately lead to gangrene

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and amputation if improperly treated [7, 8]. In addition to utilizing the appropriate diagnostic tools and determining the appropriate interventions, it is often necessary to employ a multidisciplinary approach to the treatment of PVD in the diabetic patient in order to achieve the ultimate goals of limb salvage and cardiovascular risk reduction.

Pathogenesis of Atherosclerosis in Diabetes Mellitus

Vascular cell biology is altered in diabetes, leading to accelerated atherosclerosis and its complications. Specifically, the vascular endothelium plays an active role in the dynamic process of homeostasis that involves regulating vascular tone, platelet function, the coagulation cascade, and importantly, vascular smooth muscle cell proliferation and migration. The endothelium regulates vascular homeostasis through the release of various signaling molecules. One of the most potent vasodilatory and anti-inflammatory molecules constitutively produced by healthy endothelium is nitric oxide (NO) via endothelial nitric oxide synthase (eNOS) [9, 10]. NO reduces production of proinflammatory chemokines and cytokines through inhibition of inflammatory transcription factors, limiting vascular cell injury, platelet activation, and atherogenesis. Common risk factors for atherosclerosis—hypertension, hypercholesterolemia, smoking, and diabetes—are associated with diminished release of NO into the arterial wall due to impaired synthesis or excessive oxidative degradation. Decreased bioavailability of NO in the vasculature promotes an environment that is prone to vascular injury, atherogenesis, and its sequelae.

The altered phenotype of vascular cells in diabetic patients, namely a proinflammatory and atherogenic state, is promoted by the pathologic milieu of metabolic derangements—hyperglycemia and insulin resistance—hypercoagulability, and oxidative stress, which results in the destruction of the protective endothelial cell layer and dysfunction of underlying vascular smooth muscle cells. The formation of atherosclerotic lesions is triggered by local inflammation in the vascular wall, induced by dyslipidemia, particularly high levels of LDL cholesterol. This process starts by oxidative stress that injures vascular endothelial cells which then upregulate production and expression of adhesion molecules (i.e., ICAM-1, VCAM-1) that facilitate the binding of monocytes to the vascular wall and result in their translocation to the subendothelial layer, eventual maturation to macrophages that take up LDL cholesterol infiltrates, with subsequent formation of foam cells [10]. Oxidized lipids then trigger the production of growth factors by the endothelium, which then act on vascular smooth muscle cells, shifting their normally quiescent, contractile phenotype, to a pathologic, proliferative, secretory, and migratory one. When vascular smooth muscle cells migrate into the intimal later, it is there that they proliferate and produce extracellular matrix proteins, cause intimal thickening, sclerosis, and contribute to atherogenesis.

Clinical Presentation

PAD exists on a spectrum, ranging from claudication to rest pain and critical limb-threatening ischemia with tissue loss. Claudication is defined as ischemic muscle pain that occurs as the result of inadequate blood flow. The affected muscle groups will typically be just distal to the area of arterial occlusion. Patients will experience intermittent, cramping, pain that occurs with and is exacerbated by exercise and is relieved with rest. Clinically, providers can track the progression of patient disease over time by quantifying the distance the patient can ambulate before symptom onset. The majority of patients with claudication will not have progression of disease over time. In fact, symptoms can be improved with optimal medical management alone, including smoking cessation and monitored exercise programs.

Rest pain usually indicates more severe occlusive disease and is characterized by burning pain that involves the forefoot or region of the metatarsal heads. In contrast to the intermittent nature of claudication, rest pain is constant. It classically occurs at night and is relieved by placing the foot in the dependent position. Patients will describe the need to dangle their leg over the side of the bed at night to get symptomatic relief.

PVD that progresses to the point of critical limb ischemia may present in distinct ways, including ulceration and gangrene. Gangrene is a hallmark of severe, end-stage, vascular occlusive disease, characterized by black, insensate skin, with loss of motor function. Without superimposed infection, "dry gangrene" is not an immediate threat to the patient. However, with coexisting infection, "wet gangrene" is considered an emergency and is typically associated with signs and symptoms of systemic infection, including fever, leukocytosis, purulent drainage, abscess, and osteomyelitis, necessitating urgent debridement or amputation.

The natural sequence of disease progression described above, occurs reliably in patients without diabetes. However, in patients with diabetes, the normal evolution of signs and symptoms can differ greatly. For example, diabetic patients may not ever develop rest pain or its early signs can be incorrectly attributed to underlying peripheral neuropathy. Additionally, given that the distribution of PVD in diabetic patients is generally in the distal infrapopliteal and pedal arteries, patients will not usually complain of classic claudication type symptoms. Rather than a classic progression of disease signs and symptoms, diabetic patients with PVD often present with infection superimposed on a chronic non-healing foot ulcer or gangrene (Fig. 21.1). Often aggressive and polymicrobial in nature, these infections can cause significant tissue destruction and commonly lead to amputation in the diabetic patient. As such, it is important to recognize that the signs and symptoms of infection in the diabetic patient may be subtle. With an altered immune response that occurs in diabetes, the only early indication of infection in a diabetic patient with PVD may be worsening hyperglycemia or increased insulin requirements.

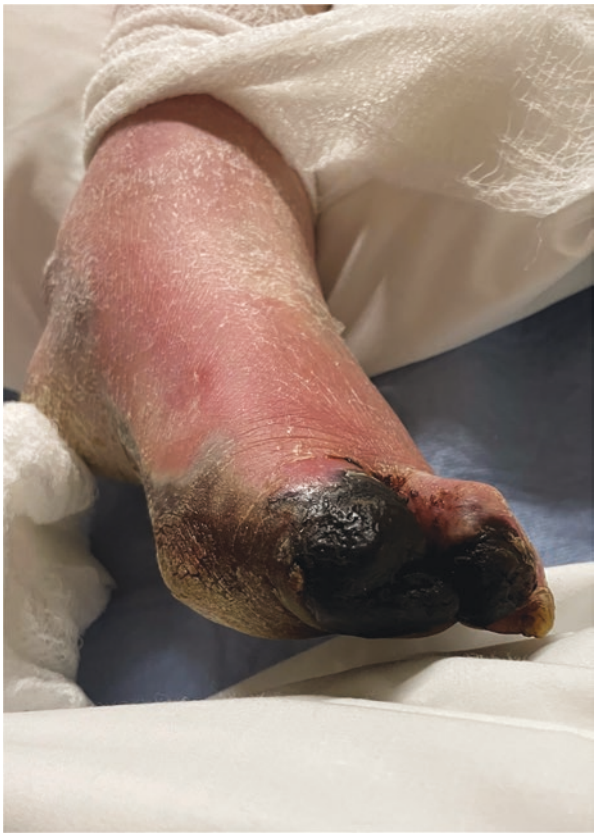

Fig. 21.1 Patient with Type II diabetes and gangrene of the foot

Principles of Evaluation

A comprehensive vascular examination of patients with diabetes begins with a thorough foot exam with a focus on inspection for signs of skin changes, hair loss, ulcers, and infections. Sensory and motor examination should then be performed, as the presence of neuropathy in patients with diabetes is an important risk factor for the development of ulcers and potential future amputation [11]. Diabetic peripheral neuropathy is characterized by symmetric sensorimotor dysfunction that begins distally and progresses more proximally in a classic "stocking and glove" distribution. Patients with peripheral neuropathy can experience a burning sensation, tingling, and pain that radiates; however, the severity of these symptoms does not predict the degree of nerve damage [12]. The current recommendation is for patients with diabetes to have yearly peripheral nerve examinations.

Patients with diabetes should have ankle-brachial index (ABI) measurements performed at least by the age of 50 years, if not sooner, and yearly if they have a prior history of diabetic foot ulcer, known CAD, prior abnormal vascular exam, or prior intervention for PVD (Fig. 21.2) [13]. Complicating the results of noninvasive

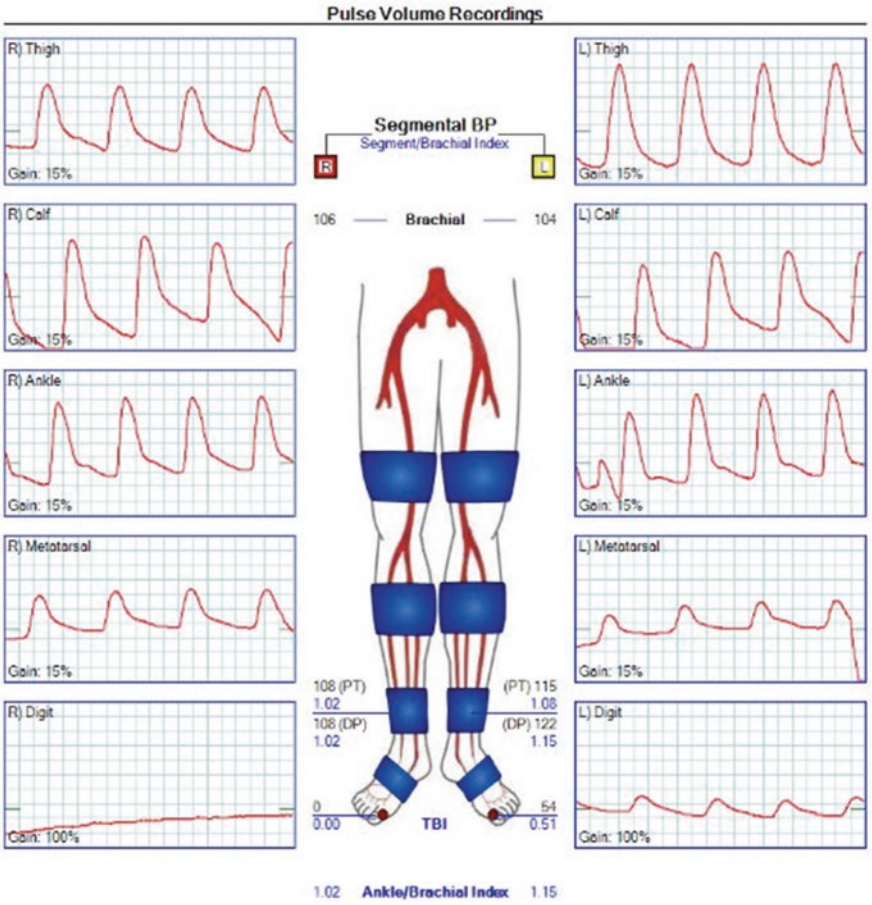

Fig. 21.2 Patient with normal arterial physiologic studies

testing is the calcification of blood vessels in diabetics, leading to noncompressibility, and falsely elevated values, which often belies the reality of severely compromised blood flow (Fig. 21.3).

Complete and precise imaging of the arterial circulation is necessary for the successful management of PVD in patients with DM, who require revascularization. Imaging in this patient population can prove challenging, given the multilevel disease distribution especially in the distal tibial and peroneal arteries, which are often calcified in the setting of diabetes. Additionally, it is not uncommon for patients with DM and PAD to have underlying renal insufficiency, further complicating comprehensive imaging evaluation of the arterial circulation. Currently available imaging modalities to evaluate the arterial circulation include intra-arterial digital subtraction angiography (DSA), contrast-enhanced magnetic resonance angiography (MRA), and computerized tomographic angiography (CTA). Not only does angiography have the advantage in the evaluation of diabetic arterial

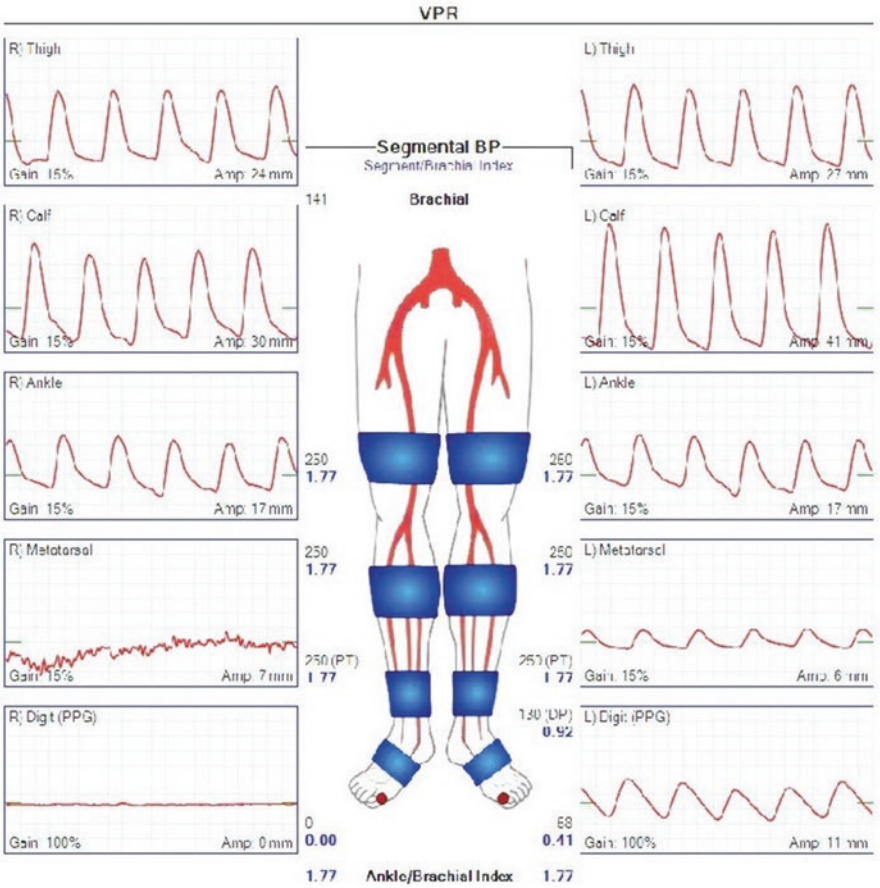

Fig. 21.3 Patient with physiologic studies showing a typical pattern of disease for a patient with diabetes and peripheral arterial disease

occlusive disease to visualize distal small caliber vessels, but also it allows the simultaneous ability to intervene with angioplasty techniques. In diabetic patients who have underlying renal insufficiency, added acute insult such as contrast-induced nephropathy is a concern, and alternatives like carbon dioxide and gadolinium can be used in place of conventional contrast. Unlike angiography, CTA is noninvasive and can be rapidly performed. It has better spatial resolution compared to MRA, though it has the disadvantage of requiring the largest volume of contrast and exposing the patient to high doses of radiation. Both CTA and MRA can be used to evaluate arterial anatomy in patients in whom surgical bypass is the planned intervention. In patients in whom an endovascular approach is indicated, angiography immediately followed by catheter-based therapy during the same procedure is preferred.

Principles of Therapy

When treating patients with PAD and DM, the ultimate goals are improving limb salvage outcomes and decreasing the potentially fatal risks of comorbid cardiovascular and cerebrovascular disease. Approach to treatment of these patients begins with aggressive optimization of medical therapy and lifestyle risk factor modification, including smoking cessation programs, exercise rehabilitation programs, maximal medical therapy to treat hypertension, hyperlipidemia, and to control hyperglycemia [14]. Optimizing non-surgical/non-interventional management of diabetic patients with PAD includes preventive foot care with emphasis on proper hygiene, careful screening, and early intervention [15]. The Society for Vascular Surgery and American Podiatric Medical Association recommends for the prevention of diabetic foot ulceration: annual foot examination by a trained provider, including peripheral neuropathy assessment, patient and family education, specialized footwear in high-risk patients with significant neuropathy, foot deformities, or prior amputations, as well as maintenance of hemoglobin A1c < 7% [16, 17]. Beyond maximizing pharmacologic therapy with use of beta blockers in patients with concomitant CAD, ACE inhibitors, and antiplatelet drugs, equally aggressive interventional therapies should be utilized in diabetics who present with ulceration or gangrene, with or without infection.

Medical Treatment for PAD

Exercise Therapy

Randomized controlled trials of supervised exercise training programs have proven the benefit of exercise therapy on improving the symptoms of intermittent claudication [17, 18]. In patients who present with symptoms of intermittent claudication in the absence of any signs of limb-threatening ischemia, they should first be referred for a trial of monitored exercise therapy and smoking cessation prior to any attempt at surgical or endovascular intervention. The optimal exercise programs include walking for ≥30 min, >3 times/week for 6 months, based on a meta-analysis by Gardner et al. In another meta-analysis by Bendermacher et al., supervised walking programs resulted in an average improvement in maximum walking time of 6.5 min compared to medications used to treat claudication symptoms. The benefit of a supervised walking regimen compared to percutaneous endoluminal revascularization has been studied in the CLEVER Trial (Claudication: Exercise versus Endoluminal Revascularization), which randomized patients with aortoiliac disease to optimal medical therapy, optimal medical therapy with supervised exercise program, or optimal medical therapy with endovascular intervention. The primary endpoint was graded treadmill test at 6 months compared to baseline. Of note, about

25% of those randomized patients had DM as well. While the supervised exercise group had the greatest improvement in walking time, the quality-of-life assessment questionnaire indicated a larger improvement in the stenting group. Further research is necessary to reach conclusive data, and the SUPER Trial is currently ongoing, looking at outcomes with supervised exercise therapy vs immediate PTA in patients with iliac artery occlusive disease specifically. Patients with diabetes typically have more severe impairments at baseline compared to patients with PAD alone. However, exercise programs have been shown to benefit patients who have both PAD and DM. In addition, the randomized controlled trials that exist, thus, far to evaluate the optimal therapy for intermittent claudication, are performed in patients with arterial disease distribution that does not generally reflect that seen in diabetic patients. Thus, a supervised exercise regimen, or a home-based exercise program of similar intensity as described above, is the current treatment modality recommended for patients with PAD with diabetes.

Antiplatelet Therapy

Antiplatelet therapy is a key component to the management of patients with diabetes and PVD [19]. Based on several trials, low-dose aspirin therapy (75–162 mg/day) is recommended for the primary prevention of cardiovascular events in patients with diabetes in whom the baseline risk of gastrointestinal bleeding is not increased. As included in this patient population for whom low-dose aspirin is recommended, they are men >50 years and women >60 years with one or more risk factors for cardiovascular disease, including smoking, hypertension, dyslipidemia, renal impairment, and family history of early onset cardiovascular disease. Numerous studies exist, which have shown a benefit of antiplatelet therapy for reducing cardiovascular events in patients with known PVD. The CAPRIE Trial compared clopidogrel versus aspirin in patients with NSTEMI, ischemic stroke, or PAD. Importantly, in a subset of those patients with diabetes, there was a 12.5% risk reduction in major cardiovascular events with clopidogrel versus aspirin. Current recommendations from the Society for Vascular Surgery include antiplatelet therapy with either aspirin 75–325 mg or clopidogrel 75 mg daily in patients with PAD. Despite this recommendation, the specific benefit of antiplatelet therapy for cardiovascular risk reduction in patients with PAD and concomitant DM needs further evaluation, as recent data questions the benefit of antiplatelet therapy specifically in patients with diabetes (JPAD, POPADAD trials).

Statins

High-dose statins are a mainstay of medical management of patients with PAD to reduce cardiovascular events. Statins have also been shown to improve walking distance and reduce progression of symptomatic claudication and are currently recommended by the SVS as therapy for patients with symptomatic PAD [20].

Cilostazol

Cilostazol is a phosphodiesterase-3 inhibitor that has been shown to improve the symptoms of claudication through its vasodilatory, antiproliferative, and antiplatelet effects. While it has not shown any mortality benefit, several RCTs found that it significantly increases walking times in patients with stable intermittent claudication. The Society for Vascular Surgery recommends a 3-month trial of cilostazol 100 mg twice daily to alleviate symptoms and improve walking distances in patients with lifestyle-limiting claudication. While it is generally well tolerated in most patients, it is contraindicated in patients with heart failure, renal, or hepatic impairment. Many patients with diabetes and macrovascular disease also have microvascular disease, including nephropathy. In those specific patients, other medications along with supervised exercise programs can be utilized.

Pentoxifylline

Pentoxifylline is a methylxanthine derivative that inhibits phosphodiesterase and has similar effects as cilostazol. While evidence to show improvement in walking distances with pentoxifylline versus placebo are lacking, a trial of this medication can be used in patients with intermittent claudication in whom cilostazol is contraindicated.

ACE Inhibitors

Small RCTs evaluated the role of ACE inhibitors in the medical management of symptomatic PAD, and meta-analysis of these studies showed a benefit in maximum walking distance and pain-free walking distance in patients treated with ACE inhibitors versus placebo. The greatest benefit was seen specifically with the use of ramipril [21, 22].

Surgical Treatment for PAD

For patients with PAD and DM who present with ulceration or gangrene with superimposed infection, early and thorough debridement of any underlying abscesses and necrotic tissue is paramount. The goal is the eradication of any septic foci, which can sometimes be accomplished with daily bedside debridements with or without local anesthetic, or may require multiple surgical debridements or amputation in the operating room [23, 24]. Once the infection is treated, the next priority is in establishing adequate flood flow to the affected extremity. Indications for revascularization are the same in patients with diabetes and PAD as they are in patients with PAD alone, including lifestyle-limiting claudication with failed medical management,

critical limb ischemia with rest pain or tissue loss, and non-healing foot ulcers. The ultimate goal is to restore pulsatile blood flow to the foot, which can be accomplished endovascularly or with open surgical bypass depending on the characteristic of the lesion(s) in question, as well as on patient-specific factors.

Summary

Diabetes increases the risk of vascular disease, including cardiovascular, cerebrovascular, peripheral vascular, and microvascular diseases. CAD is responsible for the majority of the deaths in patients with diabetes, but stroke, claudication, critical limb ischemia, diabetic foot ulcers, retinopathy, and nephropathy all contribute to the overall morbidity and mortality in patients with diabetes. Several metabolic, thrombotic, and vascular derangements occur in diabetes that account for the accelerated atherosclerosis and increased rate of thrombosis characteristic of diabetic vascular disease. Treatment of PAD in patients with diabetes involves therapies to improve symptoms, and aggressive risk factor modification is aimed at improving cardiovascular outcomes and overall mortality.

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Chapter 22
Epidemiology of Peripheral Vascular Disease

Stephanie G. Wheeler and Edward J. Boyko

Abbreviations

ABI	Ankle-brachial index
CAD	Coronary artery disease
CBD	Carotid/vertebral artery disease
HR	Hazard ratio
PAD	Peripheral artery disease

Introduction

This chapter will focus on the epidemiology of peripheral artery disease (PAD) and occlusive arterial disease affecting the extremities and will also include a discussion of associated conditions and mortality. The focus of this chapter is primarily on the most common type of occlusive peripheral arterial disease due to atherosclerosis affecting primarily the arterial intima. Other types of occlusive peripheral arterial disease such as fibromuscular dysplasia, vasculitis, or thromboangiitis obliterans occur rarely and will not be discussed.

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Epidemiologic Principles Relevant to the Study of Arterial Diseases

Measurement of disease prevalence and incidence is best conducted in a population-based sample of study subjects. Typically, such samples are obtained from defined populations, such as all residents of a certain geographic area, or using some other characteristic to define the population, such as enrollees of a health plan. Populations obtained from clinic-based or other medical care settings are likely to overestimate the prevalence and incidence of arterial diseases because associated conditions that put such persons at higher risk of arterial diseases are likely to be present in a higher proportion in these subjects who seek care rather than a random population-based sample.

In addition to measurements of disease incidence and prevalence, several methods are used by epidemiologists to assess whether an exposure (for example, smoking or diabetes) is related to a change in risk of disease. Further methods are employed to determine if such an association may be causal, or instead due to confounding, selection, or measurement bias. Cross-sectional study designs provide weak information regarding causality. Retrospective study designs tend to be less compelling in establishing whether an exposure is related to a change in disease risk, since the passage of time between the onset of the exposure and disease development may result in inaccurate exposure classification or a different mortality rate related to exposure and disease that may induce bias in the estimates of association. Prospective research is less likely to be biased due to differences in the probability of subject selection based on arterial disease and risk factor presence. Prospective research is a stronger study design with regard to inferring the possibility of causation, since the presence of risk factors may be determined prior to arterial disease onset. Many prospective studies exist on the epidemiology of CAD, but fewer have covered the topic of PAD.

The problem of measurement error in the assessment of the presence or absence of vascular disease is well recognized. Even coronary angiography for the diagnosis of CAD is likely to result in some degree of misclassification, for reasons described previously [1]. A similar situation holds for the diagnosis of PAD. For example, it is likely that in some instances, claudication will occur even with a normal or high ankle-brachial index (ABI), if noncompressible, calcified vessels result in falsely high readings of the ankle systolic blood pressure [2]. This misclassification issue is even more problematic when a test result is used to formulate a clinical plan for an individual patient, as compared to epidemiologic analysis where population statistics are the result of interest. When misclassification of PAD status occurs nondifferentially with regard to exposure (randomly), the net result is the bias of any observed difference toward the null value [3]. The same holds true for exposures that are nondifferentially misclassified with regard to PAD. Therefore, observed differences found in an epidemiologic analysis of risk factors for PAD validly reflect potential causative factors for this complication, but probably underestimate the magnitude of the risk increase. Epidemiologic studies may therefore draw valid

conclusions regarding risk factors for PAD even if the techniques used to measure either vascular disease or the potential risk factor are prone to nondifferential misclassification.

The American Diabetes Association produced a consensus statement in which they recommended using ABI to screen for peripheral artery disease in patients with diabetes over the age of 50 [4]. The issues of screening and misclassification and the limitations of the ABI were acknowledged. However, the problems were not felt to detract from the clinical usefulness of the ABI to screen for and diagnose PAD in patients with diabetes. The American Heart Association (AHA) and American College of Cardiology Foundation (ACCF) guidelines issued in 2011 for management of patients with peripheral artery disease recommend that ABI results should be uniformly reported, with noncompressible values defined as greater than 1.40, normal values 1.00 to 1.40, borderline 0.91 to 0.99, and abnormal 0.90 or less [5–7].

The Prevalence and Incidence of Peripheral Artery Disease

Peripheral artery disease affects a high proportion of older persons in general populations located in developed countries. Meijer et al. presented age- and gender-adjusted results for nine population-based surveys of the prevalence of low ABI using different definitions (<0.75 to <0.94) that ranged from 5.5% to 26.7% [8]. In very elderly (85–93 years) Japanese-American men living in Hawaii, the prevalence of PAD was somewhat higher, at 27.4% [9]. In a population of patients chosen because they were over age 70 or over age 50 but with a history of tobacco use or diabetes, the prevalence of PAD was 27% [10]. An observational study of 25,083 Danish men aged 65–74 years found the prevalence of PAD to be 10.9% [11]. Fowkes, et al. performed a systematic review and analysis of 34 community-based studies since 1997 to produce global estimates of prevalence of peripheral artery disease in 2000 and 2010 [12]. This analysis estimated prevalence of PAD in women age 65 to 69 years of age to be 10.08% in high-income countries and 9.91% in low- and middle-income countries and in men to be 10.33% in high-income countries and 6.74% in low- and middle-income countries. Among those aged 85–89 years, prevalence in women in high-income countries was 18.38% and 15.22% in low- and middle-income countries and in men to be 18.83% in high-income countries and 14.94% in low- and middle-income countries [12].

Claudication is an insensitive measure of peripheral artery disease, with symptomless diminished arterial flow estimated to occur at least two to five times as frequently as claudication [13]. The Rose questionnaire has been used by investigators to assess claudication prevalence, but it has been shown to have only moderate sensitivity (60–68%) in capturing persons with this clinical diagnosis [14]. In the Edinburgh Artery Study, the prevalence of claudication in men increased from 2.2% in the 50–59 year age category to 7.7% in the 70–74 year age category [15]. Meijer et al. reviewed 13 population-based screening surveys for the presence of claudication and reported age- and gender-adjusted estimates ranging from 0.6% to 7.4%,

with one additional study finding a prevalence as high as 14.4% [8, 16]. Although it has been written that men are affected with symptomatic PAD between two to five times as frequently as women, in the review of Meijer, a twofold or higher prevalence of claudication was seen in only one of the 13 studies [8].

Peripheral artery disease in people with diabetes is both morphologically and physiologically distinguished from nondiabetic atherosclerosis [17]. The femoropopliteal segments are most often affected, as in nondiabetic patients, but smaller vessels below the knee, such as the tibial and peroneal arteries are more severely affected in patients with compared to those without diabetes [18, 19]. In practical terms, diabetes is associated with a high prevalence of distal arterial disease, a propensity to earlier calcification, increased thrombogenicity, and generally poorer prognosis.

Among patients who have type 1 diabetes, peripheral artery disease is more common than for the general population. In the Pittsburgh Epidemiology of Diabetes Complications Study of childhood onset type 1 diabetes, women who had type 1 diabetes for 30 years were found to have a prevalence of peripheral artery disease greater than 30% compared to only 11% for men when determined by ABI less than 0.8 at rest or after exercise [20]. The Epidemiology of Diabetes and Complications (EDIC) study, the long-term follow-up of the Diabetes Control and Complications Trial (DCCT), identified those patients with ABI < 0.9. The EDIC study found that intensively treated participants, with an average duration of type 1 diabetes of about 14 years, had a prevalence of peripheral artery disease of 8.8% among women and 4.6% among men [21].

Patients with type 2 diabetes in the UK Prospective Diabetes Study (UKPDS) had a prevalence of PAD of 1.2% (95% confidence interval 0.9–1.5%) at the time of diagnosis of their diabetes [22]. Peripheral artery disease in the UKPDS was defined as the presence of any two of the following: (1) ABI < 0.8; (2) absence of both dorsalis pedis and posterior tibial pulses to palpation in at least one leg; and (3) claudication. At 6 years of follow-up in the UKPDS, 2.7% of participants (95% confidence interval 2.2–3.2%) had peripheral artery disease according to these criteria that were not present at diagnosis and 10.6% had at least one of these three abnormal measures. The prevalence of PAD increased to 12.5% in the smaller subgroup of participants followed for 18 years (95% confidence interval 3.8–21.1%) [22]. In the Rotterdam Study, diabetes was associated with a twofold higher odds of PAD (odds ratio 2.0, 95% CI, 1.6–2.5) [23]. The Framingham Offspring Study examined 1554 males and 1759 females for peripheral artery disease. In this population-based study, the odds ratio for peripheral artery disease was 2.3 (95% confidence interval 1.5–3.6) among participants with versus those without diabetes [24]. This odds ratio associated for developing peripheral artery disease with diabetes and also the odds ratios associated with hypertension, current smoking and each additional 10 years of age, are shown in Fig. 22.1. In a meta-analysis using data from seven cohort studies, the adjusted relative risk (RR) for developing PAD associated with diabetes compared with no diabetes was 1.96 (95% CI, 1.29–2.63) in women and 1.84 (95% CI, 1.29–1.86) in men [25]. In the Health Professionals Follow-up Study, a prospective study of 44,985 men followed for 25 years, the Hazard Ratio (HR) for PAD was

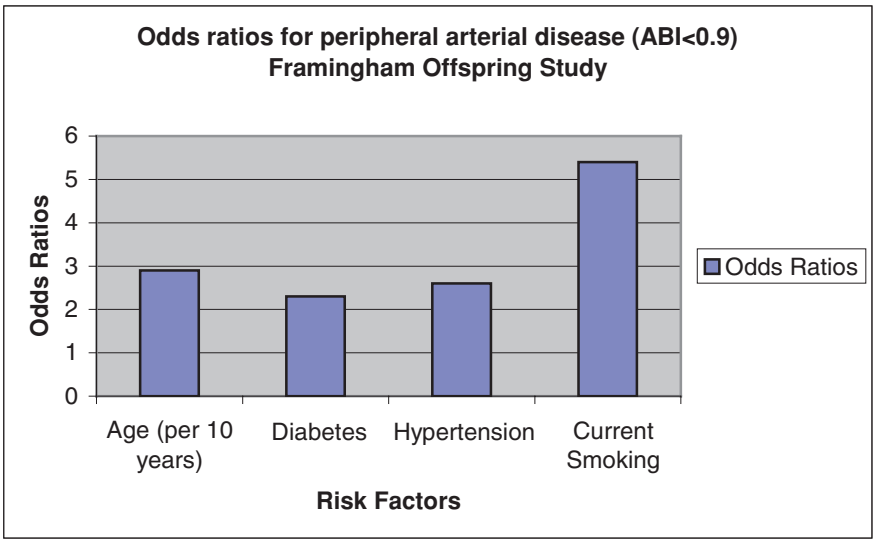

Fig. 22.1 Odds ratios for risk factors for peripheral artery disease. Peripheral artery disease was defined as an ankle-brachial index less than 0.9. Data taken from the Framingham Offspring Study, a population-based study of peripheral artery disease and its risk factors

2.55 (95% CI,1.60–4.06) for type 2 diabetes among never-smokers [26]. The National Health and Nutrition Examination Survey (NHANES) 1999–2002 found that the prevalence of PAD was higher with greater hemoglobin A1c, even among those without diabetes. The odds ratio of peripheral artery disease for those with a hemoglobin A1c of 5.7–6.0% was 1.57 (95% CI, 1.02–2.47) compared to hemoglobin A1c < 5.3%. For participants with diabetes, the odds ratio was 2.33 (95% CI, 1.15–4.70) for those with A1c < 7% and 2.74 (95% CI, 1.25–6.02) for those with A1c ≥ 7% [27].

There are racial differences in the complications of diabetes, including peripheral artery disease. The reasons for these differences are likely due to the social determinants of health rather than genetic differences. Genetic research finds more variation within racial groups than between them [28]. The NHANES 1 Epidemiologic Follow-up Study (NHEFS) found that among subjects with incident diabetes mellitus during the study follow-up period, 3.4% of Blacks had lower extremity amputations compared to 1.4% of Whites [29]. The authors of the study speculated that a combination of social and environmental factors may account for the apparent ethnic difference. To examine the question of whether the observed differences in complication rates were due to disparate access to health care, a study of an ethnically diverse population with uniform health care coverage was undertaken by the Kaiser Permanente Medical Care Program in Northern California. The study observed 63,432 health plan members with diabetes, which included 12% Asians, 14% Blacks, 10% Latinos, and 64% Whites, for 4 years, and measured nontraumatic lower extremity amputation and end-stage renal disease among other

outcomes. Age-and sex-adjusted incidence rates of lower extremity amputation did not differ significantly between Whites and Blacks or Latinos, whereas Asians had a rate 64% lower than that of Whites [30]. By comparison, age- and sex-adjusted incidence rates of end-stage renal disease were significantly higher for Blacks, Asians, and Latinos relative to Whites (112%, 44%, and 41% higher, respectively).

Nationally representative U.S. data on the prevalence of PAD are available from the National Health and Nutrition Examination Survey (NHANES) that has been conducted in an ongoing manner for over three decades. Results of the frequency of ABI < 0.9 shown in examination cycles that conducted this measurement by age, sex, race/ethnicity, smoking status, and diabetes mellitus are shown in Fig. 22.2a–d [31]. Prevalence of ABI < 0.9 is higher with greater age; similar in men and women; higher in non-Hispanic Black participants than non-Hispanic White participants; lower in Mexican American participants than other race/ethnic groups; and higher

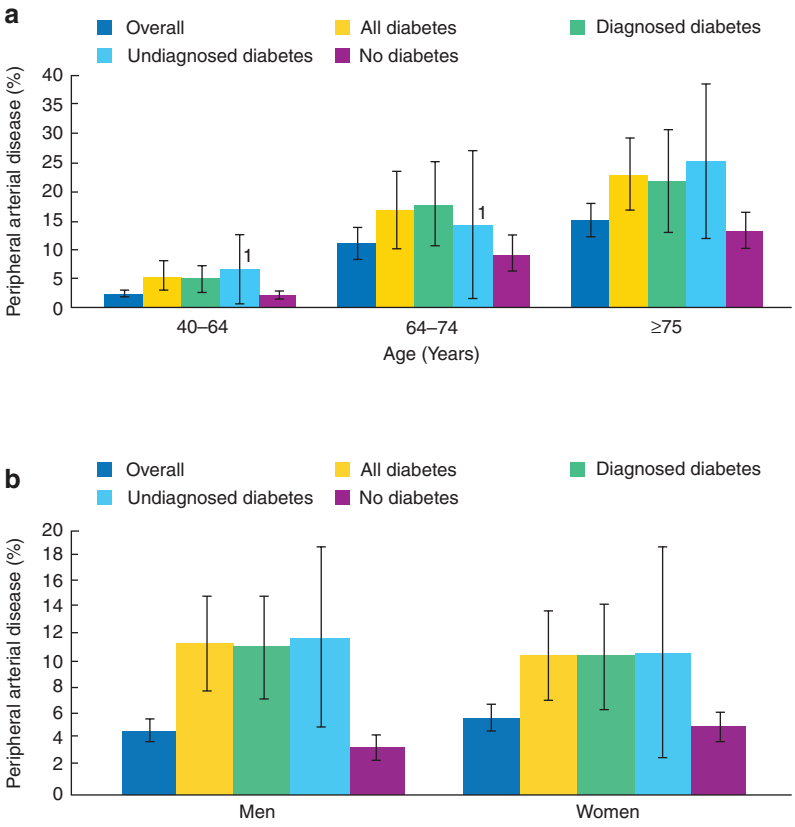

Fig. 22.2 (**a–d**) Prevalence of peripheral artery disease as defined by ankle-brachial index <0.9 on either leg among adults age ≥40 years by the presence of diabetes mellitus defined as A1c ≥6.5% or fasting plasma glucose ≥126 mg/dL and age (**a**), sex (**b**), race/ethnicity, and smoking status (**d**) from the National Health and Nutrition Examination Survey (NHANES), 1999–2004

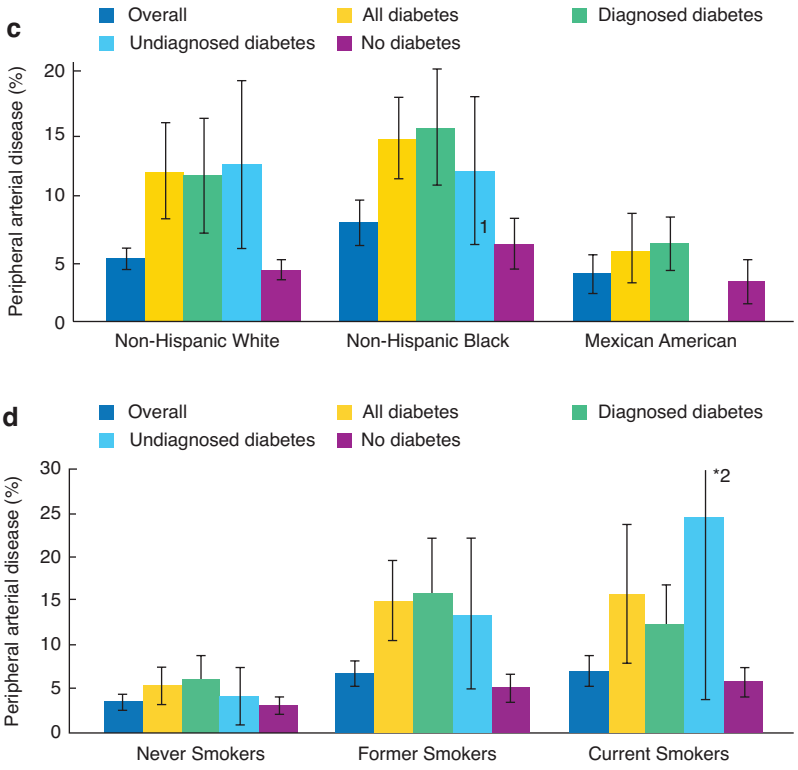

Fig. 22.2 (continued)

in former and current smokers. Prevalence of ABI < 0.9 is similar in diagnosed and undiagnosed diabetes in men and women. In all subgroups defined by the categorizations in Fig. 22.2a–d, the point estimate of ABI < 0.9 prevalence is higher in all persons with diabetes compared to all without diabetes.

Risk Factors for Peripheral Artery Disease

Smoking is one of the strongest risk factors for PAD. The Framingham Heart Study found a strong relationship between the number of cigarettes smoked and the incidence of intermittent claudication. A multivariate analysis identified smoking as the strongest single risk factor for development of symptomatic obstructive arterial disease, regardless of gender [32]. The occurrence of intermittent claudication is twice as frequent in smokers as nonsmokers. In the Edinburgh Artery Study, peripheral artery disease prevalence was strongly and positively related to lifetime cigarette smoking [15]. Smoking was related to a higher relative prevalence of peripheral artery disease (range of odds ratios, 1.8–5.6) than heart disease (range of odds ratios,

1.1–1.6). A prospective analysis of this cohort over 5 years revealed an incidence of new claudication of 2.6% among nonsmokers, 4.5% in moderate smokers (≤25 pack-years), and 9.8% in heavy smokers (>25 pack-years) [33]. The Rotterdam Study found that cigarette smoking was associated with an odds ratio for PAD of 2.8 (95% CI, 2.3–3.4) [23]. The Health Professionals Follow-up Study found that the risk of PAD among the heaviest smokers compared with never-smokers was higher, with a HR of 12.89 (95% CI, 8.59–19.34). In this study, compared with all former smokers, the HR for incident PAD among current smokers was 3.81 (95% CI, 3.00–4.84) [26].

Hyperglycemia was found to be associated with a higher risk of incident peripheral artery disease, independent of other risk factors including age, elevated systolic blood pressure, low HDL-cholesterol, smoking, prior cardiovascular disease, peripheral sensory neuropathy, and retinopathy. Each 1% increase in hemoglobin A1c was associated with a 28% increased risk of peripheral artery disease (95% confidence interval, 12–46) [22]. A cohort of 10,624 patients with diabetes in the ADVANCE trial was found to have an incidence of PAD of 5.8% over 5 years of follow-up. Microvascular disease was found to be associated with PAD after adjustment for established risk factors. Microalbuminuria was associated with a hazard ratio for PAD of 1.91 (95% CI, 1.38–2.64). Retinal photocoagulation therapy was also associated with a hazard ratio of 1.6 (95% CI, 1.11–2.32) [34].

Multiple randomized clinical trials have been conducted among persons with both type 1 and type 2 diabetes aimed at improving glucose control, but PAD typically is not a major outcome of interest and few reports exist of the results of such trials as they pertain to PAD development. The Diabetes Control and Complications Trial (DCCT) and the post-trial follow-up of these participants in the Epidemiology of Diabetes Interventions and Complications (EDIC) study evaluated the association between approximately 6.5 years of intensive glycemic control during the DCCT with the development during EDIC of low ABI (<0.8 or <0.9) or elevated ABI (>1.3) suggesting arterial calcification [35, 36]. Intensive control was associated with a significantly lower risk of developing arterial calcification during EDIC follow-up (HR 0.72, 95% CI, 0.55–0.94) but not occlusive disease as reflected by low ABI. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) randomized clinical trial tested the effects of intensive glycemic control on complications in persons with type 2 diabetes [36]. Randomization to intensive glycemic control was associated with a significant reduction in risk of lower extremity amputation (HR 0.69, 95% CI, 0.48–0.99). Given the important role of PAD in the occurrence of lower extremity amputation in diabetes, this finding suggests a potential reduction in the development of significant lower limb ischemia with intensive glycemic control in type 2 diabetes [37].

Many conditions associated with diabetes mellitus may help explain the higher prevalence of PAD seen in persons with this condition, such as other PAD risk factors that comprise features of the metabolic syndrome (hypertension, dyslipidemia) [38]. Increased levels of hemostatic factors such as fibrinogen, von Willebrand factor, t-PA, fibrin, D-dimer, and plasma viscosity explained in part the higher prevalence of PAD in subjects with diabetes or impaired glucose tolerance in the Edinburgh Artery Study [39].

Patients with hypertension in the Framingham Study showed a three-fold increased risk of intermittent claudication at a 16-year follow-up [40]. Limb arterial obstructive disease occurs twice as frequently as CAD among hypertensive individuals, and hypertension has been reported in 29 to 39 percent of patients with symptomatic peripheral artery disease [41]. The Cardiovascular Health Study reported about a 50% higher prevalence of an ABI < 0.9 associated with hypertension in a multivariate analysis adjusted for age, smoking, diabetes, and dyslipidemia [42]. Observational data analysis among 3642 patients in the UKPDS showed that the aggregate endpoint of amputation or death from peripheral vascular disease was associated with a 16% decrease per 10 mmHg reduction in systolic blood pressure, adjusted for age at diagnosis of diabetes, ethnic group, smoking status, presence of albuminuria, hemoglobin A1c, high- and low-density lipoprotein cholesterol, and triglyceride [43]. A study of 4.2 million adults registered in a primary care practice for at least 1 year in the United Kingdom found that 20 mmHg higher than usual systolic blood pressure was associated with a hazard ratio for PAD of 1.63 (95% CI, 1.59–1.66). The association was not modified by sex or smoking status [44].

The association of hypercholesterolemia with atherosclerosis of the lower extremities has been known for nearly 100 years [45]. The prevalence of claudication in patients with serum cholesterol levels over 260 mg/dl is on average over twice as high as in those with a concentration below this level. The prevalence of hyperlipidemia in patients with clinical manifestations of lower extremity arterial occlusive disease ranges in various studies from 31% to 57%. The Edinburgh Artery Study reported a higher prevalence of PAD in association with higher serum cholesterol and lower high-density lipoprotein cholesterol in multiple logistic regression analysis [15]. The Cardiovascular Health Study reached similar conclusions among its sample of 5084 subjects aged 65 years or older, with PAD defined as an ABI < 0.9 [42].

Other risk factors have been shown to be associated with a higher prevalence of peripheral artery disease. Higher circulating levels of homocysteine have been demonstrated in this condition [46], as have parallel low levels of folate in red blood cells and circulating vitamin B6, which raises the possibility that supplementation with these vitamins may reduce the incidence of peripheral artery disease [47]. One small, randomized, placebo-controlled study of secondary prevention has shown that oral therapy with folic acid, vitamin B12, and vitamin B6 decreased the need for revascularization in patients receiving percutaneous coronary intervention [48]. Higher levels of various hemostatic factors have been demonstrated in persons with lower ABI, suggesting that a hypercoagulable state predisposes to the development of PAD [49, 50]. The prevalence of low ABI (<1.05) was highest among persons with a birthweight <6.6 pounds, a demonstration of the “thrifty phenotype” hypothesis that postulates fetal growth retardation as a cause of metabolic disorders and vascular disease in adult life [51, 52]. In a study of patients with diagnosed PAD, self-perceived stress during the first 12 months of follow-up was associated with an increase in all-cause mortality over 4 subsequent years, after adjustment for demographics, comorbidities, disease severity, treatment type, and socioeconomic status, with a hazard ratio of 2.12 (95% CI, 1.14–3.94) [53].

Conditions Associated with Peripheral Artery Disease

Atherosclerosis, the underlying cause of peripheral artery disease, is a multifactorial, progressive condition that begins in childhood and involves multiple biologic processes and foci. Therefore, it is not surprising that patients with peripheral artery disease often have extensive CAD and CBD. The prevalence of CAD among persons with claudication in the general population is between two and four times higher than in those without claudication [54]. Around 50% of persons experiencing claudication also suffer from angina, while patients with angina are six times more likely to have claudication [55]. When 200 consecutive patients admitted to a vascular surgery service in an academic teaching hospital were evaluated for concomitant diseases, CAD was present in 46%, 22% had symptomatic CAD, 37% had impaired cardiac function, and 32% had carotid artery disease [20]. Both claudication and asymptomatic PAD (ABI < 0.9) in the Edinburgh Artery Study population were significantly associated with greater intima-media thickness of the carotid arteries as assessed by ultrasound [56]. Fowkes et al. in their systematic review of 34 population-based studies found that a history of cardiovascular disease, such as coronary heart disease or stroke, was associated with an odds ratio for PAD of 2.27 (95% CI, 1.98–2.59) [12].

Mortality Associated with Peripheral Artery Disease

Although peripheral artery disease rarely causes death, diminished long-term survival in these patients is well established. The causes of death associated with this condition are primarily cardiovascular. In the Framingham study, 14% of men and 18% of women died within 6 years of the onset of intermittent claudication [57]. Mortality rates appear to be related to the severity of the obstructive process. Szilagyi et al. found the cumulative six-year mortality rate was 62% in patients with symptoms sufficiently severe to require femoro-popliteal bypass [58]. In the study of DeWeese and Rob, 48% of patients with claudication, 80% of those with ischemic rest pain, and 95% of those with gangrene died within 10 years of undergoing femoro-popliteal bypass grafting [59]. In the National Health and Nutrition Examination Study (NHANES), 1999–2004, among the 7458 participants, PAD was associated with all-cause mortality, with a hazard ratio of 2.4 (95% CI, 1.9–2.9) after adjustment for age, sex, and race/ethnicity [60].

Patients with severe or symptomatic peripheral artery disease have 4 to 7 times the risk of mortality from all causes and a 15-fold higher risk of mortality from cardiovascular disease than persons who do not have peripheral artery disease [61]. Simonsick et al. demonstrated that intermittent claudication was an important predictor of mortality and cardiovascular morbidity in ambulatory older adults independent of associated coronary ischemia and cardiovascular disease risk factors [62]. Howell et al. found that independent of age or the presence or absence of

diabetes, a low ABI was strongly associated with increased mortality [63]. Although the presence of arterial obstructive disease of the legs is a hallmark of generalized atherosclerosis and therefore would be expected to confer an increased risk of cardiovascular or cerebrovascular death, extremely severe PAD appears to carry a particularly ominous prognosis. These researchers noted that patients with an ABI ≤ 0.30 had a very high six-year cumulative mortality rate (64%) [63].

Conclusions

Vascular disease of the peripheral artery beds reflects a process of generalized atherosclerosis in most cases, since co-occurrence of stenoses at multiple peripheral sites is often seen, risk factors for disease at one site usually are related to higher risk at other sites, and peripheral disease confers a higher risk of death due to CAD. Since many of these risk factors are reversible or treatable to some extent, there is hope that primary or secondary preventive interventions may yield further benefits in reducing the impacts of these diseases on mortality, morbidity, health status, and quality of life.

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Part V
Cardiovascular Disease

Chapter 23
Managing Stable Coronary Artery Disease in Diabetes

Ioannis Koulouridis and Michael Johnstone

Background

Diabetes has a direct impact not only on the life expectancy but also on the quality of life in the setting of cardiovascular disease, especially when diabetes is present from a young age [1]. In addition, cardiovascular disease and especially coronary artery disease is the leading cause of adverse outcomes in patients with diabetes [2]. The presence of diabetes mellitus along with coronary artery disease can impact the number and type of treatment choices since both the intensity and medical agents of glycemic control play a significant role on cardiovascular outcomes.

Numerous approaches to reduce the risk of adverse cardiovascular outcomes are available in patients with diabetes mellitus with the most effective measures focusing on primary prevention. Table 23.1 summarizes the guidelines among cardiology, endocrinology, nephrology, and general medicine professional societies from the world for cardiovascular risk reduction in patients with diabetes [3]. The similarity between these recommendations is noteworthy. Despite the numerous treatment strategies and the supporting evidence, their adoption in clinical practice remains low [4]. While financial and social barriers to healthcare access are definitely influential factors, neglecting to follow the guidelines plays a considerable role as well.

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Table 23.1 Comparison of type 2 diabetes guideline recommendations between medical professional societies from the world for cardiovascular risk reduction in patients with diabetes (from J Am Coll Cardiol. 2022 May, 79(18) 1849–1857)

	ACC/AHA [180–182]	ADA [183–185]	AACE/ACE [186, 187]	ESC/EASD [188]	USPSTF [189–191]	KDIGO [192–194]
Risk assessment						
Method	Pooled cohort equation and diabetes-specific risk enhancers	Pooled cohort equation and diabetes-specific risk enhancers	Framingham risk assessment tool and risk factors	Moderate, high, very high risk	Pooled cohort equation	No recommendation
Lifestyle recommendations						
Exercise	150 min of moderate-intensity activity per week	150 min of moderate-intensity activity per week	150 min of moderate-intensity activity per week	150 min of moderate-intensity activity per week	No specific recommendation	150 min of moderate-intensity activity per week
Diet	Individualized nutrition assessment; Mediterranean diet	Individualized nutrition assessment; Mediterranean diet	Individualized nutrition assessment; Mediterranean diet	Individualized nutrition assessment; Mediterranean diet	No specific recommendation	Individualized nutrition assessment; Mediterranean diet, 0.8 g protein/day if CKD
Vitamin use	No recommendation	No recommendation	No recommendation	Avoid vitamin supplementation to reduce ASCVD risk in T2DM	No recommendation	No recommendation
Blood pressure management						
BP target	<130/80 mmHg	<130/80 mmHg if 10-year ASCVD risk ≥15%; <140/90 if 10-year ASCVD risk <15%	<130/80 mmHg	<130/80 mmHg, (but not <120/70 mmHg), and 130–139 mmHg in those older than 65 year	<120/80 mmHg only for stroke risk reduction	<120/80 mmHg if concurrent CKD

First-line treatment of hypertension	Angiotensin-converting enzyme/ ARB if albuminuria	Angiotensin-converting enzyme/ ARB if albuminuria	Angiotensin-converting enzyme/ ARB	Angiotensin-converting enzyme/ ARB if albuminuria or LVH	No recommendation	Angiotensin-converting enzyme/ ARB if albuminuria
Indication for combination therapy	If BP >140/90 mmHg	Dual therapy first line regardless of BP	If BP >150/100 mmHg	If BP >160/100 mmHg	No recommendation	No recommendation
LDL-C management						
Primary prevention treatment targets	50% LDL-C lowering for those at high risk	50% LDL-C lowering for those at high risk	Numeric goal (LDL-C <55, 70, or 100 mg/dL)	Numeric goal (LDL-C <55, 70, or 100 mg/ dL)	No recommendation	No recommendation
Primary prevention in young patients	Treat if longstanding disease, end-organ damage, risk factors	Treat if longstanding disease, end-organ damage, risk factors	No recommendation	Treat if LDL-C >100 mg/dL	No recommendation	No recommendation
Secondary prevention treatment targets	Goal 50% LDL-C reduction, start meds LDL-C <70 mg/dL	Goal 50% LDL-C reduction, start meds at LDL-C <70 mg/dL	LDL-C <55 mg/dL	LDL-C <55 mg/dL	No recommendation	No recommendation
Secondary prevention second-line therapy	Ezetimibe	Ezetimibe or PCSK9i	No recommendation	Ezetimibe	No recommendation	No recommendation

(continued)

Table 36.1 (continued)

	ACC/AHA [180–182]	ADA [183–185]	AACE/ACE [186, 187]	ESC/EASD [188]	USPSTF [189–191]	KDIGO [192–194]
Hyperglycemia treatment and novel agents						
First line	SGLT2i/GLP-1RA may be beneficial regardless of background metformin	SGLT2i/GLP-1RA may be beneficial regardless of background metformin	SGLT2i/GLP-1RA may be beneficial regardless of background metformin	SGLT2i/GLP-1RA first line	No recommendation	Metformin and SGLT2i in combination for those with CKD
Relative priority of SGLT2/ GLP-1RA	SLGT2i > GLP-1RA for HF, renal disease, weight loss	SLGT2i > GLP-1RA for HF and renal disease	SLGT2i > GLP-1RA for HF and renal disease	No specific recommendation	No recommendation	SGLT2 inhibitor first, GLP-1RA second line
Aspirin recommendations						
Primary prevention	May be considered if elevated ASCVD risk without increased bleeding risk	May be considered if elevated ASCVD risk without increased bleeding risk	No recommendation	Not in moderate risk, but can be considered in high or very high risk	No significant risk reduction with aspirin in individuals with T2DM	May be considered if elevated ASCVD risk without increased bleeding risk
CKD						
Type 2 diabetes treatment	SGLT2i	SGLT2i, specifically canagliflozin	SGLT2i	SGLT2i	No recommendation	SGLT2i

Stable Coronary Artery Disease

Antiplatelets

Patients with diabetes are in a prothrombotic state that predisposes them to higher risk for adverse cardiovascular outcomes [5]. The elevated levels of insulin and glucose alter the levels of nitric oxide in the vascular bed and cause inflammation and vasoconstriction, promoting atherothrombosis. This, in turn, shortens the platelet lifespan leading to the prevalence of immature platelets, which show increased affinity to form a clot [6]. This environment is also characterized by increased expression of the glycoprotein IIb/IIIa receptors [7] and prothrombotic molecules such as von Willebrand factor and P-selectin [8]. Given this prothrombotic state, antiplatelets have been a cornerstone for secondary prevention of cardiovascular adverse events in patients with diabetes. The protective effect of antiplatelet agents is diminished compared to patients without diabetes and even more when chronic kidney disease is present [9].

Aspirin and/or Clopidogrel

Clopidogrel alone instead of aspirin is a good option in patients with diabetes and stable coronary artery disease (i.e., no stent or acute coronary syndrome in the prior year). In the CAPRIE trial (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events), clopidogrel was superior to aspirin in reducing ischemic events without increasing the risk of bleeding. In a subgroup analysis including only patients with diabetes, the clopidogrel performed even better than aspirin [10]. The overall evidence so far in stable coronary artery disease on long-term dual antiplatelet therapy with clopidogrel in addition to aspirin shows that it increases the risk of bleeding and per the American Heart Association (AHA) should be reserved only for patients that have particularly high-risk features (e.g., prior myocardial infarction, younger age, active tobacco use) with the use of a risk calculator [11] and after careful discussion with the patient [12].

Aspirin and Ticagrelor

The THEMIS trial (Effect of Ticagrelor on Health Outcomes in Diabetes Mellitus Patients Intervention Study) [13] compared dual antiplatelet therapy with aspirin and low-dose ticagrelor versus aspirin alone in patients with diabetes and coronary artery disease without a history of myocardial infarction or stroke. The aspirin plus ticagrelor group had a lower risk of the composite of cardiovascular death, myocardial infarction, or stroke over an average follow-up of 40 months but the incidence of major bleeding was higher [14]. The net clinical benefit of ticagrelor was more pronounced among patients with history of percutaneous coronary intervention [15].

Aspirin and Rivaroxaban

The COMPASS trial (Cardiovascular Outcomes for People Using Anticoagulation Strategies) [16–18] included patients with stable atherosclerotic disease and followed a factorial design (low-dose rivaroxaban plus aspirin vs rivaroxaban alone vs aspirin alone). It showed that the risk of major adverse cardiovascular events was significantly lower with the combination of low-dose rivaroxaban plus aspirin compared to monotherapy but with an increased risk of bleeding. The anti-ischemic benefits of the combination were consistent in a subgroup analysis including only patients with diabetes mellitus. In this subset of patients, low-dose anticoagulation in addition to aspirin could play a role for secondary prevention.

Blood Pressure

Hypertension is twice as common among patients with diabetes compared to the general population. Furthermore, around 70–80% of patients with diabetes have hypertension [19] which additionally increases the risk of myocardial infarction, stroke, and all-cause mortality. The increase is progressive with increasing systolic blood pressure beyond 115 mmHg [19].

Blood Pressure Target

Guideline recommendations have changed over the past decades. Earlier trials showed the benefit of systolic blood pressure control <140 mmHg. Previous guidelines at the time recommended a more aggressive target blood pressure of <130/80 mmHg in patients with diabetes (<120/75 mmHg when renal impairment is present as well). Subsequently, it was found that these aggressive targets do not prevent coronary events, even though they do decrease the risk of stroke [20–23]. As a result of these studies, guidelines changed and the recommended blood pressure target was <140/90 mmHg for patients with diabetes, with an attempt of <130/90 mmHg if it can be achieved without harm.

Intensive blood pressure control had a resurgence with SPRINT (Systolic Blood Pressure Intervention Trial) showing significant risk reduction on cardiovascular morbidity and mortality with intensive (<120 mmHg) as compared to standard (<140 mmHg) systolic blood pressure control in patients with hypertension and a high cardiovascular risk group [24]. Nevertheless, SPRINT explicitly excluded patients with diabetes and therefore generalization to these patients should not be made. It is noteworthy that intensive blood pressure control might require multiple antihypertensive agents which can potentially lead to harm [19, 20, 25–29]. This scenario is particularly important in the presence of cardiomyopathy since lower blood pressure hinders the myocardial perfusion and increases the risk of myocardial infarction, especially in the setting of pre-existing coronary artery disease.

Several randomized controlled studies [21–23] have shown that a systolic blood pressure of <120 mmHg and a diastolic blood pressure < 70 mmHg were associated with a lower risk of stroke but with a higher risk of other adverse cardiovascular outcomes. In patients with diabetes and coexisting coronary artery disease, the optimal blood pressure target is still under debate. While all patients with diabetes and coronary artery disease will benefit from blood pressure < 140/90 mmHg [19, 22, 25, 26, 29, 30], a more aggressive blood pressure target of <130/80 mmHg could be beneficial for patients with a higher stroke risk (e.g., history of prior stoke, carotid artery disease).

Choice of Antihypertensive Agents

Most patients with hypertension and diabetes will require more than one antihypertensive agent to control blood pressure. In the standard care arm in ACCORD trial, 30% required two and 39% required at least three antihypertensive agents. The potential of reducing blood pressure is similar between the different classes of antihypertensives [31]. Factors that are more important in clinical practice are potential side effects, off-target effects, cost, and frequency of dosing.

ACE inhibitors/ARBs should be the first option since they reduce both the progression of kidney disease when albuminuria is present, and reduce the risk of atherosclerotic ischemic events [32, 33]. The HOPE study (Heart Outcomes Prevention Evaluation) [34] included patients with diabetes mellitus with cardiovascular disease or at least one cardiovascular risk factor but no proteinuria or heart failure and randomized them to ramipril versus placebo. The trial showed consistent benefit of ramipril reducing the risk of myocardial infarction, stroke, and cardiovascular death. These findings were independent of changes in blood pressure. In patients after myocardial infarction [34, 35] and with reduced ejection fraction [36, 37] ACE inhibitors/ARBs are even more beneficial.

The American Diabetes Association guidelines recommend the use of thiazide-like diuretics (preferably long-acting agents such as chlorthalidone or indapamide) or dihydropyridine calcium channel blockers. Thiazide diuretics impair glycemic control by reducing insulin sensitivity and secretion [38–41] making their overall clinical effects questionable, despite their beneficial cardiovascular outcomes in blood pressure trials [39]. Mineralocorticoid receptor antagonists (spironolactone, eplerenone) can also be effective, especially in the setting of hypokalemia [42]. Most importantly, they significantly reduce morbidity and mortality in patients with left ventricular dysfunction [43, 44].

Beta-blockers are not the preferred antihypertensive agent [45, 46] 9293 for patients without coronary artery disease, but are often used for indications other than only blood pressure control. Patients who benefit the most from β-blockers are the ones with a history of prior myocardial infarction, chronic angina, left ventricular dysfunction (ejection fraction <40%), or arrhythmias. Beta-blockers did not reduce the risk of mortality or myocardial infarction in patients with stable coronary disease in the absence of left ventricular dysfunction [47]. The benefit of long-term

use of β-blockers after myocardial infarction has been questioned, with the best evidence showing that the benefit is limited to the first 30 days post myocardial infarction [48]. Therefore, β-blockers for hypertension should be reserved for patients with clear indications. And in those with poorly controlled hypertension or systolic heart failure, preferably a β-blocker with a simultaneous vasodilatory response (such as carvedilol or labetalol) should be used [49–51].

Hyperlipidemia

Diabetes promotes dyslipidemia, typically hypertriglyceridemia. Other common findings are elevated dense low-density lipoprotein (LDL) but also reduced high-density lipoprotein (HDL) caused by amplified HDL catabolism. There is also a preponderance of very large LDL particles. LDL cholesterol (LDL-C) is often similar between patients with and without diabetes but the constantly elevated triglycerides and glucose levels promote LDL oxidation and glycation, rendering the LDL particles even more atherogenic [52, 53].

Statins

Robust data from trials confirms that statins are beneficial in both primary and secondary prevention of coronary artery disease [54–65]. Their ability to lower LDL levels and minimize relative cardiovascular risk is similar between patients with and without diabetes [55–65]. The absolute cardiovascular risk reduction is actually more pronounced in patients with diabetes because of their higher underlying risk. Numerous studies and meta-analyses have confirmed that the use of statins is associated with slightly higher incidence of diabetes type II [66]. This modest risk is dwarfed by the cardiovascular protection of statins [66–68]. Besides, this increase in glucose is modest, only in the order of a 0.12% increase in mean glycated hemoglobin (HbA1c) [69].

Non-statin LDL-C–Lowering Treatments

Many patients cannot tolerate aggressive statin therapy, mainly due to side effects. Also, even with intensive statin dosing, some do not meet the expected LDL-C levels. After a median follow-up of 6 years, ezetimibe in addition to simvastatin within 10 days of an acute coronary syndrome lowered LDL-C levels further and was associated with an overall small but statistically significant further reduction in the primary composite end point of cardiovascular death, major coronary event, or stroke [54]. Subgroup analysis restricted to the cohort with diabetes mellitus showed an

even more significant reduction mainly due to a lower incidence of myocardial infarction and stroke among this population [70] with this treatment combination.

The FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) randomized 27,564 participants with atherosclerotic cardiovascular disease to either placebo or evolocumab with simultaneous statin therapy. Among the participants, 11,031 had diabetes mellitus [71] and, when compared to the patients without diabetes, there was a similar LDL-C reduction and risk for the primary composite end point of cardiovascular death, myocardial infarction, stroke, and hospitalization for unstable angina or revascularization [72]. The incidence of diabetes remained unaffected, same as with fasting plasma glucose and HbA1c levels.

In the ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab), 18,924 adults with a recent acute coronary syndrome (5444 with diabetes mellitus) were randomized to either alirocumab every 2 weeks or placebo in addition to maximally tolerated statin therapy [73]. The combination of alirocumab and statins resulted in an absolute risk reduction for time to first MACE of 2.3% in the group with diabetes mellitus relative to 1.2% for those without [74].

Although neither PCSK9 inhibitor reduced cardiovascular mortality in the FOURIER and ODYSSEY OUTCOMES trials, their cumulative data suggest that PCSK9 inhibitors effectively lower LDL-C and cardiovascular risk in individuals with coronary artery disease regardless of the presence of diabetes mellitus. They also support the 2010 Cholesterol Treatment Trialists' Collaboration suggestion toward a more aggressive LDL-C lowering strategy for cardiovascular risk reduction. This statement was driven from a meta-analysis of 26 randomized trials with 170,000 participants showing that every 39 mg/dL (1.0 mmol/L) reduction in LDL-C was associated with a 10% decrease in all-cause mortality, driven primarily by lower cardiac-related deaths without elevating the risk of adverse events [75]. For this aggressive LDL-C reduction approach, additional agents may be needed beyond statin monotherapy. In high-risk patient such as those with both diabetes and coronary artery disease, particularly when LDL-C levels are >70 mg/dL despite maximally tolerated statin, the addition of ezetimibe and PCKS9 inhibitors should be considered.

Non-LDL Target Therapies

Combinations of statins with fibrates, niacin, or fish oil have not demonstrated further cardiovascular benefits compared to statin monotherapy [76–80]. An exception is aggregate data from several fibrate trials that, when stratified by lipid profiles, show that individuals with hypertriglyceridemia and low HDL levels benefit from a reduction in cardiovascular risk with the addition of fibrates to statins [78, 81–83]. Also, fibrates or fish oil are indicated for patients with very high triglyceride levels

(>500 mg/dL) to reduce the risk of pancreatitis [30]. Icosapent ethyl is the first non-LDL target therapy that demonstrated cardiovascular benefit and should be considered as a first line for patients with diabetes and coronary artery disease whose triglycerides remain elevated (>135 mg/dL) despite maximally tolerated statin and lifestyle changes. This is supported by the recommendations of the American Diabetes Association Standards of Medical Care [30]. The data on Icosapent ethyl originates from the REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl-Intervention Trial), which randomized individuals with cardiovascular disease or diabetes, elevated triglycerides, and one additional risk factor, to icosapent ethyl vs. placebo [84]. Icosapent ethyl showed a significant reduction in the risk of the composite outcome of cardiovascular death, non-fatal myocardial infarction, stroke, coronary revascularization, or unstable angina. These findings were independent of diabetes status.

Lifestyle Modifications

Weight loss if overweight or obese, exercise, smoking cessation if a smoker, heart-healthy diet, sleep, and stress management are fundamental for patients with diabetes or coronary artery disease. There are limited randomized trials for lifestyle modifications on patients with diabetes and comorbid coronary artery disease. The evidence to support these measures on this population has been extrapolated from primary prevention trials and from subgroup analyses among studies on secondary prevention of coronary artery disease [19, 85].

Smoking

There is strong evidence supporting a causal link between cigarette smoking and numerous adverse health outcomes [86–88]. Smoking cessation reduces the risk of recurrent cardiovascular events, declining to the level of risk of non-smokers within approximately 3 years after cessation. The benefit is similar for those with and without diabetes [89]. Cessation of smoking may induce weight gain that in result might pose some cardiovascular risk but data shows that the cardiovascular risk reduction resulting from smoking cessation outweighs this risk [90].

Diet

For patients with diabetes, particular emphasis on consumption of fruits, vegetables, and low-fat dairy foods is recommended by the American Diabetes Association Standards of Medical Care in Diabetes [30]. There are no specific recommendations when coronary artery disease is a comorbidity, mainly because most data originates

from primary prevention trials [91, 92]. The PREDIMED trial (Prevención con Dieta Mediterránea) randomized Mediterranean diet supplemented with either extravirgin olive oil or mixed nuts versus a control diet and is the largest randomized controlled trial focused on primary prevention through dietary modifications on patients at high risk of cardiovascular disease [93]. In this study, 48.5% of the participants had type II diabetes. The trial was stopped early because of a 30% reduction in the primary composite outcome of cardiovascular death, myocardial infarction, or stroke with the Mediterranean diet. The results were similar in the subgroup of patients with diabetes.

Evidence suggests that low-carbohydrate and low-glycemic-index diets may improve both glycemic control and cardiovascular risk [94, 95]. Further investigations are needed to assess the role of the glycemic index for primary and secondary prevention of coronary artery disease.

Psychosocial Factors and Sleep

Depression exacerbates the risk of macrovascular complications in people with diabetes. This is supported mainly by observational studies. In the REGARDS prospective cohort study (Reasons for Geographic and Racial Differences in Stroke), people with T2DM who reported depression or psychological stress had a significantly increased incidence of stroke and acute cardiovascular disease [96]. These findings were corroborated with the results from the Denmark arm of the ADDITION study (Anglo-Danish-Dutch Study of Intensive Treatment in People With Screen Detected Diabetes in Primary Care). Psychological stress assessed by the Mental Health Inventory was associated with higher risk of a cardiovascular event (non-fatal myocardial infarction, non-fatal stroke, revascularization, or amputation) [97]. It is unknown whether cardiovascular events are more likely when depression and psychosocial stress are coupled with diabetes and whether treatment will improve the course of coronary artery disease.

Inadequate sleep is underdiagnosed in patients with diabetes and promotes endothelial dysfunction through sympathetic activation, and inflammation. Insufficient sleep in patients with diabetes is mainly due to obstructive sleep apnea but any sleep disorder has similar ramifications. Improving sleep is beneficial in terms of controlling hypertension and metabolic variables such as serum lipids and insulin resistance, despite a lack of clear benefit on cardiovascular outcomes [98].

Physical Activity

Patients with diabetes exercise less [99] and have lower exercise tolerance [100, 101] compared to patients without diabetes and these variables are associated with a higher risk of cardiovascular events [99, 100]. The American Diabetes Association

guidelines recommend that prolonged sitting should be interrupted with light activity every 30 min and that patients should engage in at least 150 min/week of moderate-to-vigorous physical activity [102]. The guidelines for patients with stable coronary artery disease recommend the same amount of weekly exercise at first diagnosis to cardiac rehabilitation, which includes supervised exercise training with a comprehensive secondary prevention program [103]. Supervised exercise training has been shown to be more effective than home training in improving HbA1c, body mass index, waist circumference, blood pressure, exercise capacity, muscle strength, and cholesterol levels [102, 104]. Patients with diabetes who enroll in cardiac rehabilitation have more cardiovascular risk factors, lower exercise capacity, are less likely to complete cardiac rehabilitation, and have a higher mortality compared to patients without diabetes [105–107].

Data regarding exercise regimens in patients with diabetes and coronary artery disease are conflicting. Some studies have shown improvements in outcomes such as exercise capacity, waist circumference, endothelial function, blood pressure, HbA1c, and cholesterol [99, 108–110], whereas others failed to show improvements in HbA1c, or exercise capacity [99, 111]. Of note though, the latter studies suffered from poor adherence. It seems that patients with coronary artery disease benefit from cardiac rehabilitation irrespective of the presence of diabetes when assessing exercise capacity [106, 112] as well as reductions in hospitalizations and mortality [113].

Weight

Clinicians should refer patients with obesity and diabetes to a dietician and a structured weight loss program since these patients have particular difficulty in losing weight [114, 115]. Medical therapy has limited efficacy 172 and evidence regarding the safety of these agents in patients with coronary artery disease is scarce. There are two agents that have been studied: lorcaserin which had a no effect on MACEs [116] and liraglutide which reduced MACEs [117].

Bariatric surgery, such as Roux-en-Y gastric bypass and sleeve gastrectomy, is also an option that guidelines support [118]. Evidence consistently shows that bariatric surgery is associated with an improvement in cardiovascular risk factors including better glycemic control, lower blood pressure, higher high-density lipoprotein cholesterol, and lower triglyceride levels [119–121]. Observational studies have shown consistent reduction in cardiovascular risk with these procedures but randomized clinical trials so far have been unable to demonstrate benefit in cardiovascular events and mortality, possibly due to inadequate power [120, 122, 123]. There are certainly risks associated with such procedures [122], especially among patients with coronary artery disease, but careful selection targeting a subset of patients with morbid obesity (body mass index $\geq$35 kg/m^2) offers benefits that outweigh these risks.

Stable Angina

Evaluation

Various non-invasive tests are available for evaluation of patients with stable coronary artery disease that provide a better assessment of prognosis. Two large studies have compared computed tomography angiography (CTA) versus functional testing in this setting. The SCOT-HEART trial (Scottish Computed Tomography of the Heart) [124] was an open-label study showing that the composite end point of death caused by coronary artery disease or non-fatal myocardial infarction was lower in the computed tomography angiography group, primarily due to a lower rate of non-fatal myocardial infarction. The findings were similar in the subgroup of patients with diabetes mellitus. Revascularization rates were not affected. The authors hypothesized that these findings were probably due to a better use of preventive therapies in the computed tomography angiography group rather than due to test results. In contrast, the PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) [125] showed no difference in outcomes between computed tomography angiography and functional testing. However, in a post hoc analysis, there was a significant interaction by diabetes mellitus when patients with diabetes were randomized to computed tomography angiography, they had a lower risk of cardiovascular death or non-fatal risks [126]. There was no significant difference in these outcomes in the absence of diabetes mellitus [127]. Summarizing the above, computed tomography angiography may be superior to stress testing among patients with diabetes mellitus, mainly due to its ability to diagnose non-critical coronary lesions.

Medical Therapy

Patients with angina and type II diabetes often have more extensive coronary artery disease that may not be amenable to revascularization [128, 129]. Persistent angina post revascularization is also common [130–132]. Therefore, antianginal medical management plays a particularly important role. Strategies involve either increasing myocardial oxygen supply (nitrates, calcium channel blockers) or decreasing demand (β-blockers, calcium channel blockers, ranolazine, ivabradine). Per guidelines β-blockers or calcium channel blockers constitute the first-line therapy, whereas long-acting nitrates and ranolazine are considered second and third lines, respectively [133, 134]. None of these agents has been shown to reduce mortality or myocardial infarction [47, 135–138]. Beta-blockers, calcium channel blockers, and nitrates have similar effects on angina and exercise duration [138, 139]. Thus, the clinician should select these agents according to the desired effect rather than follow a strict algorithm, especially as it relates to diabetes.

Beta-blockers reduce heart rate and myocardial contractility that may lead to compensatory peripheral vasoconstriction, which in turn can lead to insulin resistance and hyperlipidemia [49, 50, 140]. This does not hold true for β-blockers that have a concomitant vasodilatory effect (e.g., carvedilol, labetalol, nebivolol) [49–51] and seem to be associated with significant decreases in HbA1c, improved insulin sensitivity, lower cholesterol levels, less weight gain, and less progression to microalbuminuria [49, 141–143] compared to non-vasodilatory β-blockers. Clinicians should also consider the impact of ranolazine on glycemic control. Ranolazine is the only antianginal that has been found effective in patients with type II diabetes [144]. Interestingly, ranolazine can reduce HbA1c by reducing glucagon secretion [145]. The metabolic impact of β-blockers and ranolazine on HbA1C, however, is modest at best.

Revascularization

The preferred management of patients with type II diabetes and coronary artery disease remains optimal medical therapy and modification of cardiovascular risk factors [146, 147] in addition to revascularization when necessary [146, 148–150]. Outcomes on both surgical and percutaneous revascularization are suboptimal in the presence of diabetes with higher incidence of complications and restenosis [151–153]. In patients with multi-vessel coronary artery disease, left main disease, and complex coronary anatomy, coronary artery bypass grafting (CABG) is superior to percutaneous approach (PCI) but with a somewhat increased risk of early stroke [154, 155], which is around 1.8% for CABG versus 0.3% for PCI [156]. The main advantage of CABG compared to PCI is reduced need for repeat revascularization, especially the first few years after revascularization [155].

The importance of the internal mammary artery is highlighted with the results of the BARI (Bypass Angioplasty Revascularization Investigation) Trial comparing CABG with PCI [157, 158], (Fig. 23.1). The subgroup of patients with diabetes mellitus who were randomized to CABG had a significant survival benefit extending beyond 10 years of follow-up [159]. A durable bypass of the entire proximal vessel plays a key role in patients with left internal mammary artery grafts [149, 160, 161]. The use of bilateral left internal mammary arteries for grafting approach is usually avoided due to increased sternal infection rates [162–164]. This is despite retrospective studies implying that it is not only safe [165, 166] but possibly superior to unilateral left internal mammary artery approach [164]. This is the only large-scale randomized trial to date has failed to demonstrate superiority of bilateral compared to single approach [167, 168].

Percutaneous intervention (PCI) with drug-eluting stents in patients with diabetes is associated with a higher incidence of restenosis [169], which ranges approximately 15% within 2 years after bare metal stenting [170]. The use of drug-eluting stents reduced this risk by 60–70% [171]. With newer technologies available, the

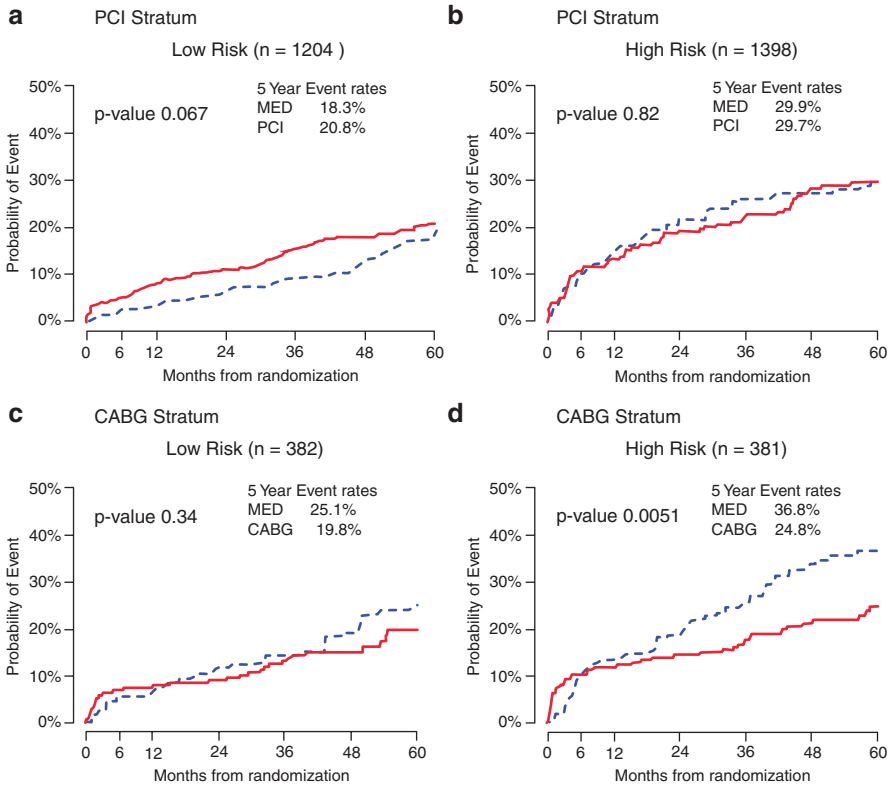

Fig. 23.1 The main findings of the BARI 2D trial. Patients are stratified according to the intended revascularization stratum and angiographic risk score. Each panel shows the Kaplan–Meier event rates for the composite outcome death/MI/stroke for patients randomized to medical therapy (MED; blue dotted line) and prompt revascularization (PCI, red continuous line) with the log-rank *p*-value. (From Circulation 2012;126:2115–2124)

benefit of CABG over PCI in patients with type II diabetes has been reduced. The FREEDOM trial (Future Revascularization Evaluation in Patients With Diabetes Mellitus: Optimal Management of Multivessel Disease) [156] was the first large-scale investigation of contemporary CABG compared with drug-eluting stent PCI (first-generation paclitaxel- or sirolimus-eluting stents) among patients with diabetes and coronary artery disease. The primary composite outcome of 5-year rates of death from any cause, non-fatal myocardial infarction, or non-fatal stroke was more frequent in the PCI group compared to CABG (26.6% versus 18.7%; $P = 0.005$), (Fig. 23.2). Similarly, the individual outcomes of death and non-fatal myocardial infarction were also more common in the PCI group. Of note, stroke was more common in the CABG group (5.2% versus 2.4%; $P = 0.03$) [156, 172, 173]. Extended follow-up for a mean of 7.5 years confirmed similar findings. The survival curves diverged at 2 years and continued to broaden, implying a solid long-term benefit [174].

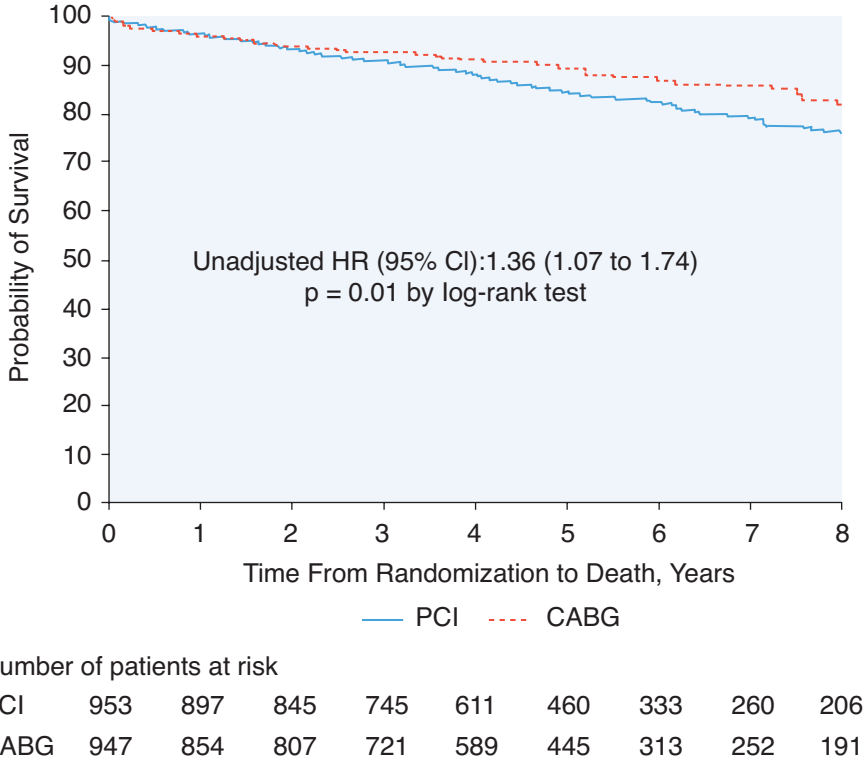

Fig. 23.2 Survival curves on the total cohort of patients according to the revascularization strategy in the FREEDOM Follow-On Study. Coronary artery bypass grafting (CABG, dotted red line) results in a long-term survival benefit in patients with diabetes and multivessel coronary disease when compared with percutaneous coronary intervention (PCI, continuous blue line) using drug-eluting stents. (From J Am Coll Cardiol. 2019 February 19;73(6):629–638)

A meta-analysis [154] has shown that there is a 5-year survival advantage with CABG compared to PCI among patients with diabetes mellitus. This meta-analysis used patient-level data from three major studies including patients with diabetes mellitus [146, 147, 156] showed a reduction in the 5-year incidence of death, myocardial infarction, or stroke with optimal medical therapy and CABG compared to optimal medical therapy and PCI. When need for subsequent revascularization was assessed with another meta-analysis [175], the optimal medical therapy and CABG were again superior.

Diabetes mellitus is associated with vascular injury, which in turn raises cardiovascular risk and the propensity for new ischemic events. This association is stronger in patients with advanced disease and poor glycemic control [148, 176]. Prevention remains the cornerstone of therapy. If revascularization is needed, PCI with new-generation drug-eluting stents or CABG with internal mammary artery are the preferred approaches [177]. In the setting of multi-vessel coronary artery

disease and diabetes, CABG along with optimal medical therapy are recommended by the current US and European society guidelines [178, 179].

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Chapter 24
The Management of Hyperglycemia and DM in Patients with an Acute Coronary Syndrome

Tatiana Joseph and Michael Johnstone

Introduction

Both type 1 and type 2 diabetes mellitus are associated with hyperglycemia. However, hyperglycemia is more common even in otherwise controlled diabetic patients during stress situations [1]. Patients presenting with the acute coronary syndrome (ACS) can have elevations in their glucose levels, be they nondiabetic or diabetic [1]. Regardless of whether patients have diabetes or not, patients with uncontrolled glucose levels have an increased risk of in-hospital complications. Hyperglycemia is also a powerful predictor of survival; studies [2] have shown that there is a relationship between hyperglycemia levels and in-hospital mortality for patients admitted with myocardial infarction (MI). Prolonged exposure to hyperglycemia promotes changes at the cellular level of vascular tissues that accelerate the atherosclerotic process [3].

Maintenance of strict glycemic control improves short- and long-term complications in patients with type 1 and type 2 diabetes mellitus [4–6]. However, in the setting of an acute MI, data are limited and inconsistent on whether strict glycemic control with insulin therapy improves outcomes.

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Hyperglycemia Defined in Relation to Acute Coronary Syndrome

Hyperglycemia can be evaluated by a glucose metabolism profile which involves a fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), or glycated hemoglobin (HbA1C). The criteria for diagnosis of diabetes include FBG ≥126 mg/dL (7 mmol/L) or a 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT or a HbA1C ≥6.5.

Although there are formal values in terms of blood glucose levels/glycated hemoglobin for diagnosis of diabetes, there is no uniform definition of what constitutes hyperglycemia in ACS. There is still controversy about the cut-off glucose value that should be used as a diagnostic marker of hyperglycemia in ACS. Furthermore, it has been unclear whether the glucose index value should be that taken on admission, after overnight fasting or doing an oral glucose tolerance test [3, 7].

According to the American Heart Association (AHA) 2013 guidelines for management of hyperglycemia in the setting of an ACS, blood glucose levels should be maintained below 180 mg/dL if possible while avoiding hypoglycemia [3]. Concerns about overly aggressive glycemic control in critically ill patients were raised by the NICE-SUGAR (Normoglycemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation) trial. In this study of medical and surgical intensive care unit patients, tight glucose control (81–108 mg/dL) compared to modest control (<180 mg/dL) was associated with increased mortality rate, primarily from cardiovascular causes as well as increased episodes of hypoglycemia [8].

Prevalence of Elevated Glucose Levels in Acute Coronary Syndrome

Numerous studies have shown that hyperglycemia is a commonly encountered issue in critically ill patients in the intensive care setting, with many of those who previously did not have a diagnosis of diabetes mellitus [9].

The Euro Heart Survey on Diabetes and the Heart analyzed the prevalence of abnormal glucose metabolism in patients with ACS. Among patients with no previous history of diabetes, impaired fasting glucose or new hyperglycemia was found in 58% of patients [10].

The mechanism of hyperglycemia in this stressful setting is thought to be the result of sympathetic nervous system activation and the hypothalamic–pituitary axis which consequently raises the production of catecholamines and cortisol that

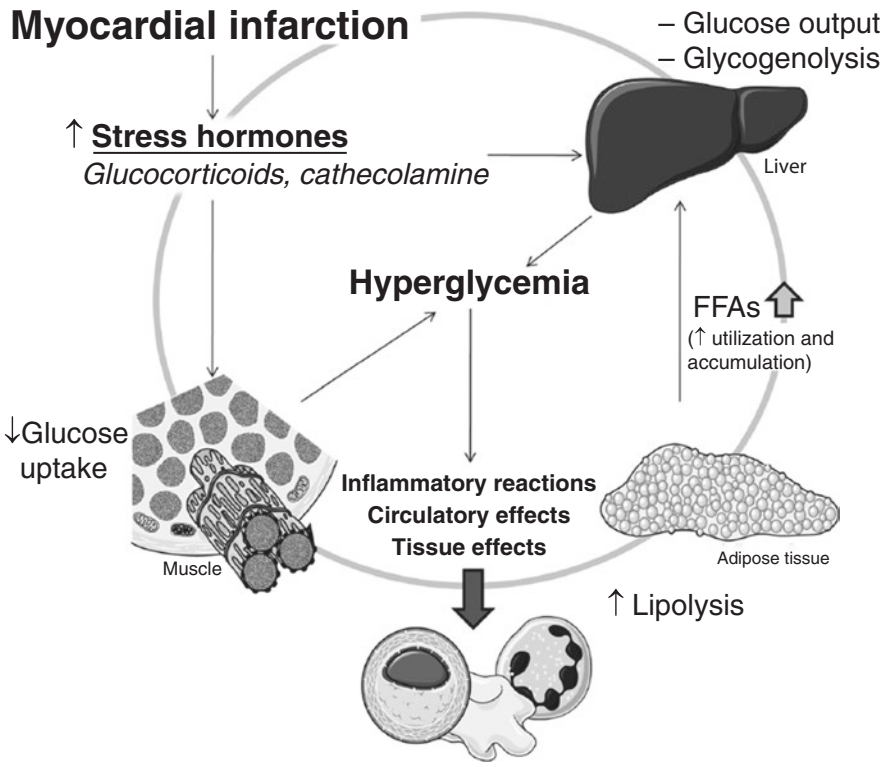

Fig. 24.1 Generation of hyperglycemia in an acute myocardial infarction

stimulate processes of gluconeogenesis, glycogenolysis, and lipolysis [11, 12] (Fig. 24.1).

It has been shown that hyperglycemia increases the release of inflammatory and vasoconstrictive factors which impair coronary endothelial function, contributes to the production of reactive oxygen species with consequent oxidative stress, and increases platelet aggregation [14, 15].

Acute or stress hyperglycemia has a role in the development of ACS and accentuates the consequence of cellular damage caused by acute myocardial ischemia [16]. Acute hyperglycemia can lengthen the QT interval which may increase the risk of ventricular arrhythmias as a result of ischemia [17]. It can also alter platelet function which can contribute to atherothrombotic complications [18] (Fig. 24.2).

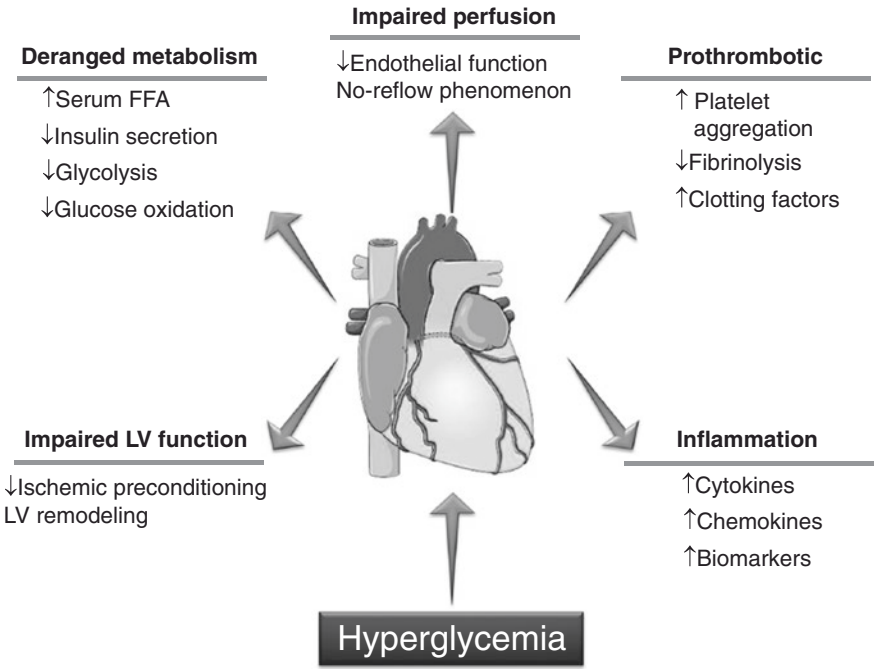

Fig. 24.2 Cardiovascular effects of hyperglycemia during the acute phase of myocardial infarction. Hyperglycemia is associated with some circulatory and tissue effects, including oxidative stress and increased inflammation, hypercoagulability, reduced endothelial function, volume depletion, acid base disturbances, and altered immunity. *FFA* free fatty acid [13]

Relationship Between Glucose Levels and Mortality in Acute Coronary Syndrome

It has been well documented that elevated glucose levels are associated with increased in-hospital and long-term mortality in ACS [3, 19]. The relationship between hyperglycemia and outcomes has been elevated in terms of short-term predictive value of the admission serum glucose in patients with and without diabetes and the long-term risk in patients with diabetes.

In a study by Pinto and colleagues, a review of 224 patients in trials of fibrinolysis or primary PCI in patients with STEMI revealed that the 30-day mortality rate in patients with hypoglycemia [blood glucose level <81 mg/dL (4.5 mmol/L)] was 4.6, euglycemia [blood glucose level of 81–99 mg/dL (4.5–5.5 mmol/L)] was 1.0, and severe hyperglycemia, [blood glucose values >199 mg/dL (11.0 mmol/L)] was 4.7. The 30-day rate of recurrent MI or death in the three groups was 10.5, 4.2, and 7.2%, respectively [20]. Even when adjustments were made for baseline differences, the relative risk for mortality was significantly increased, compared to the

euglycemic group, in patients with hypoglycemia (odds ratio 3.37) or severe hyperglycemia (odds ratio 3.09). The risk was also significantly increased in patients with blood glucose values between 150 and 199 mg/dL (8.3 and 11.0 mmol/L, odds ratio 2.93).

This U-shaped relationship was seen in both diabetic and nondiabetic patients.

Higher mortality with both hyperglycemia on admission and hypoglycemia during hospitalization has been observed in other observational studies [1, 21].

The long-term outcomes after an acute MI are worse in diabetic patients than nondiabetic patients with ACS. Increases in mortally and non-fatal cardiovascular endpoints such as reinfarction or heart failure were seen. According to Franklin and colleagues, diabetic patients tend to be older and have a greater prevalence of comorbidities compared to patients without diabetes. However, the increase in risk persists after adjustment for these differences [22].

Dynamic Changes in Glucose Levels in Acute Coronary Syndrome

Glucose levels are usually highest upon first presentation of an acute MI then gradually decrease as the stress response subsides [23]. Goyal et al. analyzed acute MI patients with baseline and 24-h glucose data from the CARDINAL trial database and found that both higher baseline glucose and the failure of glucose levels to decrease in the first 24 h after an acute MI predicted higher mortality in nondiabetic patients. Similarly, in a study of approximately 8000 patients hospitalized with ACS in the United States who had hyperglycemia (glucose >140 mg/dL) on arrival, glucose normalization after admission was associated with better patient survival, even after adjustment for confounders [24]. Improved survival was noted regardless of whether glucose normalization occurred as the result of insulin therapy or happened spontaneously.

In another study done by Okada and colleagues, which involved 57 patients with ACS, integrated backscatter intravascular ultrasound (IB-IVUS) and gray scale IVUS were performed before balloon dilatation or stent implantation in the culprit vessel. Standard IVUS indices were evaluated for volume index (volume/length) and plaques component were measured by IB-IVUS for percent tissues volume. Blood glucose variability (fluctuations in blood glucose levels over a given interval of time) was determined by calculating the mean amplitude of glycemic excursions (MAGE) using a continuous glucose monitoring system. This study demonstrated that higher blood glucose variability was strongly associated with increased lipids and decreased fibrous content, making plaques more vulnerable to disrupt as well as a larger plaque burden in the culprit vessels of ACS. The study concluded that higher blood glucose variability was an independent risk factor for plaque instability [25].

Clinical Trials of Glucose Control in Patients with Acute Coronary Syndrome

Patients with ACS presenting with hyperglycemia are at an increased risk for adverse outcomes, but it is unclear whether hyperglycemia is a direct mediator of poor outcomes or a marker indicating a greater disease severity. A few trials evaluating the optimal glycemic target for ACS patients have been performed (Table 24.1).

Table 24.1 Optimal glycemic target for ACS patients

Clinical trial	Glucose level on admission	Glucose targets	Achieved glycemic target (intervention vs. control) (mg/dL)	Primary endpoint	Result
DIGAMI (1995)	~280	126–180 mg/dL vs. usual care acutely 90–126 mg/dL fasting BG vs. usual care afterward	173 vs. 211 mg/dL during 24 h; difference in hemoglobin A_{1C} but not fasting BG afterward	Mortality at 3 months	No significant difference in mortality
DIGAMI 2 (2005)	229	126–180 mg/dL in hospital vs. usual care acutely 90–126 mg/dL fasting blood glucose (group 1 only) vs. usual care afterward	164 vs. 180 at 24 h, no difference afterward	All-cause mortality difference between groups 1 and 2	No significant difference in mortality
HI-5 (2006)	≥140	72–180 mg/dL vs. usual care	149 vs. 162 during the first 24 h	Mortality at in-hospital stage, 3 and 6 months	No significant difference in mortality
Marfella (2009)	≥140	72–180 mg/dL vs. usual care	163 vs. 192 mg/dL	Left ventricular ejection fraction (LVEF), oxidative stress, apoptosis	Higher LVEF, decreased oxidative stress and apoptosis
Marfella (2012)	≥140	80–140 vs. 180–200 mg/dL or GIK	161 vs. 194 vs. 182	Myocardial regeneration	Increased myocardial regeneration

Table 24.1 (continued)

Clinical trial	Glucose level on admission	Glucose targets	Achieved glycemic target (intervention vs. control) (mg/dL)	Primary endpoint	Result
Marfella (2012)	≥140	80–140 mg/dL for intervention arm 180–200 mg/dL for control arm	145 vs. 191 mg/dL	In stent restenosis	Decreased in-stent stenosis
Marfella (2013)	≥140	80–140 vs. 180–200 mg/dL	144 vs. 201 mg/dL	Myocardial salvage	Increase in myocardial salvage
Recreate (2012)	≥144	90–117 mg/dL vs. usual care	117 vs. 143 mg/dL	Difference in mean glucose levels at 24 h	Significant difference in glucose levels between intensive and standard group; no difference in mortality
BIOMArKS2 (2013)	≥140	85–110 mg/dL during day, 85–139 mg/dL at night vs. <288 mg/dL	112 mg/dL vs. 130 mg/dL	High-sensitivity troponin T 72 h after admission	No significant difference in high-sensitivity troponin T

The DIGAMI trail was one of the first randomized clinical trials to evaluate the effect of intensive glucose control in acute MI patients. Patients presenting within 24 h of ACS were randomized to an intervention arm with insulin-glucose infusion followed by multidose subcutaneous insulin and a control arm with conventional therapy. The trial enrolled 620 patients, 80% of whom had previously diagnosed diabetes. The primary endpoint was all-cause mortality at 3 months. Patients in the insulin arm had significantly lower glucose levels compared to the control arm during the interventional period. Although there was no difference between two treatment groups for the primary outcome, reduced all-cause mortality was observed in the insulin arm at both 1- and 3.4-year follow-up points [26]. It is unclear whether acute or chronic intensive glucose control contributed more to the reduced mortality since the insulin treatment lasted 3 months. Other similarly designed studies were subsequently carried out, DIGAMI was the only trial demonstrating any survival benefit from intensive glucose control [27], albeit much later than anticipated in the original study.

The following DIGAMI 2 study was performed comparing the effects of three different treatment strategies in diabetic patients with AMI. Unexpectedly, no difference in the glucose control was achieved between the treatment groups, and specifically, it failed to demonstrate early and continued insulin-based intensive glucose reduced mortality [28].

The -HI-5 or Hyperglycemia: intensive Insulin Infusion In Infarction Study was a prospective mulicenter randomized controlled trial that examined glycemic control among hyperglycemic) or diabetic patients admitted with an AMI using an insulin—dextrose infusion for glycemic control. Patients included subjects who were hyperglycemic without known diabetes (40% of the subjects) and were randomized to receive either insulin-based or conventional therapy. There was no difference in the mean 24-h blood glucose level between the two treatment arms. Despite a lower incidence of cardiac failure and reinfarction in the intervention arm within 3 months, HI-5 failed to demonstrate a reduced mortality either at the early in-hospital stage or later at 3 or 6 months post MI [29].

In the RECREATE trail, 287 patients with an acute MI and hyperglycemia were randomly assigned to either tight glucose control or usual care. At 24 h, patients from the tight glucose control arm had significantly lower glucose levels compared to those in control arm, yet the 90-day mortality did not differ between the two arms [30].

In a study by Marfella et al., 50 hyperglycemic patients diagnosed with AMI were randomized to intensive (target glucose level 80–140 mg/dL) or conventional glycemic control for almost 3 days before surgery. Compared to the control group, patients in the intensive group had higher ejection fractions, less oxidative stress, and less inflammation in peri-infarcted specimens. In their follow-up studies, tight glucose control in hyperglycemic patients with a STEMI resulted in an increase in myocardial salvage and a reduction in in-stent restenosis at 6 months after onset [4–6].

In light of the conflicting results of the studies mentioned above, de Mulder and colleagues hypothesized that the reason may be the glucose target. In their randomized trial BIOMARCS-2, a total of 294 patients with ACS and hyperglycemia were randomized to either intensive glucose control (85–110 mg/dL) or conventional management (<288 mg/dL). The primary endpoint was high-sensitive troponin *T*-value 72 h after admission. Glucose levels in the intensive arm were significantly lower than that of control arm within 36 h, but equalized by 72 h. In contrast, there were higher rates of mortality at both 30 days and long-term (a median follow-up of 5.1 years), suggesting that intensive glucose control in the early phase of AMI resulted in persistent harmful effects [31].

Insights from the cardiovascular outcome trials of the new glucose-lowering drugs, including Glucagon-Like Peptide 1 Receptor Agonists (GLP-1 RA's) and Sodium–Glucose Co-Transporter 2 (SGLT-2) inhibitors, have ushered a new management strategy on hyperglycemia which focused on clinical outcomes directly instead of just glucose control itself [32–34]. Although the protective effect of GLP-1RAs and SGLT-2 inhibitors has been demonstrated on myocardial ischemia in animal models, few trials have been performed in the ACS setting [35–37].

Relationship Between Glucose Variability and Patient Outcomes During Acute Coronary Syndrome

Fluctuations in blood glucose levels over a given interval of time are known as glycemic variability. Glycemic variability can have destructive effects on the endothelial function and increase the oxidative stress which theoretically can impact the prognosis of patients after an acute MI [38].

Whether glycemic variability is a predictor of adverse cardiovascular outcomes remains controversial with previous studies demonstrating conflicting results [1, 39, 40].

A post hoc analysis of data [41] from the HI-5 [29] suggested that acute glycemic variability in patients admitted immediately post-acute MI is associated with a higher risk of major adverse cardiovascular events. However, several other studies were unable to demonstrate that glycemic variability had an association with major adverse cardiovascular events [40, 42].

The Prognostic Importance of Hypoglycemia in Patients with Acute Coronary Syndrome

Those treated with glucose lowering treatment regimens are frequently complicated by hypoglycemia, which potentially may have long-lasting detrimental side effects. One group [21] found that low blood glucose levels (≤55 mg/dL or ≤3 mmol/L) during hospitalization was an independent predictor for death within 2 years compared to patients with normal glucose values throughout hospitalization, making it a possible useful marker for identification of diabetes patients with a poor prognosis.

There are possible clues for the underling mechanism in which in-hospital hypoglycemia is linked to long-term risk for adverse outcomes In one small study, six diabetic patients without known coronary artery disease were intentionally subjected to insulin-induced hypoglycemia. Ischemic EKG changes were noted in five out of the six patients, with significant elevations of catecholamines and declines in serum potassium, each of which may be associated with adverse cardiac consequences in the post-ACS period [43]. In a more recent study of 19 diabetic subjects, hypoglycemia detected by continuous glucose monitoring was linked with symptoms of angina and ischemic EKG changes recorded by Holter monitor [44].

Whether hypoglycemia is directly harmful in patients with ACS, or whether it is simply a marker for the most critically ill patients was evaluated in a large observational study. The risk associated with low blood glucose was confined to those who developed hypoglycemia spontaneously, most likely as the result of severe underlying illness. In contrast, hypoglycemia that occurred after insulin initiation was not associated with worse survival. These underlying illnesses that may cause hypoglycemia and, in turn, adversely affect outcomes include type 1/type 2 diabetes, renal insufficiency, diagnosed or occult malignancy [45]. Both the DIGAMI-2 and

CREATE-ECLA trials found no significant association between hypoglycemia and mortality after adjustment for cofounders [46, 47].

This relationship between hypoglycemia and adverse outcome and mortality may not be linear. Several studies suggest that glucose values in the hypoglycemic range demonstrate a J-shaped relationship between average glucose values during hospitalization and in-hospital mortality [1].

Current Patterns of Glucose Control in Acute Coronary Syndromes

As a general consensus, glucose levels above 180/200 mg/dL (10/11 mmol/L) should be treated, and hypoglycemia should strictly be avoided. In terms of in-hospital therapy, continuous intravenous regular insulin infusion should be initiated when blood glucose levels are greater than 180 mg/dL in critically ill patients [48–50].

There are many insulin infusions protocols available including the Yale insulin infusion protocol [51] and Leuven protocol [52]. There is no established protocol deemed most effective for optimal glycemic control; however, each insulin infusion protocol should be tailored to the subset of patients being treated and to local resources. Initial monitoring of blood glucose should be done on an hourly basis and insulin dose should be titrated to blood glucose levels [48–50].

Both subcutaneous insulin and oral anti hyperglycemic regimens should be avoided due to an increased risk of hypoglycemia.

Summary and Recommendations

In hospitalized patients, the correction and prevention of hyperglycemia are the standard of care [53–59]. To date, it remains unclear whether tight control of hyperglycemia significantly reduces morbidity and mortality. In large part, this a consequence that the trials examining target-driven glucose control in ACS lack sufficient statistical power to detect clinically important difference in mortality and other adverse clinical outcomes.

The best data that support glycemic control come from trials involving the critically ill and/or ACS patients who are not critically ill.

According to the American Heart Association (AHA), blood glucose levels should be maintained below 180 mg/dL if possible while avoiding hypoglycemia [3].

Hyperglycemia during ACS is a common finding and is associated with increased risk of immediate and long-term complication in patients both with and without diabetes mellitus [53–59]. While hyperglycemia is a predictor of a worse outcome, many knowledge gaps remain including the target glucose level and the underlying mechanism for adverse outcomes [3]. Therefore, further intervention trails are needed to optimize the definition of hyperglycemia in the setting of ACS and to established guidelines and goals of glucose lowering treatment.

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Chapter 25
Diabetes and Percutaneous Interventional Therapy

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Section A: Coronary Interventions in the Diabetic Population

Introduction

For the past two decades, the epidemic of obesity and diabetes mellitus have steadily increased in the United States and globally. In the year 2000, approximately 170 million people worldwide were estimated to have diabetes, and in the year 2019, this number increased to an estimated 463 million. The World Health Organization now lists diabetes in the top ten causes of death worldwide. In 2016, 1.6 million deaths were directly attributed to diabetes compared to less than one million in 2000. Even more worrisome is the fact that diabetes is a major risk factor for all forms of cardiovascular disease and contributes to the top two causes of death worldwide—ischemic heart disease and stroke (15.2 million out of 56.9 million deaths in 2016) [1]. The leading cause of death worldwide for greater than 30 years (greater than 50 years in the developed world) is ischemic heart disease, which is a direct result of atherosclerotic coronary artery disease [2].

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Diabetes Mellitus and Atherosclerosis

Atherosclerosis is a well-studied process that begins in the normal coronary artery wall and develops gradually over many years. The basic architecture of the coronary arterial wall has three principle layers: the intima, media, and adventitia, divided by an internal and external elastic lamina. Each layer has its own function and unique cellular and intercellular matrix components. The inner layer, the intima, composed of the endothelial cell lining and the subendothelial layer (fibroblasts and collagen), is usually only 1–2 cell layers thick and functions as a barrier to the vasoactive substances in the blood and also has specific paracrine functions. The middle layer, the media, is composed of layers of smooth muscle cells. The outermost layer is the adventitia, which consists predominantly of fibrous connective tissue adding support to the vessel while also housing the vasa vasora and sympathetic and parasympathetic nerve fibers (Fig. 25.1). In concert, the layers of the arterial wall function not only as a simple barrier but also mediate local constriction or dilation of the vessel, serve a regulatory role in inflammation via the

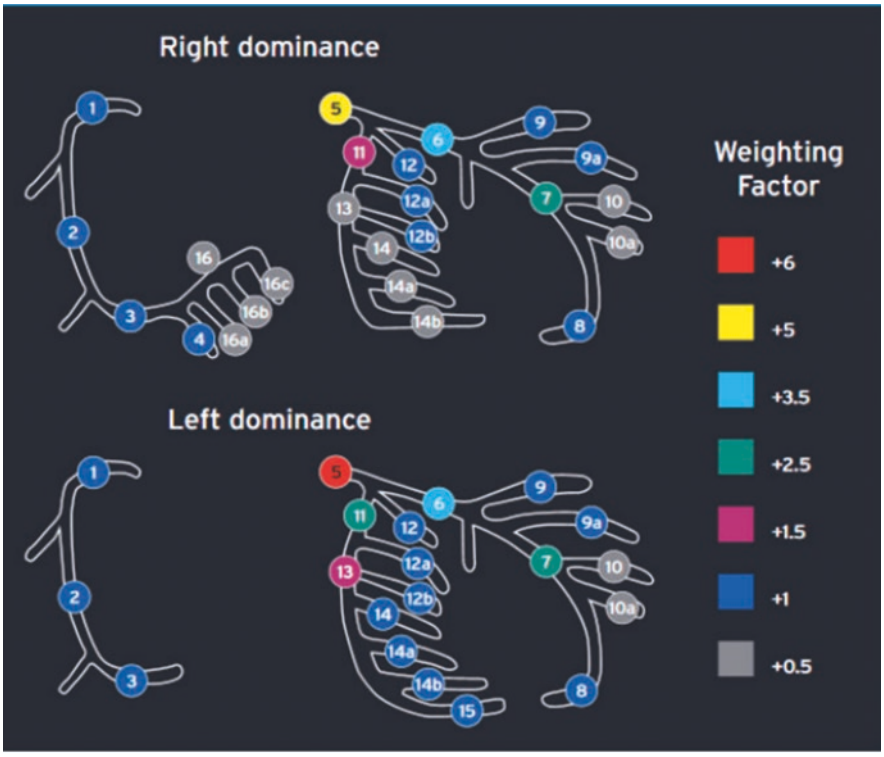

Fig. 25.1 A guide for calculating the SYNTAX score. (Adapted from the 2018 ESC/EACTS Guidelines on myocardial revascularization [3])

production of specific pro-inflammatory and anti-inflammatory molecules, regulate vascular remodeling, and maintain an equilibrium between the coagulation and fibrinolytic pathways [4].

The process of atherosclerosis begins when the endothelium becomes dysfunctional by any number of pathways/disease states. Among these causes of dysfunction are insulin resistance, hyperglycemia, elevated low-density lipoprotein (LDL) cholesterol levels, hypertension, low high-density lipoprotein (HDL) cholesterol levels, increased vascular production of reactive oxygen species, hyperuricemia, and high triglyceride levels. The pathophysiology of hyperglycemia may contribute to endothelial dysfunction before overt diabetes occurs. Once the endothelium is dysfunctional, LDL cholesterol circulating in the blood begins to accumulate within the intima. As the cholesterol accumulates within this plaque precursor, it begins to oxidize, which subsequently signals monocytes to migrate into the intima and convert to macrophages—this accumulation is known as a fatty streak. The macrophage cells enlarge and engulf cholesterol, but can become overwhelmed and subsequently undergo apoptosis, leaving behind foam cells (the remnants of cholesterol-filled macrophages), as well as releasing inflammatory cytokines. Atherogenesis is further propagated by this resultant cellular necrosis within the forming plaque, promoting inflammatory mediator expression, intimal thickening, and migration of more macrophage cells.

Initially, atheromatous plaques develop in an outward direction, with enlargement of the external radius of the artery, thereby maintaining the inner luminal diameter and thus blood flow [5]. This is referred to as "positive remodeling" or "Glagov" phenomenon of remodeling; first described in the coronary arteries and also present in the peripheral arteries. Luminal obstruction and vascular calcification are both later stages of atherogenesis. As the process continues, a well-defined core of extracellular lipids forms—at this stage, the collection is called atheroma or a fibrous plaque. Signals from the apoptotic macrophages prompt smooth muscle cell migration from the media, thus accelerating plaque formation by multiple actions, including secretion of collagen and elastin forming a protein fibrous cap, deposition of calcium, and releasing signals for neovascularization. In diabetic patient, excessive insulin and hyperglycemia further promote smooth muscle cell proliferation and migration [6]. During atherogenesis, the vasa vasorum, the network of microvessels that originate primarily from the adventitia, extends through the media into the thickening intima promoting the continued development of the atheroma.

In a multitude of pathways, the diabetic milieu further contributes to lesion progression. Hyperinsulinemia coincides with vascular dysfunction and provokes proinflammatory and prothrombotic tendencies [7, 8]. Inhibition of endothelial nitric oxide synthetase (eNOS) and altered nitrous oxide production result in vasomotor dysfunction [9]. Impaired vascular reactivity, endothelial dysfunction, and impaired vascular reactivity are seen with endogenous hyperinsulinemia [10–12]. Increased arterial intima and media layer thickness are frequently present in diabetic patient [13]. Vascular smooth muscle cell proliferation and migration are promoted by hyperglycemia [6]. These pathways among many others contribute to the progression of atherosclerosis in the diabetic patients.

Impact of Diabetes Mellitus on Ischemic Vascular Disease

Diabetes mellitus is a major contributor to ischemic vascular disease. In both the Framingham Heart Study and the Multiple Risk Factor Intervention Trial, diabetes remained a major cardiovascular risk factor independent of age, hyperlipidemia, hypertension, or smoking history [14, 15]. The significance of diabetes as a risk factor for myocardial infarction (MI) has been well studied in the INTERHEART Trial and the Atherosclerosis Risk in Communities (ARIC) Study [16, 17], and it is established that diabetic patients without previous myocardial infarction have an equal risk of future myocardial infarction as nondiabetics patients with prior myocardial infarctions [18].

In 2010, The Emerging Risk Factors Collaboration Group published a meta-analysis of 102 studies including data for 697,782 patients and found that the adjusted hazard ratio for diabetes was 2.00 (95% CI 1.83–2.19) for coronary artery disease. This study concluded that diabetes confers a twofold excess risk for a wide range of vascular diseases independent from other conventional risk factors [19].

Cardiovascular outcomes for patients with known atherosclerosis and diabetes are markedly worse than those patients without a diagnosis of diabetes. The poorer prognosis for the diabetic population, even after intervention, has been hypothesized to be secondary to a larger burden of comorbidities (hypertension, chronic renal disease, hyperlipidemia, heart failure), well-documented higher risk of stent-related complications (stent thrombosis and restenosis), and more complex coronary anatomy. The Reduction of Atherosclerosis for Continued Health (REACH) Registry followed 45,224 patients with a high risk of atherothrombosis or established atherothrombosis. Within the REACH Registry, 43.6% (n = 19,699) had diabetes at baseline; 4-year event rates were assessed from the international cohort. Endpoints included cardiovascular death, myocardial infarction, stroke, hospitalization for ischemia, and hospitalization for heart failure. In the REACH Registry, the hazard ratio of diabetes for cardiovascular death, nonfatal myocardial infection, or stroke was 14.8% (13.31–16.21). The hazard ratio for cardiovascular death was 7.7% (6.6–8.81) and the hazard ratio for nonfatal myocardial infarction was 4.1% (3.24–4.89) [20]. As dedicated high-level evidence to guide revascularization strategies in the diabetic population in the ACS setting are lacking, current guidelines are still based on publications for stable ischemic heart disease and expert opinion [21].

Management of Diabetic Coronary Artery Disease

Regardless of the diagnosis of diabetes, there are two Class I indications for coronary angiography: (1) A high likelihood of CAD based on the clinical characteristics and results of noninvasive testing when the benefits are deemed to exceed the risks. (2) In patients who, despite guideline directed medical therapy (GDMT), have unacceptable ischemic symptoms and are candidates for coronary revascularization

[22]. The risks of coronary angiography are elevated in diabetic patients. Coronary angiogram is not without risks, including but not limited to death, myocardial infarction, allergic reaction, bleeding, and contrast nephropathy. The overall risk of death during coronary angiography is approximately 0.1%, but this increases slightly in the presence of diabetes. In patients with normal renal function, the risk of contrast nephropathy is low unless large amounts of contrast, i.e., >3–5 mL/kg, are given. Diabetic patients may have an increased risk for contrast nephropathy. Importantly, metformin should not be administered if there is baseline renal dysfunction as there is an increased risk of lactic acidosis [23–25].

Once coronary artery disease has been diagnosed in the diabetic patient, treatment can broadly be divided into three categories: guideline-directed medical therapy (GDMT), percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG). Regardless of the treatment strategy, aggressive risk factor modification is mandatory including antiplatelet and lipid lowering agents, especially statins and/or PCSK9 inhibitors. Treatment should be aimed toward improving symptoms and quality of life, and reducing adverse events. The decision to proceed with a revascularization strategy, via either PCI or CABG, as opposed to continuing medical therapy alone, is thought to be appropriate in three groups of patients: (1) Patients with lifestyle-limiting symptoms despite maximum medical therapy; (2) patients who are intolerant to medical therapy; and (3) patients with anatomy for which revascularization has a proven survival benefit.

Determining the best revascularization strategy, PCI versus CABG, for the diabetic patient with advanced coronary artery disease has been the topic of much research evolving for the past 40 years. Although PCI technique, pharmacology, and stent technologies have continued to advance over this time period, there has been a consistent signal toward better overall outcome for diabetic patients with CABG. One of the earliest revascularization strategy studies was the Bypass Angioplasty Revascularization Investigation (BARI) Trial, which compared percutaneous coronary angioplasty (PTCA) vs. CABG for treatment of severe angina or ischemia with multivessel disease. This trial enrolled patients over a 3-year period between 1988 and 1991. In this trial, the 5-year survival rate was statistically nonsignificant but trended toward better outcome in the CABG group; 89.3% in the CABG group compared with 86.3% in the PTCA. Within this study in the subgroup of patients with treated diabetes mellitus, there was a noted difference that favored bypass surgery. The estimated 7-year survival greatly favored CABG over PTCA in the diabetic patients (76.4% in the CABG group vs. 55.7% in the PTCA group $p = 0.0011$). Interestingly, this benefit was observed in diabetic patients who received at least one IMA graft (7-year survival with IMA was 83.2%, $n = 140$ vs. 54.5%, $n = 33$ for those who received only saphenous vein grafts). In fact, 7-year survival in a diabetic patient was equivalent between the PTCA group and the CABG group who did not receive an IMA graft. One caveat to keep in mind when looking at the data from the BARI Trial is that neither newer antiplatelet therapy nor stents were used for the patients treated in the PTCA arm [26].

The BARI trial was the first study to suggest that diabetic patient outcomes post intervention varied greatly from nondiabetic patients. The Optimal Medical Therapy

With or Without PCI for Stable Coronary Disease (COURAGE) Trial, published in 2007, enrolled patients with stable CAD from 1999 to 2004, with the goal of determining whether an initial management strategy of PCI with optimal medical therapy was superior to optimal medical therapy (OMT) alone at reducing the risks of cardiovascular events. Of the patients enrolled, 33% were diabetic patients. In the subgroup of diabetic patients with stable coronary disease, there was no difference in the long-term outcome (risk of death, myocardial infarction, or other major cardiovascular events) for patients in whom the initial management was PCI plus optimal medical therapy versus optimal medical therapy alone. It should be noted, however, that at a medium follow-up of 4.6 years, there was high crossover in the OMT arm: 21.1% of patients in the PCI group had additional revascularization as compared with 32.6% of those in the medical therapy group ($p < 0.001$). Another limitation of the study was the randomization after angiography was performed which may have led to a selection of patients at lower risk. Lastly, PCI throughout this study was performed with bare-metal stents [27].

The Randomized Trial of Therapies for Type 2 Diabetes and Coronary Artery Disease (BARI 2D) Trial, published in 2009, enrolled patient from 2001 to 2005 with a goal of establishing optimal therapy for patients with both type 2 diabetes and stable ischemic heart disease. The study was not designed to compare CABG with PCI but rather to compare coronary revascularization with intense medical therapy; thus, direct comparison of PCI to CABG cannot be made in this study. BARI 2D Trial demonstrated that in patients with diabetes and stable coronary artery disease prompt revascularization by PCI or CABG failed to demonstrate superiority to intense medical therapy over a 5-year period. The primary endpoint was all-cause mortality at 5 years and the secondary endpoint was combination of death, MI, or stroke at 5 years. In a subgroup analysis, freedom from cardiovascular events was greater with CABG than medical therapy. No such advantage was observed for patients treated with PCI [26].

The next landmarks trial comparing PCI to CABG came in the late 2000s. The Percutaneous Coronary Intervention versus Coronary-Artery Bypass Grafting for Severe Coronary Artery Disease: Synergy Between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery (SYNTAX) Trial published in 2009 with 5-year and now 10-year follow-up published in 2019, randomized patients with three-vessel or left main coronary artery disease to PCI with paclitaxel-eluting stents (DES) versus CABG. Randomization was stratified according to presence or absence of left main coronary artery disease and medically treated diabetes. A SYNTAX score was generated to capture atherosclerotic burden and disease complexity and was based on calcification, thrombus, bifurcation, tortuosity, chronic total occlusion, vessels involved, left main disease, number and location of lesions, and dominance (Fig. 25.1). Of the 1800 patients randomized, 25.1% were diabetic patients. The primary endpoint was a composite of major adverse cardiac or cerebrovascular events (MACCE) (i.e., death from any cause, stroke, myocardial infarction, or repeat revascularization). At 12 months, there was a lower incidence of MACCE events in the CABG arm compared with PCI (12.4% vs. 17.8%, $p = 0.002$). This difference was driven primarily by a lower incident of repeat revascularization

in the CABG arm compared with PCI arm (5.9% vs. 13.5%, $p < 0.001$). In the diabetic patients, the trial found no survival difference between PCI and CABG at 10 years. However, there was a lower 12-month MACCE event rate in the diabetic patients who underwent CABG when compared to the PCI arm (14.2% vs. 26.0%, $p = 0.0025$). When stratified by SYNTAX score, PCI versus CABG for low score (<22) 3VD disease yielded equivalent outcomes. The SYNTAX trial concluded "CABG remains the standard of care for patients with complex lesions" (high or intermediate syntax scores). SYNTAX trial also demonstrated better outcomes in the diabetic patients with CABG primarily due to a reduction in repeat revascularization (2.0% versus 7.3%, $p = 0.013$) [28]. For patients with less complex disease (low syntax scores), PCI is an acceptable double alternative [29, 30].

Subsequently, the SYNTAX II score was developed to guide heart team decisions on myocardial revascularization strategies in patients with complex de novo three-vessel CAD (SYNTAX I score >22) and published in the SYNTAX II Study in 2017. The SYNTAX II score incorporated the original SYNTAX I score with clinical variables (age, sex, kidney function, ejection fraction, presents of COPD, or presents of peripheral arterial disease) to risk stratify patients for both CABG and PCI approaches. This was a multicenter, prospective, single-arm, open-label trial of patients with the above-described disease and a SYNTAX II score compared against matched patients with a similar SYNTAX II score from the original SYNTAX I trial (Fig. 25.1). The SYNTAX II score for all the patients in the study suggested equipoise between PCI and CABG. Patients were randomized to either PCI or CABG. The PCI group then underwent physiological stenosis interrogation using an iFR or FFR system prior to intervention. The 1-year data from this study suggests that using a SYNTAX II strategy was associated with improved clinical results compared with the percutaneous coronary interventions guided solely by the SYNTAX I score in comparable patients from the original SYNTAX I trial [31].

In 2009, the Coronary Artery Vascularization and Diabetes Trial (CARDIA) was published, which compared the safety and efficacy of PCI versus CABG in diabetic patients with symptomatic multivessel CAD. This was a randomized trial, with a non-inferiority design that also excluded left main disease. Primary endpoint of death, myocardial infarction, and stroke at 12 months were similar between CABG and PCI (10.5% vs. 13%, $p = 0.39$). Unfortunately, the study ultimately appeared to be underpowered given a much-reduced event rate than that used to calculate the sample size and as such did not meet the non-inferiority endpoint [28].

The Strategies for Multivessel Revascularization in Patients with Diabetes (FREEDOM) Trial, which enrolled patients from 2005 to 2010, compared revascularization via CABG versus PCI (first-generation DES) in patients with diabetes and multivessel disease. Importantly, left main disease was excluded. In 1900 diabetic patients who were enrolled, 83% were having three-vessel disease. This trial showed that revascularization via CABG resulted in lower rates of death and nonfatal MI but higher rates of cerebrovascular accidents (CVA). The primary outcome of composite death from any cause, nonfatal MI, or stroke occurred more frequently in the PCI group at a rate of 26.6% versus 18.7% in the CABG group ($p = 0.005$). Importantly, the benefit within the CABG revascularization strategy group was driven by

reduction rate of both myocardial infarction and death from any cause. Subgroup analysis reported similar outcomes independent of angiographic complexity (according to the SYNTAX score), insulin-dependent versus non-insulin dependent diabetes or renal function. Follow-up at 8 years showed continued benefit of CABG over PCI in diabetics with multivessel disease (significant reduction in all-cause mortality). The FREEDOM trial concluded that CABG, compared to PCI reduces mortality in patients with diabetes mellitus and multivessel CAD for which revascularization is likely to improve survival (3-vessel CAD or complex 2-vessel CAD involving the proximal LAD), particularly if a LIMA graft can be anastomosed to the LAD artery [32, 33].

Left Main Disease

With the exception of the SYNTAX trial, left main disease had been excluded from many of the aforementioned studies. The Everolimus-Eluting Stents or Bypass Surgery for Left Main Coronary Artery Disease (EXCEL Trial) enrolled 1905 patients with left main CAD and low or intermediate Syntax scores to a revascularization strategy of PCI or CABG, with diabetes present in 29% of the patients enrolled. The study was designed as a non-inferiority trial with the primary endpoint being a composite of death from any cause, stroke, or myocardial infarction at 3 years. The primary endpoint occurred in 15.4% of patients in the PCI group compared to 14.7% of patients in the CABG group ($p = 0.02$ for non-inferiority, upper 97.5% confidence limits). The study concluded that in patients with left main coronary artery disease and low or intermediate syntax score, revascularization strategy with PCI was non inferior to CABG. Furthermore, subgroup analysis reveals this non-inferiority of PCI to CABG held true regardless of diabetic status [34].

The Nordic-Baltic-British Left Main Revascularization (NOBLE) Trial was published in the same year as the EXCEL Trial with a very similar design. This study compared revascularization strategies for left main disease; CABG versus PCI with 1184 patients enrolled. Diabetes was present in 15.5% of patients enrolled. Both trials utilized second-generation drug-eluting stents in the PCI arms. PCI was done with the biolimus drug-eluting stent, whereas the EXCEL trial use everolimus-eluting stent. Similar to the EXCEL trial, the NOBLE trial was designed with a non-inferiority end-point. Primary endpoint was a composite of all-cause mortality, non-procedural MI, stroke, and (unlike the EXCEL Trial) repeat revascularizations. Unlike the EXCEL Trial, the NOBLE Trail found CABG to be superior to PCI for the primary composite endpoint ($p = 0.0002$). The Kaplan-Meier 5-year estimates for the primary endpoints were 28% for the PCI group versus 19% for CABG group (HR 1.58 [95% CI 1.24–2.01]); the HR exceeded the limit for non-inferiority of PCI compared to CABG. The difference was driven primarily by non-procedural Mis and repeat revascularization. All-cause mortality was similar between the two groups. Non-procedural MI was estimated in 8% after PCI versus 3% after CABG

(HR 2.99 [95% CI 1.66–5.39]; $p = 0.0002$); and repeat revascularization was estimated at 17% after PCI versus 10% after CABG (HR 1.73 [95% CI 1.25–2.40]; $p = 0.0009$). Similar to the EXCEL Trial, there was no identified interaction with diabetes for patients with left main coronary artery stenosis [35].

In 2018, a pooled meta-analysis of patient-level data "Mortality After Coronary Artery Bypass Grafting versus Percutaneous Coronary Intervention with Stenting for Coronary Artery Disease" was published. This analysis included 11 randomized trials with a total of 11,581 patients. Mean SYNTAX score was 26 points, with 22.1% of patients having a SYNTAX score of 33 or higher. All-cause mortality at 5 years was significantly different between the revascularization strategies (11.5% after PCI vs. 8.9% after CABG; HR 1.28; $p = 0.0019$) with this difference largely limited to the diabetic cohort (15.5% vs. 10.0%; HR 1.48; $p = 0.0004$), but not significant in those without diabetes (8.7% vs. 8.0%; HR 1.08; $p = 0.49$) [36].

The American College of Cardiology (ACC) and the American Heart Association (AHA) published guidelines for stable ischemic heart disease in 2012. These guidelines were released prior to the publication of the FREEDOM trial and prior to the 5-year and 10-year follow-up data for the SYNTAX trial, all of which provide insight into the management of CAD in the diabetic patients. Furthermore, the major trials guiding left main coronary artery disease revascularization (EXCEL, NOBLE) were published after the 2012 ACC/AHA guidelines. Based on the data from BARI, BARI 2D, CARDia, and SYNTAX trials among others, the 2012 ACC/AHA guidelines state that "CABG might be associated with lower risk of mortality in patients with diabetes mellitus and multivessel disease than PCI, but this remains uncertain." As such, the revascularization strategy for patients with diabetes and those patients without diabetes remained the same in the ACC/AHA 2012 guidelines. However, within the guidelines table, there is a qualifying statement, "In patients with multivessel disease who also have diabetes mellitus, it is reasonable to choose CABG (with LIMA) over PCI, Class IIa indication." This exception is noted in four categories: (1) unprotected left main revascularization, (2) 3-vessel disease with or without proximal LAD artery disease, (3) 2-vessel disease with proximal LAD artery disease, and (4) 2-vessel disease without proximal LAD artery disease [37].

The European Society of Cardiology (ESC) published the "Guidelines on Myocardial Revascularization" in 2018 and subsequently, in 2019, the "Guidelines on Diabetes, Pre-Diabetes and Cardiovascular Disease." These guidelines incorporated many of the above-mentioned trials in the recommendations for revascularization strategies within the diabetic patient population. Most striking is a category for patients with 3-vessel CAD and diabetes mellitus. Within this patient population, the ESC recommends CABG as a Class I indication for all patients. PCI was a Class IIb recommendation for patients with a low syntax score (0–22) and a Class III recommendation for diabetic patients with 3-vessel disease with intermediate or high syntax scores (>22) [3, 38].

Regardless of diabetic status, the indications for revascularization for patients with coronary artery disease remain the same in both the ACC/AHA 2012

guidelines and the ESC 2018 guidelines: (1) improved survival and/or (2) relieve symptoms. Both guidelines state that for all patients with complex CAD or planned unprotected left main interventions a heart team approach is a Class 1 indication. The heart team approach takes into consideration the individual cardiac and extracardiac characteristics of the patient as well as the patient's preference when choosing the most appropriate revascularization strategy for the individual patient [3, 37, 38].

Section B: Peripheral Arterial Interventions in the Diabetic Population

Introduction

Peripheral arterial disease (PAD) is a broad term encompassing all arterial atherosclerotic disease with the exception of coronary artery disease. PAD is estimated to affect approximately 8.5 million Americans above the age of 40 years. This translates to an age-standardized prevalence rate of 185.6 per 100,000 in 2010, which is minimally changed since 1990 [39]. The prevalence of PAD is well documented to increase with age, cigarette smoking, and diabetes [40].

Patients with PAD are at almost sixfold higher risk for acute myocardial infarction (MI), ischemic stroke, and/or death compared to general population [41, 42]. As PAD advances, there comes a higher cardiovascular mortality: with stable claudication 5-year mortality is 15–30%, with critical limb ischemia 1-year mortality equals 25% [39]. In a study of cardiovascular event rates in female patients, a higher cardiovascular risk was found when both PAD and diabetes were present compared to the cohort with neither diagnosis (76.9% vs. 14.9%, $p < 0.001$) [42].

Among PAD risk factors, smoking and diabetes have the highest relative risk (OR 4.46, 95% CI 2.25–8.84 and OR 2.71, 95% CI 1.03–7.12, respectively) [43]. Approximately, 20–30% of PAD patients have comorbid diabetes mellitus [44]. Furthermore, the duration and severity of diabetes correlate with the incidence and extent of PAD [45].

The pathophysiology of PAD is similar to atherosclerosis within the coronary arteries described earlier in this chapter. The presence of diabetes promotes PAD by multiple mechanisms including inflammation, endothelial cell dysfunction, smooth muscle cell migration, altered platelet function, and hypercoagulability. These mechanisms suggest that diabetes shares similar pathogenic pathways to atherosclerosis and PAD [46]. Patients with diabetes are more likely to have calcified plaques compared to patients without diabetes [47]. PAD can be more challenging to treat in the diabetic patients due to the severity of the disease, location of the disease, and calcifications found in diabetic patients. Medial calcification is frequently found on histologic review of diabetic patients with PAD [48].

Lower Extremity PAD

PAD is most commonly detected within the arteries of the lower extremities. Untreated peripheral arterial disease in the lower extremities can lead to major limb amputation or death. It is well documented that limb loss leads to early mortality [49]. In the United States, every year more than 73,000 amputations of the lower limb unrelated to trauma are performed on patients with diabetes, which equates to greater than 60% of all nontraumatic amputations [50]. Diabetes is associated with greater severity and more diffuse PAD relative to nondiabetics [51].

Overall, diabetic patients with coronary artery disease have higher risk of cardiovascular and limb events when compared to the nondiabetic populations [44, 52]. Diabetes and, in particular poorly managed diabetes, has been shown to negatively affect a patient's cardiovascular outcomes. The EUCLID trial, designed to compare treatment with either ticagrelor versus clopidogrel in symptomatic PAD (patients who either had a lower extremity revascularization procedure in the last 30 days or patients with ankle–brachial index of 0.80 or less) showed worse MACE outcomes based of hemoglobin A1C. In the 13,885 patients (5345 or 38.5% of whom were diabetic patients), in the EUCLID trial, a subgroup comparison between diabetics and nondiabetics showed a 14.2% ($p < 0.0001$) increased relative risk for MACE for every 1% increase in HbA1c, even for patients on contemporary therapy [53].

Owing to the ability of the vascular bed to recruit robust collaterals, a vast majority of patients, up to two-thirds of US adults with PAD, are asymptomatic [39]. As a result, PAD presentations can vary widely and ultimately infrequently present with classic intermittent claudication. Claudication represents approximately 10% of presenting symptoms, atypical leg pain representing roughly 50% of symptoms at presentation, and the remaining 40% having no leg pain at all [54]. Given the strong association of diabetes with PAD, the severity of PAD within the diabetic population and the potential for patients to remain asymptomatic in the setting of advanced disease, the American Heart Association/American College of Cardiology (ACC/AHA) have incorporated diabetes into the PAD screening algorithm. The ACC/AHA guidelines advise screening all asymptomatic diabetic patients aged 50–64 and any patients aged <50 with diabetes and one additional risk factors for PAD. The most recent ACC guidelines recommend screening for PAD with ankle-brachial index (ABI) even without history or physical examination findings suggestive of PAD (Class IIa indication) [55].

Rutherford, and later Baker, developed the Rutherford Classification as a standard for reporting chronic lower extremity ischemia in 1986 with revisions in 1997 [56]. This classification combines a clinical description as well as objective noninvasive data. There are seven Rutherford Baker (RB) stages (0–6) with stage 0 for asymptomatic patients. The clinical description are as follows: Stage 0 denotes the asymptomatic patient with no hemodynamically significant occlusive disease. Stages 1, 2, and 3 represent mild, moderate, and severe claudication, respectively. Stage 4 is indicative of ischemic rest pain, while Stages 5 and 6 are categorized by minor and major tissue losses, respectively [56, 57] (Table 25.1).

Table 25.1 Rutherford classification for chronic limb ischemia

Stage	Patient presentation
0	Asymptomatic
1	Mild claudication
2	Moderate claudication
3	Severe claudication
4	Rest pain
5	Ischemic ulceration not exceeding ulcer of the digits of the foot
6	Severe ischemic ulcers or frank gangrene

Acute limb ischemia (ALI) has a separate Rutherford classification system, which categorizes the acute limb into viable, threatened, or irreversible [56, 57]. Acute limb ischemia is a common complication in diabetics patients with PAD [54]. A popular theory to guide revascularization strategies in ALI is the concept of arterial perfusion via angiosomes. Angiosome directed revascularization revolves around anatomical units of tissue (skin, subcutaneous tissue, fascia, muscles, nerves, and bone) that are supplied by a major vessel; these units are called angiosomes. There are three vessels that supply five angiosomes in the foot [58, 59]. The anterior tibial becomes the dorsalis pedis and supplies the dorsum of the foot. The peroneal artery provides a collateral vessel that supplies the lateral ankle and heel. The posterior tibial artery divides into the medial and lateral plantar arteries. These three vessels then supply the medial ankle, and the medial, and lateral plantar surface of the foot and digits. Angiosome directed revascularization (sometimes referred to as direct revascularization) is not always possible. Therefore, the conventional endovascular approach to treating foot ulcers and gangrene has been to improve flow in whichever vessel is easiest to recannulize, thereby allowing wound healing via collateral flow (indirect revascularization). To date, no randomized control trials have been performed to evaluate direct versus indirect revascularization strategies on wound healing. A meta-analysis, published in 2014, included nine studies comparing direct versus indirect revascularization which showed significantly improved wound healing (HR, 0.64; 95% CI, 0.52–0.80), lower risk of amputation (HR, 0.72; 95% CI, 0.50–1.04), and higher limb salvage rates (HR, 0.43; 95% CI, 0.24–0.77) with a direct revascularization approach (Table 25.2) [60].

Revascularization Approach: Surgery Versus Endovascular Intervention

Chronic lower extremity ischemia (CLI) is initially managed with smoking cessation, guidelines-directed medical therapy (GDMT), structured exercise therapy, and care to minimize tissue loss. If patient has persistent lifestyle-limiting claudication (RB3) or critical limb ischemia (RB4–6), then revascularization is appropriate [53]. Revascularization can be achieved by surgical endarterectomy, endovascular revascularization, or a hybrid approach [61]. The Bypass versus Angioplasty in Severe

Table 25.2 Rutherford classification for Acute Limb Ischemia

Category	Description/prognosis	Findings		Doppler signals	
		Sensory loss	Muscle weakness	Arterial	Venous
I—Viable	Not immediately threatened	None	None	Audible	Audible
II—Threatened					
a—Marginally	Salvageable if promptly treated	Minimal (toes) or none	None	Inaudible	Audible
b—Immediately	Salvageable with immediate revascularization	More than toes, associated with rest pain	Mild, moderate	Inaudible	Audible
III—Irreversible	Major tissue loss or permanent nerve damage inevitables	Profound, anesthetic	Profound, paralysis (rigor)	Inaudible	Inaudible

Ischemia of the Leg (BASIL Trial) published in 2010 was designed as a randomized controlled trial to investigate revascularization strategies (surgical bypass versus endovascular therapy) in patients with severe leg ischemia defined as rest pain and/or tissue loss (ulcer and/or gangrene) of arterial etiology present for more than 2 weeks. The trial enrolled 453 patients with critical limb ischemia and randomized to surgical bypass (n = 228 patients) versus PTA (n = 224 patients) and followed amputation-free survival at 3–7 years and operative survival. The BASIL trial demonstrated that endovascular revascularization is an effective treatment option for infrainguinal PAD patients presenting with CLI as compared with open surgical treatment. The primary endpoint of amputation-free survival was the same in the endovascular and surgical arms [62, 63].

Revascularization via open lower extremity bypass (LEB) versus endovascular peripheral vascular intervention (PVI) was studied in 2019 by Hicks et al., in a prospective trial randomizing 195 revascularizations in 120 diabetic patients presenting with critical limb-threatening ischemia. The majority (65.6%) of the disease detected was multilevel. In the LEB cohort, 67.9% of targets were infrapopliteal and in the PVI cohort 63.4% of interventions were isolated to or involved the tibial vessels. At 4 years postoperatively, there was no significant difference in crude (unadjusted) primary patency for PVI versus LEB (34.5 ± 6.6% vs. 49.6 ± 8.1, $p = 0.89$). Secondary patency was better for the LEB group (50.3 ± 7.4% vs. 55.4 ± 7.5%; $p = 0.04$), and amputation-free survival was similar (65.1 ± 6.7% vs. 60.9 ± 9.7%; $p = 0.79$). Notably, perioperative complications occurred and 52.8% in LEB verses 12.0% in the PVI cohorts ($p < 0.001$). When the data was adjusted for baseline differences between groups, primary patency (HR: 0.61; 95% CI: 0.34–1.10) and amputation-free survival (HR: 1.51; 95% CI: 0.71–2.34) remained similar for both interventions, but secondary patency was persistently lower for PVI (HR: 0.35; 95% CI: 0.14–0.90). This study adds to the data showing that endovascular interventions for treatment of lower extremity PAD have equivalent long-term amputation-free survival, improved secondary patency, and significantly lower perioperative complications when compared to open surgery, in the diabetic patient [64].

Endovascular Therapies in the Diabetic Patient

Initial data from the early 2000s suggested that diabetic patients had worse outcomes after peripheral revascularization compared to a nondiabetic cohort. A retrospective cohort study from 2005 of 65 consecutive patients with lower extremity PAD who underwent long-segment (≥10 cm) femoropopliteal stent implantation using self-expanding nitinol stents after initial failure of plain balloon angioplasty compared diabetic versus nondiabetic patients. The study tracked cumulative freedom from restenosis at 6 and 12 months where restenosis was defined by duplex and confirmed angiographically (>50% diameter reduction). Rates of restenosis at 6 months and 12 months were 84% and 71% in nondiabetic patients ($n = 41$) versus 68% and 22% in diabetics ($n = 24$) (adjusted hazard ratio 3.8, $p = 0.01$). The results from this study strongly suggested that diabetic patients have worse outcomes after endovascular stent revascularization when compared to the nondiabetic population [65]. A later retrospective study, published in 2008, studied 291 patients with RB stages 3–6 disease who underwent a total of 385 infrainguinal interventions and compared the outcomes between the diabetic and nondiabetic patients. Interventions included angioplasty, cryoplasty balloon angioplasty, bare-metal stent placement, and laser and excisional atherectomy. The results from this study suggested that there was a reduced but non-significant difference between diabetic and nondiabetic patients: for nondiabetics, primary patency was 88 ± 2%, 71 ± 4%, and 58 ± 4% at 6, 12, and 18 months, while for diabetics, it was 82 ± 2%, 53 ± 4%, and 49 ± 4%, respectively ($p = 0.05$) [66].

A study published In 2017 looked at patients from the Multi-center Registry for Peripheral Artery Disease Interventions and Outcomes (XPLAD) involving 1906 patients after undergoing an indexed endovascular procedure for symptomatic lower extremity PAD (2426 limb procedures). The interventions included superficial femoral, popliteal, peroneal, anterior tibial, or posterior tibial arteries. The study found that diabetes increases the risk of major amputation and all-cause death at 12 months following endovascular revascularization. These risks are especially heightened in patients presenting with CLI [67].

In 2020, Lee et al. published a prospective study of 765 patients post endovascular therapy for symptomatic PAD to evaluate the long-term impact of diabetes in patients with PAD. PTA was performed preferentially with stenting reserved as a bailout therapy in the setting of an unsatisfactory angioplasty result. The primary endpoints where the 5-year rates of major adverse cardiac and cerebrovascular events (MACE) and major adverse limb events (MALE). MACE was defined as composite total death, myocardial infarction, repeat coronary revascularization, and stroke. MALE was defined as composite of target extremity revascularization or target extremity surgery including amputation or lower extremity bypass surgery. The study found similar 5-year rates of MACE both before and after propensity score matching analysis, between diabetic patients and patients without diabetes following successful endovascular revascularization: MACE (19.1% vs. 18.0%, $p = 0.793$), after propensity score matching analysis MACE (20.7% vs. 20.7%, log rank $p = 0.989$). Rates of MALE were significantly higher in the diabetic population

prior to propensity score matching analysis MALE (34.2% vs. 23.7%, $p = 0.001$) but after propensity score matching, MALE rates were similar and non-significant between both groups (19.8% vs. 24.5%, log rank $p = 0.312$) [68].

In an effort to determine if diabetic patients tend to do worse or better than non-diabetic patients post percutaneous revascularization, in 2016, Hicks et al. analyzed a large database of 2566 patients who underwent below-knee peripheral vascular intervention (bypass surgery in 19% or endovascular therapy in 81%). Within the LEB group, there were no significant differences in 1-year primary patency (74% vs. 71%; $p = 0.52$), major amputation (16% vs. 12%; $p = 0.39$), or mortality (10% vs. 6%; $p = 0.16$) between diabetic and nondiabetic patients. There were also no significant differences in 1-year primary patency (81% vs. 79%; $p = 0.36$), major amputation (14% vs. 11%; $p = 0.09$), or mortality (6% vs. 7%; $p = 0.30$) among patients with diabetes versus nondiabetic patients undergoing PVI. Multivariable analysis adjusting for baseline differences between groups demonstrated a non-significant trend toward better primary patency in the diabetic group following both LEB (hazard ratio, 1.55; 95% confidence interval, 1.00–2.42; $p = 0.05$) and PVI (hazard ratio, 1.23; 95% confidence interval, 0.97–1.56; $p = 0.09$). There were no significant differences in 1-year major amputation or mortality between the diabetic and nondiabetic cohorts for either LEB or PVI after risk adjustment ($p \geq 0.16$) [69].

Drug-Coated Balloons and Drug-Coated Stents

In the early days of endovascular peripheral interventions, percutaneous transluminal angioplasty (PTA) was the only tool available and the initial immediate success rate was excellent with PTA. Unfortunately, with PTA alone, a high incidence of short-term restenosis became well documented [70]. Subsequently, bare metal stents (BMS) were introduced, which had a greatly improved patency rate over PTA alone, yet still had a high rate of restenosis at 1 year primarily due to neointimal hyperplasia (30–50% restenosis) [71]. In an effort to control late restenosis by way of neointimal hyperplasia, drug-coated balloons (DCB), drug-eluding balloons (DEB), and drug-eluting stents (DES) were introduced. DCBs, DEBs, and DESs all function to inhibit neointimal growth by the delivery of an antiproliferative drug into the vessel wall. DCBs consist of a standard PTA catheter, an excipient that facilitates drug absorption rapidly once the balloon makes contact with the vessel wall and the drug itself. DCB angioplasty results in significant reduction of binary restenosis, target lesion revascularization, and improvement in primary patency rates [72].

An early trial of the antiproliferative therapy came in the DEBELLUM (Drug-Eluting Balloon Evaluation for Lower Limb MUltilevel TreatMent) trial, published in 2012, which randomized 50 patients with 122 lesions of the femoropopliteal or below-the-knee arteries to DEB (paclitaxel-eluting in this particular trial) vs. conventional angioplasty balloon. Of the patients enrolled, 44% were diabetic patients. The primary endpoint for the study was late lumen loss (determined via duplex at 6 months), and the secondary endpoints were target lesion revascularization (TLR),

amputation, and thrombosis. Late lumen loss was significantly lower in the DEB group (0.5 + 1.4 mm vs. 1.6 + 1.7 mm, $p < 0.01$). Furthermore, the secondary endpoints all trended toward better outcomes with DEB use: reduced TLR in the DCB group, 6.1% vs. 23.6% ($p = 0.02$), reduced amputation 3.0% vs. 7.9% ($p = 0.36$), and reduced thrombosis 3.0% vs. 5.2% ($p = 0.6$) [73].

One of the earliest DCB trials to enroll diabetic patients was the Belgian IN.PACT trial. This trial randomized 106 diabetic patients with Rutherford stage 3 to stage 5 to treatment with either Paclitaxel DCB or PTA. Lesions included in this trial were within the superficial femoral artery (SFA), popliteal or below-the-knee (BTK) arteries and defined as follows: ≥50% de novo or restenotic SFA lesions with a length of ≤10 cm or ≤5 cm occlusion, ≥50% de novo or restenotic lesions or occlusion of popliteal and BTK arteries with a length of ≤10 cm. The 6-month mean diameter restenosis was significantly lower in the DCB arm than in the PTA group (29 ± 36% vs. 46 ± 35%, $p = 0.032$) and the binary (≥50% diameter stenosis) restenosis rate was significantly lower in DCB patients compared with the PTA's (27% vs. 49%, $p = 0.03$). The primary patency was significantly better in the paclitaxel-coated balloon group (73% vs. 51%, $p = 0.03$). The 6-month adverse effects rates were similar: 5.5% in the PTA and 5.7% in the DCB arm. The IN.PACT trial results suggest that DCB treatment for lower extremity disease within the diabetic population is effective and safe [74].

In 2016, Ibrahim et al. published a retrospective study looking at diabetic patients with Rutherford stages 3–6 undergoing infrapopliteal revascularization treated with Paclitaxel DCB vs. standard PTA and found that primary patency was higher in the DCB group than in the PTA group (97.8% vs. 81.1%, $p = 0.020$) in the first 3 months. However, there was no statistically significant difference in primary patency at the 12-month follow-up (68.2% vs. 48.5%, $p = 0.131$). Likewise, at 12-month follow-up, there was no difference in clinical improvement between the groups ($p = 0.193$) [75]. These trials suggest that DCB therapy is superior to PTA and that in the diabetic population, outcomes are similar to the nondiabetic population.

The Bare Metal Stent Versus Paclitaxel Eluting Stent in the Setting of Primary Stenting of Intermediate Length Femoropopliteal Lesions (BATTLE) trial, published in 2020, randomized 181 enrolled patients with Rutherford classification 2–5 with de novo atherosclerotic femoropopliteal lesions (2–14 cm in length with a reference vessel diameter of 4–7 mm) to be treated either by BMS or polymer-free DES. The primary endpoint was freedom from in-stent restenoses at 1 year defined by duplex as peak systolic velocity index >2.4 at the target lesion. Unfortunately, diabetes was the only baseline characteristic that was not well balanced, representing 26% in the BMS group versus 48% in the DES group. Despite the unequal representation of diabetes within the two groups or possible in spite of this difference, the BATTLE trial found that DES was not superior to a BMS when measuring freedom from in-stent restenosis at 1 year (88.6% within the BMS group compared with 91.0% of the DES group ($p = 0.64$)) [76].

The IMPERIAL trial was designed to compare drug-coated stents (DCS) versus drug-eluding stents (DES). The trial enrolled 465 patients with Rutherford category 2–4 with lesion(s) in the native SFA and/or proximal popliteal artery with stenosis

≥70% by visual angiographic assessment. Patients were randomized in a 2:1 fashion to receive either Boston Scientific's Eluvia DCS ($n = 309$; 42% of which carried the diagnosis of diabetes) or Cook Medical's Zilver PTX DES ($n = 156$; 44% of which carried the diagnosis of diabetes). The primary endpoint, primary patency at 12 months by duplex ultrasound, was equivalent for both DCS and DES therapies; 86.8% vs. 81.5%, respectively, p for noninferiority <0.0001 [77].

Diabetes is a well-known predictor of in stent restenosis (ISR) in lower extremity PAD [78]. To investigate the effectiveness of DEB therapy for ISR within the diabetic population, the Paclitaxel-Eluting Balloon vs. Standard Angioplasty to Reduce Recurrent Restenosis in Diabetic Patients with InStent Restenosis of the Superficial Femoral and Proximal Popliteal Arteries: The DEBATE-ISR Study was undertaken. The DEBATE-ISR study examined the 1-year rate of restenosis (>50% diameter reduction) in diabetic patients with femoropopliteal ISR who underwent treatment with DEBs and found that recurrent restenosis, assessed by angiography (66%) or ultrasound (34%), occurred in 19.5% patients in the DEB group versus 71.8% in the PTA group ($p = 0.001$). Likewise, target lesion revascularization for symptomatic recurrent restenosis was reduced in the DEB-treated group 13.6% versus 31.0% in the PTA group ($p = 0.045$) [79].

Debulking Therapies

The DEFINITIVE LE Study, published in 2014, was a prospective study that enrolled 800 claudicant or CLI patients with infrainguinal lesions up to 20 cm and treated these patients with directional atherectomy. Within the claudicant cohort (598 patients), the 12-month primary patency rate was 77% in the diabetic subgroup versus 78% in the nondiabetic subgroup (noninferior, $p < 0.001$) [80]. The DEFINITIVE LE study suggests that diabetic patient do equally well with infrainguinal atherectomy treatment when compared to the nondiabetic cohort.

The REALITY trial (DiRectional AthErectomy + Drug CoAted BaLloon to Treat Long, CalcifIed FemoropopliTeal ArterY Lesions), published in 2020, assessed the safety and effectiveness of a vessel preparation strategy with directional atherectomy before DCB angioplasty in patients with symptomatic severely calcified femoropopliteal PAD. The study prospectively enrolled 102 patients at 13 multinational centers with 8–36 cm femoropopliteal stenoses or occlusions with bilateral vessel wall calcification treated with directional atherectomy prior to DCB angioplasty. Results included a 12-month primary patency rate of 77% (66/86) and freedom from clinically driven target lesion revascularization rate of 93% (87/94). This study suggested that vessel preparation with directional atherectomy followed by DCB is a safe and effective strategy [81].

The LIBERTY trial is an ongoing prospective, observational, multicenter study of endovascular atherectomy treatment in 1204 patients. The study included any endovascular atherectomy device, but the majority of the devices used were the orbital atherectomy system. Patients were stratified into three categories: Rutherford classification (RC) 2–3 (501 patients/599 lesions), RC 4–5 (603 patients/758

lesions), or RC 6 (100 patients/146 lesions). The 1-year results show that 30-day freedom from major adverse events (MAE) estimates were high across all groups: 99.2% in RC 2–3, 96.1% in RC 4–5, and 90.8% in RC 6. At 12 months, the freedom from MAE was 82.6% in RC 2–3, 73.2% in RC 4–5, and 59.3% in RC 6 patients. Estimates for freedom from major amputation at 12 months were 99.3%, 96.0%, and 81.7%, respectively. As expected, the prevalence of diabetes increased significantly with the increase in Rutherford classification and was highest (79%) in RC 6 [82].

Renal Interventions

Renal artery stenosis (RAS) has a prevalence of 13.6% among diabetic patients (83% unilateral, 17% bilateral; 11.7% with total thrombosis) compared to roughly 4% in the general public [83]. In diabetic patients with hypertension and or renal impairment, the prevalence increases to 33% [84]. To make the diagnosis of RAS imaging is required and may be accomplished by several modalities: duplex ultrasound, CT angiography (CTA), MR angiography (MRA), or endovascular angiography.

The ASTRAL (Angioplasty and Stenting for Renal Artery Lesions) trial randomized 806 patients with substantial anatomical atherosclerotic stenosis in at least one renal artery to percutaneous renal artery revascularization plus medical therapy or medical therapy alone. Diabetes was present in 30% of the patients enrolled. Notably, patients with need for surgical revascularization or high likelihood of needing revascularization within 6 months were excluded. Overall mortality was 25.6% in the revascularization group, as compared with 26.3% in the medically treated groups ($p = 0.46$). There was no difference in serum creatinine, systolic blood pressure, time to first renal event, or overall vascular event during follow-up (p = NS for all outcomes) [85].

The CORAL (Renal Artery Stenting in Preventing CV and Renal Events) trial, published in 2014, randomized 947 patients with atherosclerotic renal artery stenosis and hypertension or chronic kidney disease to renal artery stenting plus medical therapy or medical therapy alone. Over the course of a median 43 months of follow-up, 35.1% of patients who underwent stent implantation and 35.8% of patients who received medical therapy alone reached the primary endpoint of a composite of adverse cardiovascular and renal events that included death from cardiovascular or renal causes, myocardial infarction, stroke, hospitalization for congestive heart failure, progressive renal insufficiency, or the need for renal-replacement therapy. The difference did not reach statistical significance ($p = 0.58$). No statistically significant differences in the individual components of the composite endpoint or all-cause mortality were observed. Stenting was associated with a modest improvement in systolic blood pressure during follow-up (−2.3 mmHg; 95% CI, −4.4 to −0.2, $p = 0.03$). No interactions were observed between treatments and the pre-specified diabetic subgroup with respect to the occurrence of the primary endpoint [86].

The above trials studying renal artery revascularization were mostly negative but excluded many patients who might benefit. CORAL, STAR, and ASTRAL all showed no benefit to revascularization if blood pressure is controlled and renal functions are stable. Of note, these randomized controlled trials included only mild disease and left out the patients who seemed more unstable, i.e., patients with severe hypertension or declining renal function both of which are prevalent in the diabetic population.

Carotid Interventions

Approximately, 800,000 primary (first-time) or secondary (recurrent) strokes occur each year in the United States; the majority of these strokes being primary strokes (approximately 75%) [87]. Diabetes increases the risk of ischemic stroke in the general population [88] and can also aggravate the severity of extracranial atherosclerotic disease [89]. Carotid lesions in diabetic patients tend to have an increased frequency of echogenic and extensively calcified plaques [90].

In the Insulin Resistance Atherosclerosis Study, investigators compared the rate of progression of carotid atherosclerosis by way of internal carotid artery intimal media thickness (ICA IMT) measurements in patients with normal glucose tolerance, impaired glucose tolerance, and undiagnosed and diagnosed type 2 diabetes. After adjustment for CVD risk factors, a graded relation was observed with ICA IMT progression rates: lowest in those with normal glucose tolerance and impaired glucose tolerance (19.6 μm/year and 16.9 μm/year, respectively), intermediate in persons with diagnosed diabetes (26.6 μm/year), and highest in persons with undiagnosed diabetes (33.9 μm/year). Glucose tolerance status was significantly ($p = 0.001$) predictive of ICA IMT progression [91].

Diagnosis of carotid artery stenosis is made by way of an imaging modality. Diagnostic options include duplex, CTA, MRA, or angiogram. An angiogram may be necessary to resolve discordance between noninvasive imaging findings or during intervention.

There is a correlation between the degree of stenosis and cerebrovascular outcomes in both symptomatic and asymptomatic patients. The distinction between asymptomatic patients and patients who have experienced a stroke or a transient ischemic attack (TIA) is of importance in terms of management.

The CREST (Carotid Revascularization Endarterectomy vs. Stenting Trial) trial, published in 2010, sought to compare outcomes between carotid artery stenting (CAS) and carotid endarterectomy (CEA) in a contemporary population. Approximately, 30% of patients enrolled carried the diagnosis of diabetes. The trial included symptomatic patients with an associated carotid stenosis ≥50% by angiography, ≥70% by ultrasound, or ≥70% by CTA or MRA as well as asymptomatic patients (in the last 6 months) with carotid stenosis ≥60% by angiography or ≥70% by ultrasound or ≥80% by CTA or MRA. The primary endpoint of death, myocardial infarction (MI), or stroke at 30 days plus ipsilateral stroke up to 4 years was

similar between the CAS and CEA arms (7.2% vs. 6.8%, HR 1.11, 95% CI 0.81–1.51, $p = 0.51$). On 10-year follow-up, the primary endpoint for CAS vs. CEA was 11.8% vs. 9.9% ($p = 0.51$). No interaction was noted by symptomatic status. Postprocedural ipsilateral stroke was 6.9% vs. 5.6% for CAS vs. CEA ($p = 0.96$); major stroke was 2.7% vs. 1.1% for CAS vs. CEA ($p = 0.2$); and restenosis/repeat revascularization was 12.2% vs. 9.7% for CAS vs. CEA ($p > 0.05$) [92].

According to the ACC/AHA guidelines released in 2011, symptomatic carotid artery stenosis should be revascularized either by way of CAS or CEA in patients with greater than 70% stenosis by noninvasive imaging or greater than 50% stenosis by angiography (Class I indication). Revascularization in contraindicated in patients with a chronic total occlusion. It is reasonable to revascularize asymptomatic patients with greater than 70% stenosis if perioperative risk is low by way of CEA or CAS [93]. Efficacy of optimal medical therapy versus carotid endarterectomy versus carotid artery stenting is currently being studied in the CREST 2 trial.

The currently ongoing Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis Trial (CREST-2) is two parallel multicenter randomized trials involving patients with asymptomatic high-grade carotid stenosis. One arm of the study randomizes patients to endarterectomy versus no endarterectomy and one arm of the study randomizes patients to carotid stenting versus no stenting. All arms have uniform medical management.

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Chapter 26
Cardiac Surgery and Diabetes Mellitus

Michael P. Robich and Frank W. Sellke

Introduction

In 2018, 34.2 million or 10.5% of the US population were affected by diabetes, and 88 million Americans aged 18 and older had prediabetes [1]. These individuals carry up to eight times the risk of cardiovascular events compared to nondiabetic individuals, making cardiovascular disease the largest cause of mortality in this population [2]. The prevalence of coronary artery disease (CAD) has been estimated to be as high as 55% in the diabetic population [3]. It has been shown that diabetes is a major independent risk factor for cardiovascular disease after adjustment for other risk factors such as age, hypertension, hypercholesterolemia, and tobacco abuse [4]. Patients with diabetes appear to develop accelerated and more severe CAD and also exhibit a diminished angiogenic response to myocardial ischemia as shown angiographically [5] and in autopsy studies [6]. This diminished angiogenic response is associated with coronary microvascular and endothelial dysfunction as well as the presence of an overall anti-angiogenic milieu leading to fewer collateral blood vessels [7, 8]. Hyperglycemia, hyperinsulinemia, and insulin resistance further add to the development of CAD, cardiomyopathy, and heart failure (Fig. 26.1). This culminates in a greater tendency toward more frequent and more severe adverse cardiovascular events. The relative risk of myocardial infarction is 50% greater in diabetic men and 150% greater in diabetic women [9]. Approximately, 20–30% of patients who have undergone coronary artery bypass grafting (CABG) have diabetes mellitus [10]. Thus, diabetic patients undergoing surgical coronary

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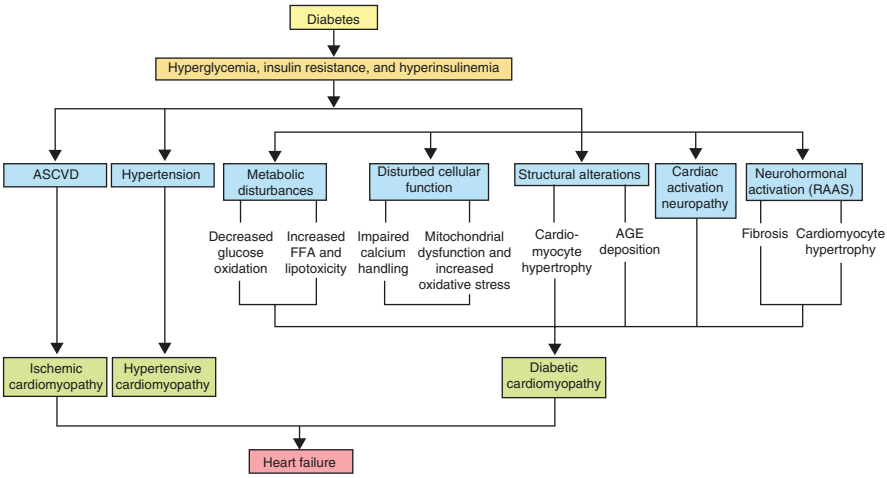

Fig. 26.1 Pathophysiologic mechanisms of heart disease in diabetes. Mechanisms by which diabetes effects multiple pathologic changes leading to coronary artery disease, cardiomyopathy, and ultimately heart failure. *ASCVD* atherosclerotic cardiovascular disease, *FFA* free fatty acids, *AGE* advanced glycation end-product

revascularization represent a large and complex patient population. There continue to be advancements in both percutaneous coronary interventions (PCI), primarily the use of drug-eluting stents, and surgical techniques, such as off-pump CABG and the use of multiple arterial grafts, that have continued to improve methods of coronary revascularization. While there is evidence to suggest that these new techniques have improved outcomes in diabetic patients [11], the optimal treatment for multivessel CAD continues to evolve for the diabetic patient population, which still suffers from worse long-term outcomes compared to the nondiabetic population.

Operative Risks of Cardiac Surgery in Diabetic Patients

In diabetic patients, CAD is not only more prevalent compared to nondiabetic patients, but also is more extensive, involves multiple vessels, and is often rapidly progressive. Patients with diabetes represent a significant proportion of the population requiring myocardial revascularization and can offer a technical challenge due to the often diffuse nature of the coronary disease. In the diabetic population, coronary artery bypass grafting (CABG) has been associated with increased rates of perioperative complications and mortality as compared to nondiabetic patients in the past (Table 26.1). More recently, diabetes has not been associated with worse post-operative or short-term complications [12], even with reduced ejection fraction [13]. Diabetes has, however, been well established as an independent risk factor for increased late mortality in patients treated with CABG [14–16]. A review of 9920 patients with diabetes and 2278 patients without diabetes from a single center over

Table 26.1 Risks associated with diabetes mellitus and cardiac surgery

Increased morbidity
Stroke
Low cardiac output syndrome
Renal failure
Wound infection
Increased mortality

15 years revealed lower survival rates in diabetics versus nondiabetics at 5 years (78% vs. 88%) and 10 years (50% vs. 71%) of follow-up [16]. One recent study of over 6000 patients having CABG showed that the pre-operative HgbA1c is predictive of long-term survival, with the risk of death increasing by 13% for every unit increase in HbA1c [11]. In addition to decreased long-term survival, patients with diabetes have been shown to have increased rates of sternal wound infection [17–19]. Diabetes appears to increase the risk of both superficial sternal wound infections and the deep space mediastinitis [20] as well as saphenous vein harvest site infections [21]. While diabetes has also been associated with increased rates of renal complications [22], more recent studies have shown that diabetes may not be an independent predictor of renal insufficiency [23]. Pulmonary complications including prolonged ventilation and reintubation occur more frequently in diabetic patients [24], with one study of 8555 patients showing only patients with undiagnosed or insulin-dependent diabetes were at increased risk [24]. While diabetic patients appear to be at increased risk for complications after cardiac surgery, it appears there has been some progress in mitigating these risks. This may be due to improved perioperative blood glucose control, enhanced cardiopulmonary bypass technology, and better post-operative critical care management. Finally, once patients with diabetes have been discharged from the hospital, evidence suggests that this group of patients is at higher risk for readmission [25]. Thus, diabetics present a challenging patient population for cardiac surgeons. Severe, multivessel coronary disease in the diabetic population often benefits from surgical revascularization, which is associated with some increased morbidity and long-term mortality.

Strategies for Myocardial Revascularization in Diabetic Patients

CABG Versus Percutaneous Angioplasty

Several large, published clinical studies have demonstrated that patients with diabetes suffering from multivessel coronary disease have an increased survival benefit when treated surgically with CABG as compared to percutaneous transluminal coronary angioplasty (PTCA). The BARI study was initiated by the National Heart, Lung, and Blood Institute (NHLBI) in 1987 to test the hypothesis that in patients with multivessel CAD, initial revascularization by PTCA does not result in a poorer

outcome than CABG during a follow-up period of five months. The study was a multicenter, randomized trial that assigned patients with multivessel CAD to an initial treatment strategy of either CABG (n = 914) or PTCA (n = 915). Average follow-up was 5.4 years. The five-year survival rate was 89.3% for patients assigned to CABG and 86.3% for those assigned to PTCA. Five-year survival rates free from Q-wave myocardial infarction were 80.4% for CABG and 78.7% for PTCA. By five years of follow-up, 8% of the patients assigned to CABG had undergone additional revascularization procedures, while 54% of patients assigned to PTCA required further revascularization. Among diabetic patients treated with either oral hypoglycemic agents or insulin, five-year survival was greater in the CABG group at 80.6% compared to 65.5% in the PTCA group [26].

Several follow-up studies to the BARI trial provided additional information. A study that examined the finding of increased survival in diabetic patients who had CABG versus PTCA determined that the improved survival was due to reduced cardiac mortality. Furthermore, the reduced cardiac mortality was confined to those that received at least one internal mammary artery graft, suggesting that long-term patency of the internal mammary artery graft contributed to the reduction in cardiac mortality [27]. In another follow-up study of myocardial infarction after CABG or PTCA, diabetic patients undergoing CABG had a greatly reduced risk of death from Q-wave myocardial infarction compared to those that were treated with PTCA. Patients with diabetes were 10 times more likely to die of their myocardial infarction if treated with PTCA as compared to CABG [28]. Overall, CABG was shown to be the treatment of choice for diabetic patients with multivessel CAD based on these results that showed improved long-term survival when compared to PTCA.

CABG Versus Stenting

Since the BARI study compared CABG with PTCA and demonstrated a survival advantage for diabetic patients treated surgically, the use of stents (bare-metal, drug-eluting (DES), and bioresorbable coronary scaffolds), rotational and orbital atherectomy, strong anti-platelet agents, and glycoprotein IIb/IIIa inhibitors have become central to the treatment of coronary disease by percutaneous coronary intervention (PCI) with demonstrated clinical benefits over PTCA [29]. Improvements in percutaneous technology and techniques including treatment of chronic total occlusions (CTO), unprotected left main lesions, bifurcation lesions, and PCI with mechanical circulatory support have been introduced. These advanced approaches have made percutaneous treatment available to more patients and prompted further comparisons with CABG. Furthermore, CABG has seen improved surgical outcomes as well, likely due the more common use of arterial conduits and better adherence to guideline directed medical management after surgery in multivessel CAD (2 vessel with proximal LAD and ≥3 vessel) [30].

The Arterial Revascularization Therapies Study (ARTS) randomly assigned 1205 patients with multivessel CAD to either stent placement or CABG. The primary clinical end points were freedom from cardiac or cerebrovascular events at one year. There was no significant difference between groups in terms of periprocedural rates of death, stroke, or myocardial infarction. However, at one-year follow-up, 16.8% of patients in the stent group required a second revascularization compared to only 3.5% in the surgery group [31]. A study of the outcomes of diabetic patients in the ARTS trial showed that diabetics treated with stenting had a 1-year event-free survival of 63.4% compared to nondiabetics at 76.2%. In contrast, patients with diabetes and without diabetes treated by CABG had similar 1-year event-free survival at 84.8% and 88.4%, revealing significantly greater event-free survival when compared to the stent group. Event-free survival of diabetic patients was lower in the PCI group at 1 year compared to the CABG group due to a higher incidence of repeat revascularization in the PCI group [32]. A follow-up study examined the two-year outcomes of the patients enrolled in the ARTS trial. Again, at two years, freedom from death, stroke, and myocardial infarction was equivalent in the stent and CABG groups. Similarly, event-free survival was greater in the CABG group (84.8%) than in the stent group (69.5%). In diabetic subgroup, the difference was more pronounced with event-free survival of 82.3% in the surgery group and 56.3% in the stent group. The follow-up study concluded that the greater need for repeat revascularization in the PCI group seen at 1 year remained essentially unchanged at 2 years, particularly in the diabetic group, suggesting that surgery is the preferable form of treatment for these patients [33].

The SYNTAX trial randomly assigned 1800 patients with three-vessel or left main coronary artery disease to undergo CABG or PCI with DES (paclitaxel-eluting stents) in a non-inferiority trial [34]. At one year, the rates of major adverse cardiac or cerebrovascular events (MACCE) were significantly higher in the PCI group (17.8% vs. 12.4% for CABG; $P = 0.002$), in large part because of an increased rate of repeat revascularization in the PCI group (13.5% vs. 5.9%, $P < 0.001$). The rates of death and myocardial infarction were similar between the two groups; stroke was more likely with CABG (2.2%, vs. 0.6% with PCI; $P = 0.003$). Non-inferiority criteria were not met in this trial. To quantitate the anatomic complexity of the CAD for this study, the SYNTAX score algorithm was utilized [35]. This tool, often used in subsequent studies, allows for risk stratifying patients with complex coronary artery disease. It is somewhat cumbersome to calculate and not often used in daily clinical practice, but has been validated [36]. The five-year analysis of SYNTAX studied subgroups with ($n = 452$) or without ($n = 1348$) diabetes [37]. This showed that MACCE rates were significantly higher for PCI vs. CABG (PCI: 46.5% vs. CABG: 29.0%; $P < 0.001$) and repeat revascularization (PCI: 35.3% vs. CABG: 14.6%; $P < 0.001$). There was no difference in the composite of all-cause death, stroke, MI (PCI: 23.9% vs. CABG: 19.1%; $P = 0.26$) or individual components all-cause death (PCI: 19.5% vs. CABG: 12.9%; $P = 0.065$), stroke (PCI: 3.0% vs. CABG: 4.7%; $P = 0.34$) or MI (PCI: 9.0% vs. CABG: 5.4%; $P = 0.20$). At five years, the authors conclude, “PCI is a potential treatment option in patients with less-complex lesions, CABG should be the revascularization option of choice for

patients with more-complex [SYNTAX score >22] anatomic disease, especially with concurrent diabetes." The 10-year analysis showed mortality after PCI of 28% and 24% after CABG (hazard ratio 1.19 [95% CI 0.99–1.43], $P = 0.066$). Among patients with three-vessel disease, 28% died after PCI vs. 21% after CABG (hazard ratio 1.42 [95% CI 1.11–1.81]), and among patients with left main coronary artery disease, 27% died after PCI vs. 28% after CABG (0.92 [0.69–1.22]) [38]. While the study demonstrated a survival advantage for three-vessel CAD, this did not appear to apply to left main CAD. The FREEDOM trial confirmed the SYNTAX findings in 1900 patients with multivessel CAD and diabetes, demonstrating comparatively worse 5-year rates of composite MACCE outcomes, including death from any cause, nonfatal MI, or nonfatal stroke, in the PCI group (27% vs. 19% in the CABG group) with any SYNTAX score [39].

Subsets of coronary artery disease, such as unprotected left main disease and reduced ejection fraction, have been further studied to understand the value of CABG and PCI. Several studies have examined the role of PCI in unprotected left main CAD. Utilizing SYNTAX trial data and five other RCTs, a recent meta-analysis of 4686 patients demonstrated similar mortality rates between PCI and CABG at a short-term follow-up of 39 months [40]. The authors noted that there was an association based on complexity of disease (SYNTAX score) and survival, with low SYNTAX scores doing better with PCI and higher SYNTAX scores benefiting from CABG. The European NOBLE RCT allocated patients to PCI with Biolimus-eluting biodegradable stent ($n = 598$) or CABG ($n = 603$) [41]. Only 15% of the patients were diabetic in this trial. At a median of 4.9 years of follow-up, the MACCE rates were 28% for PCI and 19% for CABG (HR 1.58 [95% CI 1.24–2.01]; $P = 0.0002$). CABG was found to be superior for the primary composite endpoint. This was largely based on non-procedural myocardial infarction that was 8% after PCI vs. 3% after CABG (HR 2.99 [95% CI 1.66–5.39]; $P = 0.0002$); and repeat revascularization of 17% after PCI vs. 10% after CABG (HR 1.73 [95% CI 1.25–2.40]; $P = 0.0009$).

The EXCEL trial is the largest trial of stent versus surgery in left main disease performed to date [42]. They enrolled patients with left main coronary artery disease of low or intermediate anatomical complexity (SYNTAX score ≤ 32), 948 patients in PCI group (everolimus-eluting stents) and 957 patients in the CABG group, and 29% had diabetes. At a median of 5 years of follow-up, no significant difference between PCI and CABG with respect to the rate of the composite outcome of death, stroke, or myocardial infarction was found. There have been numerous publications comparing these two studies which seemingly came to different conclusions. The EXCEL trial used a primary composite endpoint that did not include repeat revascularization. This was higher in PCI (17.2% vs. 10.5% in CABG (HR 1.79 [95% CI 1.36–2.36]; $P < 0.0001$)). When repeat revascularization was included in the composite endpoint, CABG was superior at 5 years of follow-up. While neither NOBLE or EXCEL was powered to assess mortality as a single endpoint, in EXCEL, death was significantly more common in the PCI group (HR 1.38 [95% CI 1.03–1.85]; $P < 0.0001$). Both studies were funded by the respective stent manufacturer; however, interpretation of the EXCEL trial has been further

complicated by credible concerns over data management. These include the definition periprocedural MI used (using creatine kinase-MB rather than troponin), downplaying the higher mortality rate in the PCI group, and industry influence. Abbott (the stent maker) stopped the trial early, cutting enrollment from the planned 2600 to 1906 patients when mortality concerns were raised by the data safety monitoring board.

While a number of studies have provided evidence that diabetic patients have better outcomes with surgery than PCI primarily from decreased need for revascularization, the AWESOME focused on the benefit of PCI versus CABG in diabetic patients medically refractory myocardial ischemia and high risk for surgery, defined as those with previous CABG, MI within 7 days, left ventricular ejection fraction <35%, age greater than 70 years, or intra-aortic balloon pump requirement for stabilization. This prospective, randomized trial showed that firstly, only 18.5% of eligible patients were actually randomized to PCI versus CABG and that for these patients randomized to CABG or PCI, 36-month survival rates were similar at 85% and 89%, respectively. The non-randomized patients had greater incidence of triple vessel disease and the majority underwent CABG. In the subset of diabetic patients who were randomized to CABG, there was less recurrent angina and a decreased need for repeat revascularization [43].

Many studies and meta-analyses have been performed to understand if newer percutaneous techniques and devices are superior to surgical revascularization. Drug-eluting stents have been shown to be superior to bare metal stents [44], and subsequent generations of stents are similarly scrutinized. A pooled analysis of individual patient data in the examined 11 randomized trials involving 11,518 patients assigned to PCI (n = 5753) or to CABG (n = 5765) [45]. The mean SYNTAX score was 26 ± 9 with mean follow-up of 3.8 years. In this large cohort, five-year all-cause mortality was 11% after PCI and 9% after CABG (hazard ratio [HR] 1.20, 95% CI 1.06–1.37; $P = 0.0038$), and in diabetics was 16% vs. 10.0% (HR 1.48, 1.19–1.84; $P = 0.0004$). The authors conclude that CABG has a mortality benefit, especially in diabetics and those with complex multivessel coronary disease. A summary of the major RCTs and meta-analyses published in 2018 shows advantages of CABG over PCI even with the newest stents [46] (Fig. 26.2). The authors demonstrate that the current evidence from the largest RCTs supports CABG as the superior revascularization strategy in multivessel disease and diabetic patients. SYNTAX demonstrated superiority of CABG in cases of SYNTAX scores >22 [47]. The BEST [48] and FREEDOM [39] trials showed CABG superiority, irrespective of the SYNTAX score. A large patient-level meta-analysis, combining the results of SYNTAX and BEST trials, concluded that CABG offers improved outcomes when compared to DES-PCI in both and nondiabetic and in MVD (2 or 3 vessels involved) with proximal LAD involvement [49]. CABG has been shown to have a higher stroke rate than PCI; however, this risk does not outweigh the improved survival.

In addition to randomized controlled trials which study carefully selected patients who typically are a small proportion of patients with coronary artery disease, important data can also be obtained from registries that provide information on "real world" outcomes. The New York State cardiac registry compared outcomes

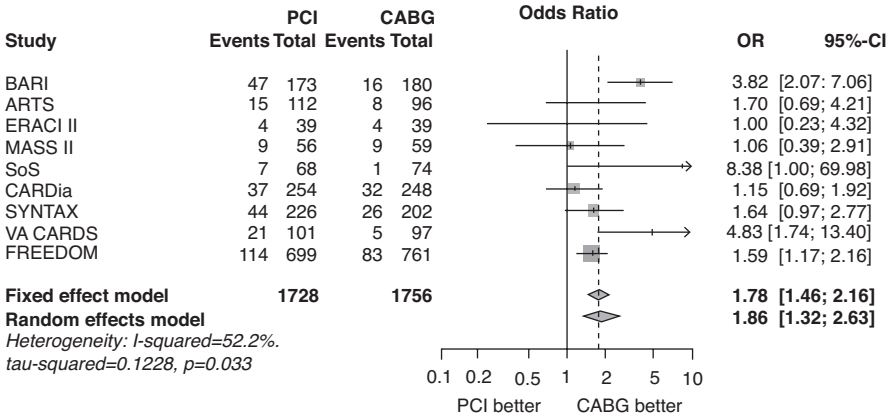

Fig. 26.2 Summary of clinical trials comparing coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI). (Originally published18 Mar 2015. https://doi.org/10.1161/CIRCINTERVENTIONS.114.001944Circulation: Cardiovascular Interventions. 2015;8)

following PCI with stenting (n = 22,102) and CABG (n = 37,212) for multi-vessel coronary disease and revealed that after adjusting for baseline differences in illness severity, 3-year survival for patients undergoing CABG was significantly higher compared to PCI. This was consistent across all anatomic and clinical sub-groups of patients. For example, diabetic patients with triple vessel disease had a hazard ratio of death 0.65 (0.49–0.85) with CABG compared to PCI [50]. Another comparison of drug-eluting stents (DES, n = 9971) versus CABG (n = 7437) in the same registry revealed that the risk of death as well as myocardial infarction was significantly lower in patients undergoing CABG and was applicable in all anatomic subsets of patients [51]. Further, a real-world Canadian study examining long-term survival of PCI versus CABG in patients with multivessel CAD and diabetes was published in 2020 [52]. Examining 4301 propensity score matched patient pairs undergoing DES PCI or CABG showed that all-cause mortality (HR 1.39; 95% CI 1.28 to 1.51; $P < 0.001$) and overall MACCE (HR 1.99; 95% CI 1.86 to 2.12; $P < 0.001$) were significantly higher with PCI compared with CABG with median and maximum follow-ups of 5.5 and 11.5 years, respectively. Finally, a multicenter, retrospective analysis performed a "real world" STITCH trial among 7 medical centers examining 955 CABG and 718 PCI patients with an ejection fraction ≤35% and 2- or 3-vessel coronary disease [53]. The analysis showed a survival benefit of CABG over PCI (hazard ratio, 0.59 [95% confidence interval, 0.50–0.71]; $P < 0.01$). Stroke and acute kidney injury were more common in CABG and repeat revascularization more common in PCI. Thus, "real-world" outcomes continue to demonstrate survival benefits of CABG over PCI, regardless of the type of drug-eluting stent.

There are clinical situations identified in which PCI is likely superior to surgical intervention, even in diabetic patients (Table 26.2). Many studies have shown that one- or two-vessel disease, focal lesions, and low SYNTAX scores are often best

Table 26.2 Management considerations in diabetic patients with coronary artery disease

Favors CABG	Favors PCI
Multivessel coronary artery disease	Severe co-morbidities
Reduced left ventricular function (EF <35%)	Advanced age
Proximal LAD involvement	Frailty and Reduced life expectancy
Left main coronary artery involvement	Significant mobility issues/poor rehab potential
Complex CAD (SYNTAX≥23)	Single or two vessel disease with focal lesion(s)
Severely calcified CAD	Low complexity CAD (SYNTAX0-22)
Incomplete revascularization with PCI	Culprit lesion PCI in acute coronary syndrome
In-stent re-stenosis (DES in major artery)	Incomplete revascularization with CABG (poor distal targets or lack of conduit)
Contraindication to duel antiplatelet therapy	Severe chest deformation or scoliosis
Need for concomitant cardiac surgery procedures	Sequelae of major (mantle) chest radiation (e.g. Porelain aorta)

served with PCI [54]. In acute coronary syndrome, with or without cardiogenic shock, it is often reasonable to percutaneously treat the culprit lesion, with a plan to address remaining lesions after the patient is stabilized and multidisciplinary discussion held [55, 56]. Additionally, patients with less than three years life expectancy are best served with PCI, as survival curves from trials (e.g., FREEDOM) cross at that point and stenting has better outcomes very early [39].

Surgical Outcomes in Diabetic Patients

While technologic advancements have largely driven improvements in PCI outcomes, refinement of technical skills and improvements in medical management have helped improve CABG outcomes. In a follow-up study of the BARI trial, the group of patients who underwent CABG and evaluated coronary artery bypass graft patency in patients with and without treated DM were examined. The results of this study showed that diabetic patients were more likely to have grafts to small (<1.5 mm) distal vessels and coronary arteries of poor distal quality. Angiographic evaluation at a mean follow-up of 3.9 years showed that graft patency was equivalent for diabetic and nondiabetic patients for both IMA grafts (89% vs. 85%, respectively; $P = 0.23$) and vein grafts (71% and 75%, respectively, $P = 0.40$). The authors concluded that despite having smaller distal target vessels of poorer quality, patients with treated DM do not have adverse effects on graft patency at an average of nearly 4 years follow-up and therefore graft patency does not explain differences in survival that has been observed in diabetic and nondiabetic patients following CABG [57]. While the findings of this study are important, a difference in mortality rate

between the diabetic and nondiabetic populations may have introduced a bias that would affect the patients that were studied by angiography, which is those that survived for follow-up.

Long-term outcomes for diabetic patients undergoing CABG have long been worse when compared to nondiabetics, but recently some studies have shown equivocal peri-operative and short-term outcomes [11]. Patients with diabetes undergoing surgical revascularization are still more likely to have comorbidities such as hypertension, obesity, and chronic kidney disease [58]. Despite these significant risk factors, short-term outcomes have improved, and survival rates for diabetics begin to diverge from nondiabetics at about three years after surgery [12, 59].

Surgical Considerations in Diabetic Patients

Operative Considerations

The effect of CABG without cardiopulmonary bypass generally has been favorable in terms of morbidity and mortality, though some conflicting data have been reported [60–63]. Early studies suggested that diabetics appear to have fewer postoperative complications including lower rates of atrial fibrillation, renal failure, and respiratory failure with off-pump CABG [64]. Recent studies have shown that long-term outcomes are not improved with off-pump CABG in diabetics [65]. In a follow-up study of 835 diabetic VA patients that underwent either off-pump (n = 402) or on-pump (n = 433) CABG showed that five-year all-cause death rates were 20.2% off pump versus 14.1% on pump (P = 0.0198) [66]. There were no differences seen in MACE, repeat revascularization, and nonfatal myocardial infarction. For all patients undergoing CABG, there has been growing concern about the outcomes of off-pump surgery due to worse outcomes. A registry study of experienced off-pump surgeons performing 6950 off-pump CABGs and 15,295 on-pump CABGs showed that off-pump CABG was associated with higher mortality (33.4% vs. 29.6% at 10 years [HR 1.11]; 95% CI 1.04 to 1.18; P = 0.002) [67]. There were also increased rates of incomplete revascularization, need for repeat revascularization, and long-term mortality. However, in the hands of surgeons who perform very high-volumes of off-pump CABG, the long-term outcomes can be comparable to on-pump [68, 69].

Another technique that likely improves outcome of CABG is the use of multiple arterial bypass grafts [70]. In diabetics, the use of arterial grafts has been suggested to improve the outcomes of survival [10]. CABG with at least one internal mammary artery (IMA) graft should be a standard practice in diabetic patients. Bilateral IMA grafts appear to be safe in patients with diabetes and may have a survival advantage [71, 72]. However, use of bilateral IMA has been shown to be a potential risk factor for sternal wound infection and mediastinitis, due to devascularization of the sternum. A multicenter, retrospective analysis of 1297 diabetic bilateral IMA

patients propensity-matched to 1297 single IMA patients showed no difference in the rate of mediastinitis, sternal dehiscence, or in-hospital mortality between groups [73]. At a median follow-up of 9.3 years, diabetic patients who received a bilateral IMA had significantly improved long-term survival when compared with single IMA patients (HR 0.75 [95% confidence interval 0.57 to 0.98], $P = 0.034$). In this context, skeletonizing of the IMA (i.e., harvesting of only the artery and leaving the venous drainage intact) has been demonstrated to preserve sternal perfusion [74] and may reduce sternal wound infections [75] and permit increased arterial revascularization in diabetic patients.

The use of the radial artery as a conduit for bypass has been researched. It has been shown to offer very good durability and improve long-term survival [76]. The multicenter Radial Artery Patency Study (RAPS) examined patients with multivessel CAD undergoing CABG with and without a radial artery graft with planned angiographic follow-up [77]. In diabetic patients at a mean follow-up of 7.7 years, the proportion of complete graft occlusion was significantly lower in the radial grafts (4.8%) than in the saphenous grafts (25.3%) ($P = 0.0004$). Multivariate analysis demonstrated that the use of the radial artery and high-grade target vessel stenosis were protective against late graft occlusion. Ultimately, diabetic patients should be considered for total arterial revascularization when possible, as this appears to offer improved long-term survival without increasing perioperative mortality [78].

Finally, diabetes has been shown to be a risk factor for wound infection of the saphenous vein graft harvest site. The technique of endoscopic vein harvesting has been shown to decrease wound infections of the lower extremity after vein harvest in high-risk patients such as those with diabetes [21, 79].

Postoperative Care

The management of patients after cardiac surgery has been closely studied to improve outcomes after CABG, especially in diabetic patients and those at higher risk for post-operative complications. The Enhanced Recovery After Surgery (ERAS) Society published guidelines that have aggregated data on best available evidence regarding peri-operative management of cardiac surgery patients [80]. In addition to treating hyperglycemia, the recommendations include screening for delirium, adjunctive non-narcotic pain management, avoidance of persistent hypothermia, deep vein thrombosis prophylaxis, maintaining chest tube patency, goal directed fluid administration, and early identification of acute kidney injury.

Hyperglycemia has been associated with adverse outcomes in patients with cardiovascular disease. In patients with myocardial infarction, glucose values in excess of 110 to 144 mg/dL were associated with a threefold increase in mortality and a greater risk of heart failure [81]. There has been considerable interest in glucose control after cardiac surgery [82]. High blood glucose can increase rates of infections and impair collagen synthesis and wound healing among patients with poorly controlled diabetes [83]. It is also associated with impaired leukocyte function,

Table 26.3 Special considerations in diabetic patients undergoing surgical coronary revascularization

Considerations for CABG in Diabetic Patients
Pre-operative
• Obtain hemaglobin A1c (HgbA1c) for risk stratification
• Maintain blood glucose levels below 180 mg/dL
Intra-operative
• Use skeletonization technique for harvest of internal mammary artery (especially if bilateral arteries are taken)
• Consider adjunctive rigid sternal fixation for patients at increased risk of sternal dehiscence. Risk factors include poor pre-operative glucose control and diabetes in addition to: obesity, active tobacco abuse or COPD
Post-operative
• Maintain blood glucose levels below 180 mg/dL with insulin drip
• Avoid prolonged hypothermia to decrease the risk of wound infection

including decreased phagocytosis, impaired bacterial killing, and chemotaxis. In addition, acute hyperglycemia results in activation and production of inflammatory cytokines [84]. In the post-operative patient physiologic stress, pain, the presence of infection or administration of vasopressors are factors that have an impact on insulin requirement. The specific goals of glucose control are debated (generally between 100 mg/dL and 180 mg/dL), but the need to avoid prolonged hyperglycemia after surgery has been established [85, 86]. A list of special considerations for diabetic patients undergoing surgical coronary revascularization is listed in Table 26.3.

Molecular Mechanisms of Pathogenesis in Diabetes During Cardiac Surgery

Endothelin-1 and Oxidative Stress

A multitude of molecular mechanisms likely contributes to the clinical manifestations of diabetes in cardiac surgery. Recently, there has been evidence to suggest that endothelin-1 and nitric oxide play important roles in the pathophysiology of diabetics undergoing cardiac surgery. The response of the myocardium in diabetics to cardiac surgery and the associated reperfusion injury is characterized by alterations in neutrophil adhesion, endothelial function, myocyte contractility, and oxidative stress. Endothelin-1 is a potent, endogenous vasoconstrictor that has been associated with endothelial dysfunction and vasospasm. In a study of 25 patients (13 diabetic and 12 nondiabetic) who underwent cardiopulmonary bypass with

cardioplegic arrest, levels of endothelin-1 in the coronary sinus effluent of diabetic patients were greater than that from nondiabetics. Furthermore, coronary microvessels from the diabetic patients showed increased vasoconstriction to endothelin-1 and diminished nitric oxide-mediated vasodilation. These responses were blocked by endothelin antagonism [87]. These results suggest that endothlelin-1 may contribute to reperfusion injury in diabetic patients as seen in dysfunction of the coronary microcirculation, which is a major determinant of myocardial perfusion. Furthermore, there has been evidence to suggest that endothelin receptors mediate reperfusion injury of cardiomyocytes under conditions of hyperglycemia [88]. These alterations in microvascular response can have implications in the patient's response to vasoactive medications [89, 90].

Oxidative stress has been shown to play a significant role in the response to cardiopulmonary bypass and reperfusion injury. In a study of patients with diabetes ($n = 20$) and without diabetes ($n = 20$) who underwent cardiac surgery, the response to cardiopulmonary bypass and cardioplegic arrest was measured in terms of oxidative stress. Cardiopulmonary bypass with cardioplegic arrest induced a greater oxidative stress in patients with diabetes compared to nondiabetics as measured by plasma lipid hydroperoxides and protein carbonyls. These results suggest that oxidative stress in response to cardiac surgery is increased in diabetic patients [91]. Considering that oxidative stress is associated with reperfusion injury, alterations in the coronary microcirculation, and myocardial contractile dysfunction, further investigation of the role of increased oxidative stress in the pathophysiologic responses of diabetics to cardiac surgery may be warranted. Decreased oxidative stress in off-pump CABG compared to CABG with cardiopulmonary bypass may represent a mechanism by which diabetics benefit from the off-pump technique [92].

Gene Expression Profiles of Diabetic Patients After Cardiac Surgery with Cardiopulmonary Bypass and Cardioplegia

As diabetes is an independent risk factor for postoperative complications and mortality after CABG, we sought to examine and compare myocardial gene expression responses to cardiopulmonary bypass and cardioplegic arrest in patients with and without diabetes by the use of cDNA array analysis. Ten atrial myocardial samples were harvested from five insulin-treated diabetic and five matched nondiabetic patients undergoing CABG, before and after cardiopulmonary bypass and cardioplegia. Each gene whose expression was uniformly modified by a median ratio of fourfold or greater magnitude was the object of a literature search and reported, along with its GenBank number, according to the current nomenclature of Online Mendelian Inheritance in Man. Of 12,625 genes examined, 851 were upregulated in

the diabetic group and 480 in the control group ($p < 0.001$). Less genes were downregulated in diabetic (443) compared to nondiabetic (626) patients ($p < 0.001$) (Fig. 26.3). There were a total of 39 genes showing greater than fourfold upregulation in the diabetic group, and 35 genes in the nondiabetic group. Of these, 17 were upregulated in both groups, while 22 and 18 were upregulated exclusively in the diabetic and nondiabetic patients, respectively, a highly significant different expression profile (Table 26.4). We concluded that the gene expression profile after cardiopulmonary bypass and cardioplegic arrest is quantitatively and qualitatively different in patients with diabetes [93]. These results have important implications for the design of tailored myocardial protection and operative strategies for diabetic patients undergoing cardiac surgery.

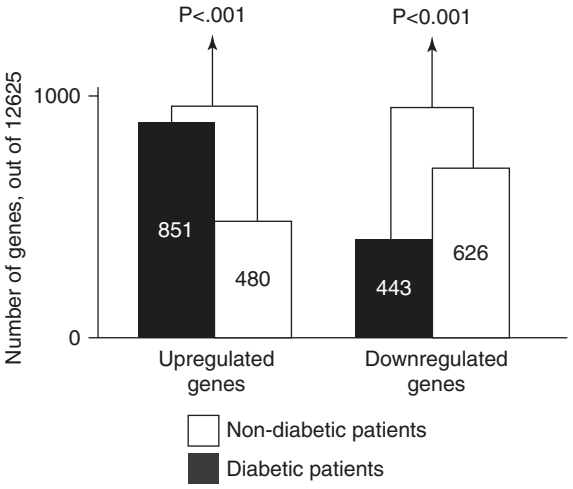

Fig. 26.3 Differential gene expression after cardiopulmonary bypass and cardioplegic arrest in myocardial tissue of diabetic and nondiabetic patients

Table 26.4 Select genes exhibiting an increase in expression in myocardial samples from diabetic patients after cardiopulmonary bypass and cardioplegic arrest

Gene name	Symbol	Fold increase	Function
Amphiregulin	AREG	16:1	Autocrine growth factor
Interleukin (IL)-1β	IL-1B	9:1	Inflammatory cytokine
Nuclear receptor subfamily 4, group A, member 3	NR4A3	8:1	Transcription factor
Regulator of G protein signaling 1	RGS1	8:1	Immediate-early response gene
Activating transcription factor 3	ATF3	8:1	Transcription factor
Insulin receptor substrate 1	IRS1	7:1	Substrate of the insulin receptor tyrosine kinase
V-maf musculoaponeurotic fibrosarcoma oncogene	MAFF	7:1	Transcription regulator
Ras homolog gene family, Member B (Rho B)	ARHB	6:1	Growth factor-responsive early gene

Table 26.4 (continued)

Gene name	Symbol	Fold increase	Function
Complement component 1, q Subcom, R1	C1QR1	6:1	Regulation of phagocytic activity
Nuclear antigen SP100	SP100	6:1	Role in autoimmunity, infections, tumorigenesis
Chemokine (CC motif) ligand4	CCL4	6:1	Chemotactic factor for monocytes
Interleukin 8	IL8	5:1	Mediates neutrophils chemotaxis and migration
Interleukin 1 receptor	IL1RN	5:1	Inhibits Interleukin 1-alpha and -beta antagonist
Oncogene MYC	MYC	5:1	Transcription factor
Vascular Endothelial Growth Factor	VEGF	5:1	Growth factor, mitogen primarily for vascular endothelial cells
Chemokine (C-X-C motif), ligand 3	CXCL3	5:1	Chemotactic factor for monocytes

Conclusions

Diabetes mellitus is a well-established risk factor for increased morbidity and mortality associated with cardiac surgery. Patients with diabetes have been shown to have worse outcomes than nondiabetics from both surgical and percutaneous catheter-based techniques of revascularization. Clinical trials have demonstrated increased event-free survival for diabetic patients treated surgically, likely in part due to the completeness of revascularization considering that freedom from repeat revascularization procedures has been the most consistent benefit shown from CABG when compared to current PCI including stenting. Furthermore, data from large registries have demonstrated that "real-world" outcomes for CABG are significantly superior to PCI with respect to survival and repeat revascularization. The best method of selection of revascularization should be based on a number of factors including the severity and extent of coronary disease, the potential for complete revascularization, the presence of co-morbid illnesses, and patient. Currently, surgical revascularization is recommended in diabetic patients with multivessel CAD, particularly for those patients with extensive coronary disease and myocardial dysfunction. However, these recommendations will continue to evolve with improving technologies and therapies. Surgical treatment options that potentially decrease morbidity associated with diabetes include multi-arterial grafting, IMA skeletonization, endoscopic saphenous vein harvest, and aggressive management of hyperglycemia with continuous insulin infusions. Research techniques with improved animal models of diabetes as well as application of genomic techniques in patients will continue to provide novel insights into the molecular mechanisms involved and identify new therapeutic targets in order to improve the outcome of diabetic patients after cardiac surgery.

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Chapter 27
Heart Failure and Cardiac Dysfunction in Diabetes

Maxwell Eyram Afari and Michael M. Givertz

Introduction

The prevalence of heart failure (HF) in American adults is estimated to be 6.2 million, with more than 600,000 new case diagnosed each year [1]. Globally, an estimated 450 million people have diabetes mellitus (DM) and the prevalence is projected to increase to nearly 700 million in 2045 [2]. HF and DM can occur independently or together, and a diagnosis of one can increase the risk of developing the other. Management requires concerted multidisciplinary interventions with a focus on guideline-directed medical therapy to improve clinical outcomes. This chapter reviews the epidemiology of DM and HF, the pathophysiology of these diseases, and the importance of their interdisciplinary management.

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Epidemiology of Diabetes and Heart Failure

Diabetes in Heart Failure

In clinical trials of ambulatory HF patients, the prevalence of DM has ranged from 15% to 54% (Fig. 27.1), with increasing prevalence over time [3]. Using data from the Get With the Guidelines Heart Failure registry, the temporal trend of hospitalizations between 2005 and 2015 shows a progressive increase of HF patients with diabetes. The overall prevalence of hospitalized patients with diabetes was approximately 44% [4]. In the recently completed VICTORIA (Vericiguat Global Study in Subjects with Heart Failure with Reduced Ejection Fraction) study, 47% of patients with worsening chronic HF had DM at enrollment [5]. The incidence of new onset DM in patients with HF has also been explored in both population-based studies and randomized clinical trials. In the CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity) and EMPHASIS-HF (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure) trials, the incidence of DM was unusually high (28 per 1000 person-years and 21 per 1000 person-years, respectively) compared to the general population (10 per 1000 person-years for adults ≥45) [6, 7]. In CHARM, hemoglobin A1c and body mass index were strong predictors of the development of diabetes [6]. Insulin-requiring DM is associated with a twofold increase in death or the composite outcome of cardiovascular death or hospitalization for HF [8]. Independent predictors of new-onset diabetes in the EMPHASIS-HF trial included longer duration of HF, higher waist circumference, and higher systolic blood pressure [7]. The mechanism of DM in patients with HF may be related to metabolic derangements and insulin resistance caused by HF [9, 10].

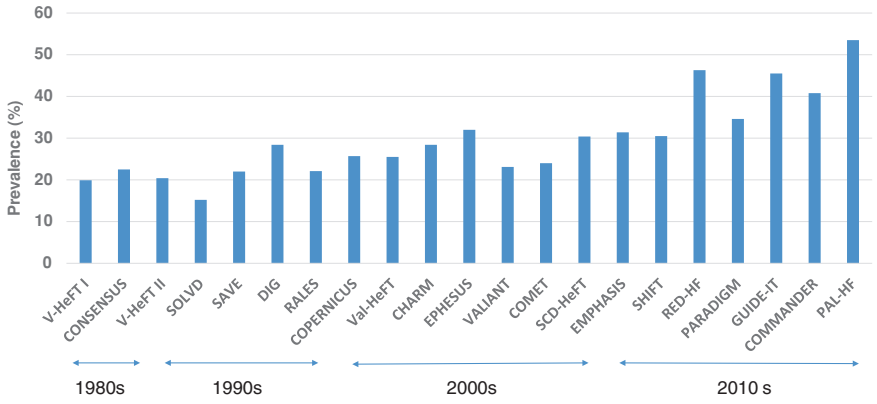

Fig. 27.1 Prevalence of diabetes in heart failure. Over the last 40 years, the prevalence of diabetes in clinical HF trials has ranged from 15% in the SOLVD (Studies of Left Ventricular Dysfunction) trial to 54% in PAL-HF (Palliative Care in Heart Failure) trial, reflecting both increasing severity of illness and demographic trends over time

Heart Failure in Diabetes

Patients with DM are more likely to develop HF compared to non-diabetics [11]. A systematic review and meta-analysis demonstrated a pooled prevalence of left ventricular diastolic dysfunction of 48% in hospitalized patients with diabetes [12]. In a Swedish registry of 35,163 participants, ischemic heart disease was more common (62%) in those with co-existent DM than those without DM (47%) [13]. In the Framingham Heart Study, DM was shown to increase incident risk of HF by twofold in men and by fivefold in women after adjustment for cardiovascular risk factors [14]. The higher incidence of HF noted in patients with DM could be due to the presence of comorbid risk factors such as hypertension, coronary artery disease, obesity, dyslipidemia, and the metabolic syndrome. For each 1% increase in hemoglobin A1c, the risk of HF increases by 30% in type 1 DM and 8% in type 2 DM independent of other risk factors [15]. DM is associated with the progression of HF in patients with asymptomatic left ventricular (LV) dysfunction [16]. Six percent of patients with newly diagnosed DM will develop HF within 5 years [17].

Prognosis of Patients with Heart Failure and Diabetes

Concomitant DM and HF are associated with poor clinical outcomes. Compared to patients without diabetes, the 1-year mortality rate in HF patients with DM is significantly higher (31% vs. 23%) [18]. Diabetes increases the risk of the composite outcome of cardiovascular mortality and HF hospitalization in patients admitted with acute HF [19]. This risk is irrespective of the type of HF (i.e., HF with reduced ejection fraction [HFrEF] vs. HF with preserved ejection fraction [HFpEF]) [20]. The risks of all-cause mortality in both HFpEF and HFrEF are similar [8], but the risk of cardiovascular death or HF hospitalization is higher with HFpEF. Compared to having a diagnosis of HF alone, having HF and DM is associated with a poorer quality of life and higher health care costs [21]. Even in patients without diabetes, the incidence of HF hospitalization increases with elevation in hemoglobin A1c [22]. In patients at high cardiovascular risk, a 1-mmol/L higher fasting plasma glucose increased HF hospitalization by 1.23-fold [23].

Diabetes and HF place significant burden on the health care system. In the Unites States, Medicare costs per person for patients with DM and HF is 18% higher than for those with HF alone, and 54% higher than for those with DM alone (DM + HF: $26,544; HF: $21,808; and DM: $12,229) [24]. The global economic burden of DM will increase to $2.5 trillion by 2030 [25], while the total cost of HF in the United States will increase to $69.8 billion by 2030 [26].

Causes of Heart Failure

In developed countries, coronary artery disease is responsible for approximately two-thirds of the cases of HF, with hypertension as a principal contributor in up to 75% and DM in approximately one-quarter. Diabetic cardiomyopathy is defined as

Table 27.1 Proposed mechanisms underlying diabetic cardiomyopathy

Resistance or lack of insulin shifts metabolism from glucose to fatty acid. The increase in myocardial oxygen utilization changes calcium homeostasis, which leads to cardiac dysfunction [29].
Depletion of myocardial catecholamine stores through autonomic dysregulation blunts contractile reserve, which causes systolic and diastolic dysfunction [30].
Insulin resistance stimulates glycation of proteins, lipids, and nucleic acids. Advanced glycation end-products increase free radicals, which inactivate nitric oxide leading to impaired endothelium-dependent relaxation and microvascular dysfunction [31, 32].
Inactive nitric oxide decreases intracellular Ca^{2+} sensitization and impairs sarcoplasmic Ca^{2+} uptake [33].
Activation of RAAS by hyperglycemia increases angiotensin II and aldosterone levels, inducing fibrosis and left ventricular hypertrophy, which are precursors to HFpEF (previously referred to as diastolic HF) [34].

HF heart failure, *HFpEF* heart failure with reduced ejection fraction; RAAS, renin–angiotensin–aldosterone system

the presence of systolic or diastolic dysfunction in a patient with DM without other obvious causes for cardiomyopathy [27, 28]. Risk factors for diabetic cardiomyopathy include impaired insulin signaling within the myocardium, hyperglycemia, obesity, inappropriate activation of renin–angiotensin–aldosterone system (RAAS), and systemic insulin resistance. The mechanisms leading to cardiac dysfunction are outlined in Table 27.1.

Clinical Presentation of Diabetic Cardiomyopathy

The clinical presentation of diabetic cardiomyopathy falls within a spectrum of disease from asymptomatic left ventricular dysfunction [33] to symptomatic heart failure. Impairment of global longitudinal strain [35] and diastolic dysfunction [36] may be precursors to HF symptoms. Left ventricular hypertrophy and/or decreased LV compliance are more prevalent in diabetic patients than non-diabetics [37], and these structural and functional changes can evolve to LV dilatation and systolic dysfunction [33]. Diabetes results in diffuse coronary artery disease, which causes patchy necrosis and fibrosis, and can in turn lead to systolic dysfunction through myocardial hibernation or stunning. Symptoms of HF such as shortness of breath, orthopnea, paroxysmal nocturnal dyspnea, lower extremity edema, abdominal bloating, and weight gain can occur in both HFpEF or HFrEF. Patients should be screened for other cardiac symptoms such as chest pain, palpitations, dizziness, syncope, or claudication.

Recently, the Heart Failure Society of America along with other international societies proposed a universal definition and classification system that recognizes HF as a clinical syndrome [38]. Patients with DM are categorized as “At-risk for HF (Stage A),” with no structural cardiac abnormality or elevated cardiac biomarkers but with risk factors such as atherosclerotic disease, obesity or hypertension. Pre-HF

Table 27.2 Heart failure type based on left ventricular ejection fraction

Type of heart failure	Definition based on ejection fraction
HF with reduced ejection fraction (HFrEF)	LVEF < 40%
HF with mildly reduced ejection fraction (HFmrEF)	LVEF 41% to 49%
HF with preserved ejection fraction (HFpEF)	LVEF > 50%
HF with improved ejection fraction (HFimpEF)	Baseline LVEF < 40%, increase of least 10 points from baseline, and second measured LVEF > 40%

HF heart failure, *LVEF* left ventricular ejection fraction

(Stage B) patients have evidence of structural heart disease, abnormal cardiac function, or elevated natriuretic peptide levels but no symptoms or signs. Stage C patients have symptoms or signs of HF caused by a structural and/or functional cardiac abnormality, while Stage D defines those with severe symptoms and/or signs of HF at rest and recurrent hospitalizations, requiring consideration for transplant, mechanical circulatory support, or palliative care. This universal definition has also brought clarity to the classification of HF based on left ventricular ejection fraction (LVEF) (Table 27.2).

Cellular and Molecular Mechanisms

The pathophysiology of diabetic cardiomyopathy is complex with a variety of cellular and molecular mechanisms supported by pre-clinical and translational studies (Fig. 27.2). A summary of these mechanisms is as follows [39]:

- Cardiac structural abnormality: Advanced glycation end-products lead to the generation of reactive oxygen and nitrogen species by endothelial cells. The resultant decrease in nitric oxide diminishes soluble guanylate cyclase activity and cyclic GMP levels. Coronary endothelial microvascular inflammation and infiltration of inflammatory cells stimulate transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), angiotensin II, and interleukins which activate pro-fibrotic responses [40]. The end result is the impairment of extracellular matrix degradation through the upregulation of tissue inhibitors of metalloproteinases. The inhibition of metalloproteinases results in fibrosis and the development of myocardial stiffness, the hallmark of diastolic dysfunction [41].
- Cardiac inflammation: Hyperglycemia, hyperlipidemia, and elevated angiotensin II levels activate infiltration of pro-inflammatory macrophages and lymphocytes. These cells secrete cytokines such as TNF-α, interleukin-1β, interferon-γ, and TGF-β, which can directly act on cardiac fibroblasts and myofibroblasts to induce cardiac injury [42]. Glucotoxicity and lipotoxicity can activate the

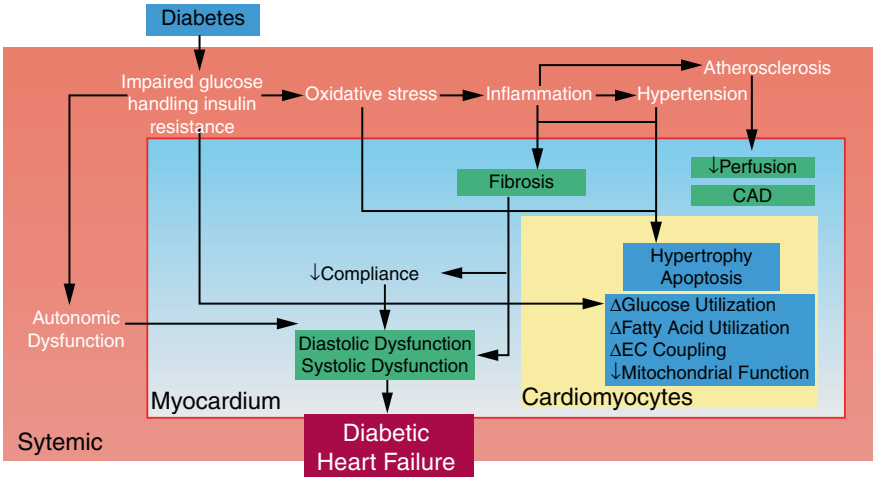

Fig. 27.2 Cellular and molecular mechanisms of diabetic cardiomyopathy. Glycemic changes (impaired glucose handling, insulin resistance) contribute to a variety of systemic effects as well as effects on the cardiomyocyte including disturbances of glucose and fatty acid utilization, mitochondrial function, and excitation contraction (EC) coupling. Other systemic effects (autonomic dysfunction, oxidative stress) lead to coronary artery disease (CAD) and abnormal myocardial structure and function. Consequent disturbances of myocardial performance at rest and with exercise result in the systemic consequence of diabetic heart failure. (Reprinted from Marwick TH et al., Implications of underlying mechanisms for the recognition and management of diabetic cardiomyopathy. J Am Coll Cardiol 2018;71:339–351. Copyright 2021, with permission from Elsevier)

12-lipoxygenase (LOX) and 15-LOX enzymes, which can mediate cardiomyocyte death, hypertrophy, and loss of contractility through oxidative stress and mitochondrial dysfunction [43].

- Cardiac oxidative stress: Lipotoxicity and glucotoxicity generate excess reactive oxygen and nitrogen species through the activation of cellular and mitochondrial NADPH oxidase. These species in turn increase oxidative stress in diabetic cardiomyopathy through myocardial inflammation [44, 45].
- Metabolic disturbances: In diabetes, free fatty acid uptake increases via CD36 translocation due to impaired signaling from hyperglycemia. In a normal heart, free fatty acid increases mitochondrial oxidative metabolism to generate ATP via the tricarboxylic acid cycle and β-oxidation. In a diabetic heart, lipid and free fatty acid accumulation exceeds the capacity to generate ATP, leading to cardiomyocyte death and impaired cardiac function [39].

Evaluation of Heart Failure in Patients with Diabetes

Evaluation of HF begins with obtaining a comprehensive medical history including the review of past and current problems. Questions regarding daily activities, diet, and sleep should elicit symptoms discussed under “Clinical Presentation of Diabetic

Cardiomyopathy." Social history should focus on risk factors for heart disease (smoking, alcohol, recreational drugs), while multigenerational family history should establish a genetic disposition for cardiomyopathy. A focused physical examination should complement the history with a search for clues to hemodynamic status. Findings such as narrow pulse pressure (low cardiac output), Cheyne–stokes respirations (advanced heart disease), and tachycardia (acute heart failure, shock, or arrhythmia) can be important clues in patient assessment. A key goal of the physical examination is to identify the presence of congestion and adequacy of organ perfusion. Volume status can be assessed through the jugular venous pressure, rales on lung examination, lower extremity edema, and ascites. Organ perfusion is manifested through the pulse pressure, mental status, skin warmth/coolness, and urine output among others. Categorization of clinical profile based on congestion and perfusion carries prognostic value [46].

Initial testing should include chest radiography, electrocardiography, hematology, and blood chemistry to identify the cause of HF. Biomarkers such as B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) provide diagnostic and prognostic information [47]. Non-invasive imaging modalities such as echocardiography, cardiac magnetic resonance imaging, cardiac computed tomography, and nuclear imaging are complementary. Exercise testing can be used to assess submaximal or peak functional capacity and look for evidence of ischemia. Invasive procedures such as right heart catheterization and coronary angiography are used to assess resting or exercise hemodynamics and rule coronary artery disease, respectively.

Treatment of Heart Failure in Diabetes

Patient-centered care is imperative for the effective management of patients with both DM and HF (Fig. 27.3) in order to alleviate symptoms, reduce morbidity and mortality, and improve quality of life. Achieving these goals requires an interdisciplinary approach involving multi-specialty health care providers, along with a social worker, dietitian, and pharmacist. Effective care teams should include physicians, advanced care providers, and nurses across specialties such as primary care, endocrine, renal medicine, cardiology/HF, palliative medicine, and mental health. Targeted referrals to specialists in weight loss management (e.g., bariatric surgery), sleep medicine, and neurology may also be indicated for select patients. In addition, the importance of diabetes-specific care (e.g., podiatry, ophthalmology, vascular medicine) cannot be overemphasized.

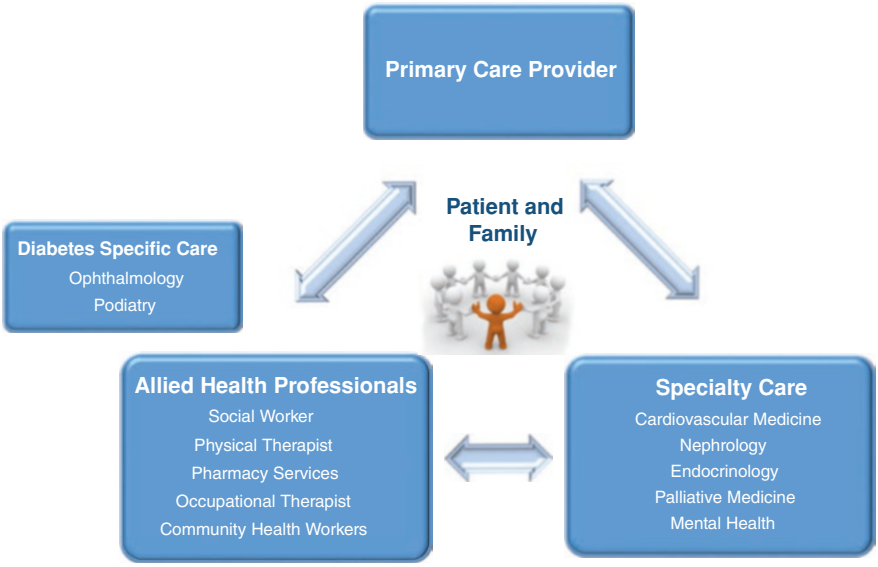

Fig. 27.3 Patient-centered interdisciplinary care. For patients with diabetes and heart failure, a multidisciplinary team (primary care, cardiology, endocrinology, nephrology, palliative care, and mental health) and inter-professional approach (physicians, nurses, allied health professionals) are central to optimizing care

Management of Diabetes in Patients with Heart Failure

Intensity of Blood Glucose Levels

Tight blood glucose control is associated with a 15% reduction in non-fatal myocardial infarction (MI) but does not reduce the risk of all-cause mortality, cardiovascular mortality, or stroke [48]. In the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial [49], intensive blood glucose control (HbA1c 6.4–7.0%) did not significantly reduce major cardiovascular events but increased mortality when compared to standard glycemic control (HbA1c 7.3–8.4%). Plausible explanations for the increased mortality in ACCORD include hypoglycemia episodes from strict glucose reduction, weight gain, and drug interactions from using multiple agents to intensify glucose management [50]. The ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) trial and VADT (Veterans Affairs Diabetes Trial) did not show significant improvement in cardiovascular outcomes with intensive glycemic control [51, 52]. Nevertheless, stringent glucose control reduces the risk and/or progression of retinopathy, nephropathy, and neuropathy [53, 54]. In patients with type 2 diabetes and either a prior cardiovascular event or increased cardiovascular risk, mortality rates associated with glycemic control follow a U-shaped curve with worsening death associated with HbA1C < 7 and >8% (Fig. 27.4) [55]. A similar relationship between HbA1c and risk of death has been demonstrated in patients with HF (Fig. 27.4).

The American Diabetes Association (ADA) "Standards of Medical Care in Diabetes" in 2020 recommend an A1c goal <7% for non-pregnant adults [56]. A

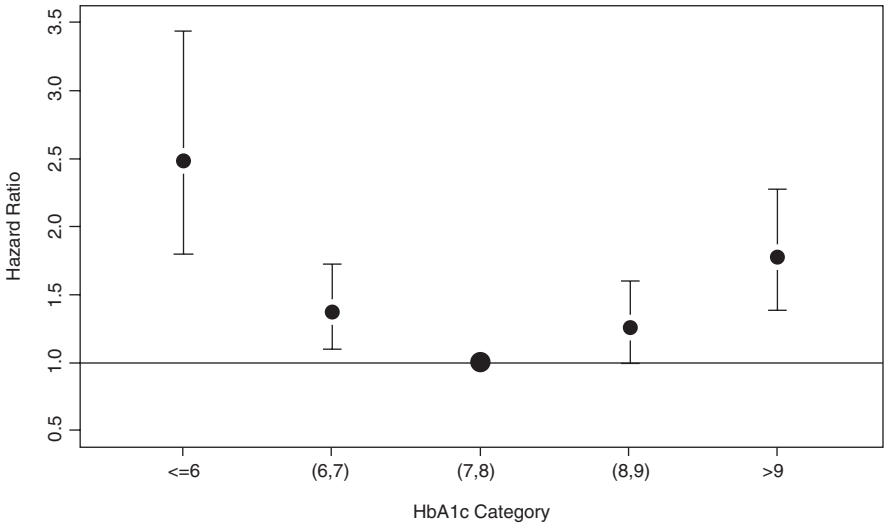

Fig. 27.4 Risk of all-cause mortality with diabetes treatment strategy. Hazard ratios for death by categories of glycosylated hemoglobin (HbA1c) in 1447 patients with type 2 diabetes who subsequently developed HF. (Reprinted from Elder DH et al., Mean HbA1c and mortality in diabetic individuals with heart failure: a population cohort study. Eur J Heart Fail 2016;18:84–102. Copyright 2021, with permission from European Society of Cardiology)

Table 27.3 Recommendations for glycemic control in patients with heart failure

Source	Recommendation	Reference
American Heart Association/Heart Failure Society of America	Optimal targets should be individualized to reflect comorbidity burden, severity of HF, and risks vs. benefits of lowering HbA1c	Dunlay et al., Circulation 2019;140:e294–e324
European Society of Cardiology	Glycemic control should be implemented gradually and moderately giving preference to those drugs that have been shown to be safe and effective	Ponikowski et al., Eur Heart J 2016;37:2129–2200
Canadian Cardiovascular Society	With the available evidence, an intensive glycemic control strategy cannot be recommended for all patients with diabetes. Instead, each individual should be assessed for his or her optimal glycemic target for the prevention of macrovascular events or HF	Ezekowitz et al., Can J Cardiol 2017;33:1342–1433

HbA1c hemoglobin A1c, *HF* heart failure

less stringent HbA1c goal (<8%) is recommended for those with limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, or long-standing diabetes. Most patients with HF fit in this latter category. A scientific statement from the American Heart Association and Heart Failure Society of America recommended a goal hemoglobin A1c of 7–8% for patients with HF [57]. Other general recommendations have recently been published by major cardiovascular societies (Table 27.3).

Pharmacologic Management of Diabetes in Patients with Heart Failure

Older Oral Agents

Biguanides such as metformin decrease gluconeogenesis and increase insulin sensitivity by increasing peripheral glucose uptake and utilization. Initial concerns regarding metformin use in patients with HF were related to risk of lactic acidosis, but this theory has subsequently been disproved [58]. The US Food and Drug Administration (FDA) removed the contraindication for use of metformin in HF in 2006. Metformin is considered the first-line therapy for patients with DM, although emerging guidelines appear to favor sodium–glucose co-transporter 2 (SGLT2) inhibitors.

Dipeptidyl peptidase-4 (DDP-4) is an enzyme involved in the rapid degradation of glucagon-like peptide 1 (GLP-1). DPP-4 inhibitors (alogliptin, linagliptin, saxagliptin, and sitagliptin) improve glycemic control by enhancing the effects of the incretin system. In large safety studies, these agents have demonstrated no cardiovascular benefit [59–61], while saxagliptin was shown to increase the risk of HF hospitalization by 27% in the SAVOR TIMI 53 (Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction 53) study [62]. This increase in risk was highest among patients with elevated levels of natriuretic peptides, previous HF, or chronic kidney disease. In 2016, an FDA safety panel added a new warning to the drug label regarding this safety issue.

Thiazolidinediones (TZDs) redirect free fatty acids into pre-adipocytes, in the process reducing intracellular triglyceride accumulation in cardiomyocytes. Pioglitazone and rosiglitazone are contraindicated in patients with New York Heart Association (NYHA) functional class III–IV heart failure due to the risk of precipitating fluid retention [63]. Given the availability of safer, alternative agents, these drugs are not recommended in any patient with HF and may increase the risk of HF events in individuals with DM without HF.

Sulfonylureas (glimepiride, glyburide and glipizide) act on ATP-sensitive potassium channels in the beta-cell plasma membrane, releasing insulin in the process. Sulfonylureas are not effective in the treatment of patients with insulin deficiency such as type 1 DM. Despite common use of these agents in patients with HF and type 2 DM, there are no randomized trials examining effects on clinical outcomes. However, several observational studies suggest that sulfonylureas may increase the risk of HF events compared to metformin or newer agents [64, 65].

Meglitinides (nateglinide, mitiglinide, and repaglinide) have a similar mechanism of action to sulfonylureas, but have a weaker binding affinity which leads to increased intracellular potassium. The latter causes depolarization, stimulating the opening of voltage-gated calcium channels which stimulates pro-insulin release. Meglitinides can be used in patients allergic to metformin or sulfonylureas.

Nateglinide and repaglinide failed to demonstrate cardiovascular benefits in the previous studies of diabetes, with no evidence of increased risk in patients with HF [66, 67].

Insulin

Insulin is recommended for patients whose diabetes is not appropriately controlled on conventional therapies. Patients with type 1 DM require multiple daily injections of prandial (50% daily requirement) and basal insulin, while patients with type 2 DM may progressively need basal insulin on top of oral hypoglycemic agents. Insulin can cause hypoglycemia which could trigger the neurohormonal axis, leading to HF exacerbation. In the ORIGIN (Outcome Reduction with Initial Glargine Intervention) trial, the rate of non-fatal myocardial infarction, non-fatal stroke, death from cardiovascular causes, revascularization, or hospitalization for heart failure was similar between insulin and standard of care [68]. Trial of different basal insulins (Degludec versus Glargine) did not affect major cardiovascular events in patients with type 2 DM at high risk for cardiovascular events [69]. A retrospective study suggested that the addition of insulin to metformin increased the risk of a composite of non-fatal cardiovascular outcomes and all-cause mortality [70], which potentially reveals the selection bias of a sicker population receiving insulin.

Cardiac-specific Diabetes Medications

In contrast to the agents described above, SGLT2 inhibitors and GLP-1 receptor agonists have demonstrated the ability to reduce cardiovascular risk [71].

Glucagon-like Peptide-1 Receptor Agonists

GLP-1R agonists (liraglutide, semaglutide, lixisenatide, and exenatide) increase glucose-dependent insulin secretion, decrease glucagon secretion, and cause early satiety by delaying gastric emptying. The LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial randomized 9340 patients with type 2 diabetes at high risk for cardiovascular disease or with cardiovascular disease (80% of study population) to liraglutide or placebo. Over a median follow-up of 3.8 years, liraglutide reduced the risk of cardiovascular death, non-fatal MI or non-fatal stroke (13% versus 14.9% [HR 0.87; 95% CI, 0.78–0.97; $p < 0.001$ for non-inferiority; $p < 0.01$ for superiority]). All-cause mortality was also lower in the liraglutide group (8.2% versus 9.6% [HR 0.85; 95% CI, 0.74–0.97;

$p = 0.02$]) [72]. Based on these data, liraglutide was approved by the FDA to reduce the risk of major adverse cardiovascular events in adults with type 2 diabetes and established cardiovascular disease. Neither HF-specific harms nor benefits have been established for GLP-1R agonists, although the FIGHT (Functional Impact of GLP-1 for Heart Failure Treatment) study showed a trend toward higher risk of HF-related events with liraglutide in patients with diabetes and HFrEF who were recently hospitalized [73]. Semaglutide was recently approved by the FDA an adjunct to a reduced calorie diet and increased physical activity for chronic weight management in adult patients with obesity (BMI 30 kg/m^2 or greater) or overweight (BMI 27 kg/m^2 or greater) with at least one comorbidity such as DM.

Sodium–Glucose Co-transporter 2 Inhibitors

SGLT2 inhibitors have recently emerged as first-line therapy for treating patients with HF and reduced ejection fraction, with or without diabetes, due to their benefits on morbidity and mortality (Table 27.4). SGLT2 inhibitors inhibit proximal sodium and glucose reabsorption, promoting glucosuria, thereby increasing both diuresis and natriuresis [80]. Additional beneficial effects on neurohormonal activation, myocardial structure and function, and renal function have also been demonstrated (Fig. 27.5).

In the EMPA-REG OUTCOME (Empagliflozin Cardiovascular Outcome Event Trial in T2DM Patients—Removing Excess Glucose) trial, a randomized double-blind placebo-control study, empagliflozin reduced HF hospitalization by 35% and cardiovascular death by 38% in 7020 patients with type 2 diabetes and existing cardiovascular disease [74, 81]. Similarly, in the CANVAS (Canagliflozin Cardiovascular Assessment Study) and CANVAS-R studies [82], canagliflozin demonstrated a 33% reduction in the risk of HF hospitalization compared to placebo. A total of 10,142 patients with type 2 diabetes and high cardiovascular risk were randomized to canagliflozin or placebo for an average of 3.6 years. Canagliflozin significantly reduced the composite outcome of cardiovascular death, non-fatal MI, or non-fatal stroke by 14% compared to placebo (HR 0.86; 95% CI, 0.75–0.97; $p < 0.001$ for non-inferiority; $p = 0.02$ for superiority).

The DECLARE–TIMI 58 (Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58) study was a randomized, double-blind, placebo-controlled phase 3b trial of dapagliflozin 10 mg once daily [83]. In 17,160 patients with type 2 diabetes who had or were at risk for cardiovascular disease, dapagliflozin reduced the risk of HF hospitalization by 27% (HR 0.73; 95% CI, 0.61–0.88) over a median of 4.2 years. The DAPA-HF (Dapagliflozin and Prevention of Adverse-Outcomes in Heart Failure) trial investigated the effect of dapagliflozin in 4744 patients with HF, LVEF ≤40%, and NYHA functional class II–IV symptoms. Compared to placebo, dapagliflozin was associated with a 25% (diabetics) and 26% (non-diabetics) reduction in the primary composite outcome of cardiovascular death, HF hospitalization, or urgent HF visit [76]. Similar impressive results

Table 27.4 Study of SGLT2 inhibitors for treating patients with HF

Target population	Study	Number	Agent	Entry criteria	Follow up	Primary endpoint	Findings
T2DM	EMPAREG-Outcome [74]	7020	Empagliflozin	T2DM Age > 18 years CVD	3.1 years	CV mortality, non-fatal MI or non-fatal stroke	Empagliflozin 10.5% vs. Placebo 12.1%; $p = 0.04$; NNT 62
HFrEF	EMPEROR-REDUCED [75]	3730	Empagliflozin	NYHA II-IV Age > 18 years	16 months	Composite of cardiovascular death and first hospitalization for decompensated heart failure	Empagliflozin 19.4% vs. Placebo 24.7% (HR 0.75; 95% CI 0.65–0.86; $p < 0.001$)
HFrEF	DAPA-HF [76]	4744	Dapagliflozin	NYHA II-IV Age > 18 years	18 months	Worsening heart failure (hospitalization or urgent visit resulting in IV therapy for HF) or cardiovascular mortality	Empagliflozin 16.3% vs. Placebo 21.2% (HR 0.74; 95% CI 0.65–0.85; $p < 0.001$)
HFpEF	DELIVER [77]	6263	Dapagliflozin	NYHA II-IV Age > 40 years	2.3 years	Composite of worsening HF (defined as unplanned hospitalization or urgent visit for HF) or cardiovascular death assessed in a time-to-event analysis	Dapagliflozin 16.4% vs. Placebo 19.5% (HR 0.82; 95% CI 0.73– 0.92; $p < 0.01$)
HFpEF	EMPEROR PRESERVED [78]	5988	Empagliflozin	NYHA II-IV Age > 18 years	38 months	Time to first event of adjudicated cardiovascular death or adjudicated hospitalization for heart failure	Median 26.2 months, Composite of cardiovascular death or hospitalization for heart failure; Empagliflozin; 13.8% versus placebo; 17.1% [hazard ratio, 0.79; 95 CI, 0.69–0.90; $p < 0.001$].
AHF	SOLOIST-WHF [79]	1222	Sotagliflozin	HF admission Treatment with diuretics	9 months	Total cardiovascular death, heart failure hospitalization, or urgent visit for heart failure	Sotagliflozin vs. placebo, 70 vs. 98 events/100 patient-years (HR 0.67, 95% confidence interval 0.52–0.85, $p = 0.0009$).

AHF acute heart failure, *CVD* cardiovascular disease, *HF* heart failure, *HFrEF* heart failure with reduced ejection fraction, *HFpEF* heart failure with preserved ejection fraction, *NYHA* New York Heart Association, *T2DM* type 2 diabetes mellitus

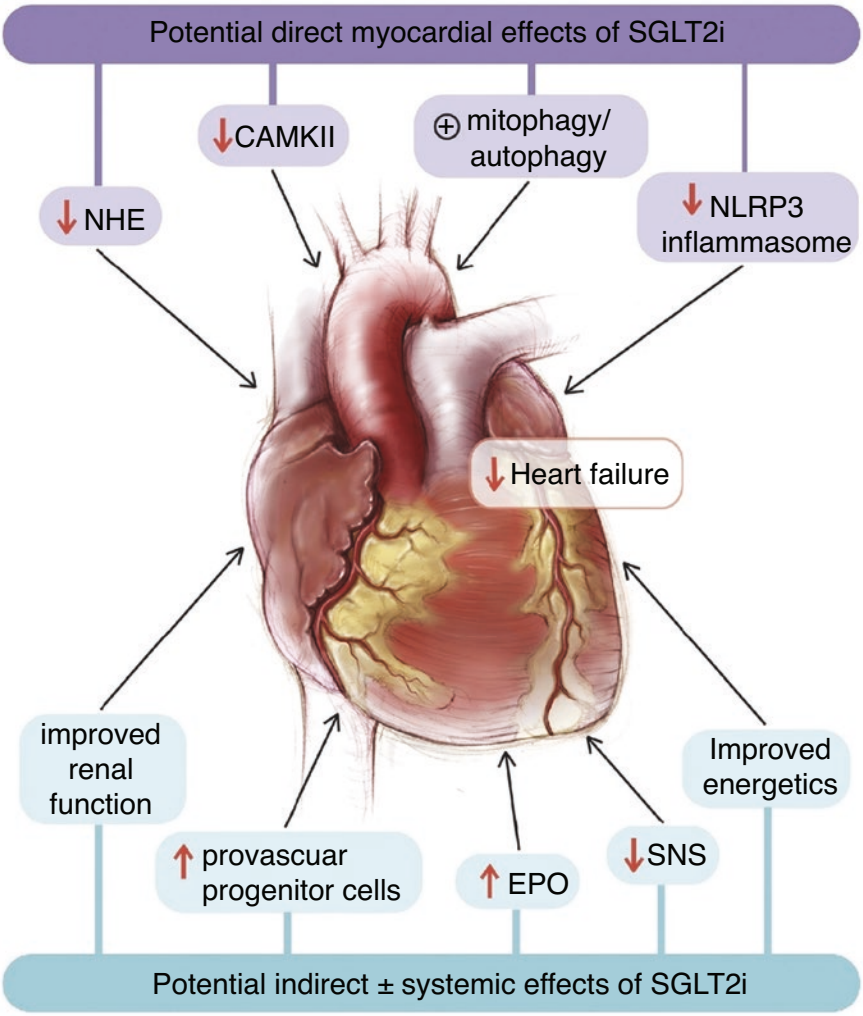

Fig. 27.5 Mechanisms of benefit of SGLT2 inhibitors in heart failure. SGLT2 inhibitors exert direct and indirect effects on the myocardium. They directly reduce cardiac NLRP3, which mediates inflammation, autophagy, and lysosomal degradation among others. SGLT2 inhibitors regulate intra-glomerular pressure which attenuates sympathetic nervous system activity, and also reduces reactive oxygen species (source: Lopaschuk GD and Verma S, Mechanisms of Cardiovascular Benefits of Sodium Glucose Co-Transporter 2 (SGLT2) Inhibitors, A State-of-the-Art Review, JACC Basic Transl Sci. 2020 Jun; 5(6): 632–644. This is an open access article under the CC BY-NC-ND license). *CAMKII* calmodulin-dependent protein kinase II, *EPO* erythropoietin, *NHE* sodium/hydrogen exchanger, *NLRP3* nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing 3, *SGLT2i* sodium–glucose co-transporter 2 inhibitor, *SNS* sympathetic nervous system

(decreased HF hospitalization and overall mortality) were observed with empagliflozin in the EMPEROR-Reduced (The Empagliflozin Outcome Trial in Patients with Chronic Heart Failure and Reduced Ejection Fraction) trials [75]. In patients with HF with or without DM and previously implanted pulmonary artery pressure sensors, empagliflozin reduced pulmonary artery diastolic pressure at 8–12 weeks (1.5 mmHg lower) and 12 weeks (1.7 mmHg lower) compared to placebo independent of diuretics [84]. Similar decreases were observed in systolic and mean pulmonary artery pressures.

SGLT2 Inhibitors in Heart Failure with Preserved Ejection Fraction

SGLT2 inhibitors have shown similar benefits in HFpEF irrespective of diabetes status. In a double-blind trial of 5988 patients with NYHA III–IV, empagliflozin reduced the combined risk of cardiovascular death or hospitalization for heart failure (Empagliflozin; 13.8% versus Placebo; 17.1%, hazard ratio, 0.79; 95% confidence interval [CI], 0.69–0.90; $p < 0.001$). The lower event rate in the Empagliflozin group was driven by lower HF hospitalization [78]. In the PRESERVED-HF Trial, compared to placebo, 12-week treatment with dapagliflozin significantly improved patient-reported symptoms (Kansas City Cardiomyopathy Questionnaire Clinical Summary Score), physical limitations and exercise function (6-min walk test) [85]. Recent results from the DELIVER Phase III trial [77] demonstrate that dapagliflozin reduces the combined risk of worsening HF or cardiovascular death in patients with HF and mildly reduced or preserved ejection fraction (Dapagliflozin 16.4% versus Placebo 19.5%, hazard ratio 0.82; 95% CI 0.73–0.92; $p < 0.001$). Approximately 45% of patients enrolled in DELIVER had DM and there was no difference in the benefits of dapagliflozin in patients with or without DM.

SGLT2 Inhibitors in Chronic Kidney Disease

The effect of SGLT2 inhibition on renal function has also been studied in patients with diabetes. The Canagliflozin Cardiovascular Assessment Study (CANVAS) showed a benefit of canagliflozin with respect to the composite outcome of a sustained 40% reduction in the estimated glomerular filtration rate (GFR), the need for renal-replacement therapy, or death from renal causes (hazard ratio, 0.60; 95% CI, 0.47–0.77) [82]. In the CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) trial, canagliflozin resulted in a 30% reduction in the composite of end-stage kidney disease, doubling of the serum creatinine level, or death from renal or cardiovascular causes [86]. In other studies, empagliflozin was associated with 44% and 55% relative risk reductions in the

doubling of creatinine and renal replacement therapy, respectively [87]. Similarly, dapagliflozin reduced the risk of sustained decline in the estimated GFR of at least 50%, end-stage kidney disease, or death from renal or cardiovascular causes in patients with chronic kidney disease [88].

Based on the totality of evidence from these trials, SGLT2 inhibitors are being recommended posed as part of the standard therapy for HFrEF and HFpEF with or without DM [89–92].

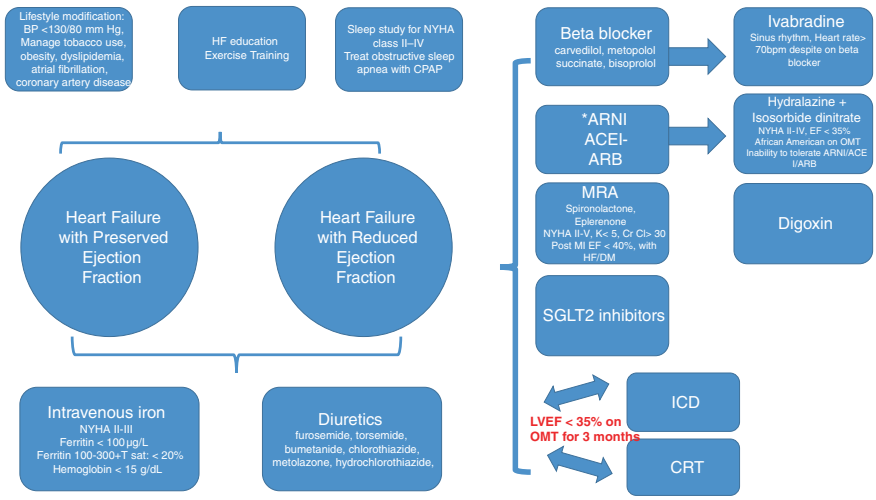

Fig. 27.6 Heart failure guideline-directed medical therapy. All patients with HF, regardless of severity of illness or ejection fraction, benefit from HF education, lifestyle modification and management of comorbidities such as obesity, sleep apnea, and iron deficiency. For patients with signs or symptoms of volume overload, diuretics are indicated to reduce systemic and/or pulmonary venous congestion. For patients with HFrEF, first-line medical therapy includes a combination of renin–angiotensin system antagonist (preferably an angiotensin–neprilysin inhibitor [ARNI]), beta-blocker, mineralocorticoid receptor antagonist (MRA), and SGLT2 inhibitor. For patients who remain symptomatic or are intolerant of one or more of these agents, adjuvant therapy with ivabradine, hydralazine and nitrates, vericiguat and digoxin should be considered. Additional indications for implantable cardioverter-defibrillator (ICD) and cardiac resynchronization therapy (CRT) devices have been published [93]. *ACE-I* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *BP* blood pressure, *CPAP* continuous positive airway pressure, *EF* ejection fraction, *HF* heart failure, *NHYA* New York Heart Association, *OMT* optimal medical therapy, *T sat* transferrin saturation

Management of Heart Failure in Patients with Diabetes

The cornerstone of HF management is neurohormonal blockade. Figure 27.6 summarizes the staged treatment approach to patients with HF based on the American College of Cardiology Foundation/American Heart Association/Heart Failure Society of America guidelines [94, 95].

General Management

Lifestyle modifications, management of comorbidities in addition to diabetes, and exercise training are essential for the management of all patients with HF regardless of ejection fraction. SPRINT (Systolic Blood Pressure Intervention Trial) demonstrated that targeting a systolic blood pressure <120 mmHg, as compared with <140 mmHg, resulted in lower rates of fatal and non-fatal major cardiovascular events and death from any cause [96]. Considering that hypertension is a potent risk factor for HF [97], blood pressure control is key to disease prevention. A blood pressure goal of <130/80 mmHg is recommended based on these data.

Comorbidities such as dyslipidemia [98], obesity [99], and tobacco use [100] are all associated with an elevated risk of HF, hence, the importance of aggressively managing these conditions. Cardiac comorbidities such as coronary artery disease and atrial fibrillation should be managed as per guidelines pertaining to these conditions. A formal sleep assessment should be offered to patients with NYHA functional class II–IV symptoms and suspicion of sleep-disordered breathing or excessive daytime sleepiness. Upon diagnosing obstructive sleep apnea, continuous positive airway pressure (CPAP) should be prescribed to improve sleep quality. Although CPAP has been shown to decrease the progression to permanent atrial fibrillation [101], lower blood pressure, and increase ejection fraction in HFrEF, other long-term cardiovascular benefits have not been established [102]. There is no role for CPAP therapy in patients with HF and central sleep apnea. A multicenter study of nocturnal oxygen therapy in patients with stable HFrEF and central sleep apnea was terminated early due to slow participant accrual [103].

Heart failure education by nurse specialists is associated with decreased hospitalization within 6 months of discharge, lower costs of care, and reduced rates of HF readmission and mortality at 6 months [104, 105]. In the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) study, exercise training modestly reduced all-cause mortality or hospitalization by 17% (HR 0.93, 95% CI, 0.84–1.02; $p = 0.13$) and cardiovascular mortality or HF hospitalization by 13% (HR 0.87; 95% CI, 0.75–1.00; $p = 0.06$) compared to usual care [106]. Modest improvements in quality of life were also observed. If tolerated,

exercise training and cardiac rehabilitation are both recommended for patients with symptomatic HF [94].

Less than 3 g sodium restriction is recommended for ACCF/AHA stage C HF patients and a more stringent 1.5 g sodium restriction for stage A and B patients, although the data to support these recommendations are limited [107]. Many clinicians also recommend that patients with HF limit fluid intake to less than 2 L daily to avoid congestion. While it is recognized that alcohol consumption is a risk factor for HF, there remains uncertainty about the amount of alcohol ingested and the likelihood of developing HF. Nevertheless, heavy alcohol use has been associated with cardiomyopathy and arrhythmias. Thus, patients should be counseled regarding alcohol intake.

Pharmacologic Therapy

Diuretics

The effect of diuretics on morbidity and mortality in HF has not been demonstrated, but these agents relieve symptoms of congestion and improve exercise tolerance. Loop diuretics such as furosemide, torsemide, and bumetanide increase sodium excretion and enhance free water clearance to achieve euvolemia. Loop diuretics inhibit the Na^+–K^+–$2Cl^-$ symporter (co-transporter) on the apical membrane of epithelial cells in the thick ascending loop of Henle. Hydrochlorothiazide, chlorthalidone, chlorothiazide, and metolazone inhibit the Na/Cl co-transporter in the distal tubule, thus blocking sodium resorption. Thiazide diuretics, including metolazone, are used to enhance the effect of loop diuretics but may be limited by worsening renal function and electrolyte abnormalities (e.g., hypokalemia, hyponatremia). At high doses, mineralocorticoid receptor antagonists (MRAs) such as spironolactone and eplerenone, which are potassium-sparing and act on the distal tubule, can function as diuretics.

In patients with HF and diabetes, use of diuretics can cause hyperglycemia. Prolonged thiazide usage may precipitate diabetes mellitus [108]. Furosemide decreases the sensitivity of glucose utilization to insulin in skeletal muscle by directly inhibiting the glucose transport process [109].

Renin–Angiotensin–Aldosterone System Inhibitors

Angiotensin receptor–neprilysin inhibitors (ARNI) have emerged as first-line therapy in patients with HFrEF and mild-to-moderate symptoms, with a class I/level of evidence A recommendation. The prototype drug combines valsartan, an angiotensin receptor blocker (ARB), with sacubitril, an inhibitor of neprilysin, which is an

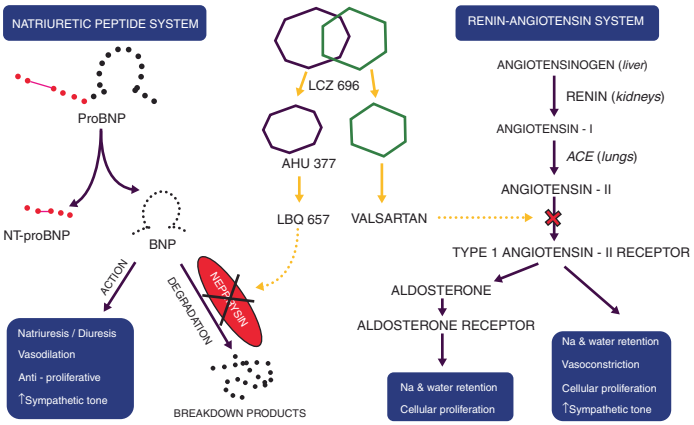

Fig. 27.7 Schematic representation of mechanism of sacubitril/valsartan on the natriuretic peptide and RAAS. Sacubitril inhibits neprilysin leading to an increase in natriuretic and vasoactive peptides. Natriuretic and vasoactive peptides promote natriuresis, vasodilation, and reduction of sympathetic tone. Valsartan inhibits angiotensin II which is responsible for vasoconstriction and activation of aldosterone. AHU 377, sacubitril; BNP, B-type natriuretic peptide; LBQ 657, sacubitrilat; LCZ 696, sacubitril/valsartan (prototype name); Na, sodium; NT-proBNP, N-terminal pro B-type natriuretic peptide; RAAS, renin–angiotensin–aldosterone system. (Reprinted with permission from Singh JSS, et al. Sacubitril/valsartan: beyond natriuretic peptides. Heart 2017;103(20):1569–1577)

enzyme that degrades natriuretic peptides, bradykinin, and other vasoactive peptides (Fig. 27.7). In the PARADIGM-HF (Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial, sacubitril–valsartan was shown to significantly decrease death or HF hospitalization by 20% when compared to enalapril [110]. Additional beneficial effects of sacubitril–valsartan on all-cause mortality, cardiovascular hospitalization [111], quality of life, and ventricular remodeling have also been demonstrated [112].

In a post hoc analysis of patients with HF and diabetes ($N = 3778$) enrolled in PARADIGM-HF, hemoglobin A1c concentrations were persistently lower in the sacubitril–valsartan group than in the enalapril group over 3-year follow-up (between-group reduction 0.14%, 95% CI 0.06–0.23, $p = 0.006$). New use of insulin was 29% lower in patients receiving sacubitril–valsartan and fewer patients were started on oral antihyperglycemic therapy [113]. There are several potential mechanisms by which inhibition of neprilysin may lead to improvement in glycemic control, with most evidence suggesting the modulation of neprilysin circulating substrates [114]. The effect of renin–angiotensin system inhibition on glucose metabolism is likely modest. Additional data from PARADIGM-HF showed that patients treated with sacubitril–valsartan had a slower rate of decline in eGFR compared with patients treated with enalapril, and the magnitude of the benefit was larger in patients with diabetes [115].

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are alternative therapies to sacubitril–valsartan in patients who cannot tolerate ARNI due to hypotension, renal dysfunction, or cost. ACE inhibitors

[116–121] and ARBs have well-established mortality benefits in patients with HFrEF [122–124], and can be used to treat hypertension in patients with HFpEF. ACE inhibition of kinase results in increased bradykinin, which can induce cough and/or angioedema, in this case ARB is an acceptable alternative. ACE inhibitors and ARBs exert renal protective effects in patients with albuminuria, which make them a good choice for patients with prediabetes [125]. The benefits of ACE inhibitors and ARBs on clinical status in patients with HF and diabetes are equivalent to those without diabetes [8, 126, 127]. Furthermore enalapril [128] and candesartan [129] have each been shown to reduce the incidence of diabetes in chronic HF.

There are limited data on the impact of ACE inhibitors/ARBs on glycemic control in patients with HF and preexisting DM. ACE inhibitors and ARBS may control glucose levels by improving insulin sensitivity through the increase of peripheral blood flow to skeletal muscle through elevation of bradykinin level or suppression of angiotensin II [130].

In clinical practice, MRAs (spironolactone and eplerenone) are used to reduce morbidity and mortality in patients with symptomatic HFrEF, creatinine clearance >30, and potassium level <5.0 mEq/L [131, 132]. MRAs are also indicated for patients after myocardial infarction with LVEF ≤ 40% and symptoms of HF or DM [133]. MRAs have consistent benefits in HFrEF patients with and without DM [134], while limited data suggest that eplerenone might have a more favorable impact on glycemic control than spironolactone [7, 135, 136].

Beta-Blockers

Historically, there has been some reluctance to prescribe beta-blockers in patients with diabetes due to concern that these agents can mask hypoglycemia and stimulate insulin resistance [137]. A meta-analysis of six pivotal studies in patients with HFrEF showed that beta-blockers significantly reduced mortality in individuals with (relative risk, 0.84; 95% CI, 0.73–0.91) and without (relative risk, 0.72; 95% CI, 0.65–0.79) diabetes [138]. Recently, a prospective cohort study demonstrated that patients with HF and diabetes were prescribed larger doses of beta-blockers than those without diabetes, and that increasing beta-blocker dose was associated with a greater reduction in mortality in patients with diabetes [139]. Patients with HFrEF should be on one of three beta-blockers (bisoprolol, carvedilol, and metoprolol succinate), since they have been shown to reduce the risk of death and the combined risk of death or hospitalization [140–144]. Non-selective beta-blockers such as carvedilol reduce HbA1c and fasting insulin, and are associated with better glycemic effects [145, 146]. In a non-HF population of patients with hypertension and diabetes, carvedilol was shown to improve insulin sensitivity and glycemic control compared to metoprolol tartrate [146], however, further studies are needed to validate these findings in HF.

Adjunctive Pharmacologic Therapy

In self-identified African Americans with NYHA functional class III–IV HFrEF receiving optimal therapy with ACE inhibitors and beta blockers, the combination of hydralazine and isosorbide dinitrate should be considered as additive therapy [147]. This combination could also be considered in patients with symptomatic HFrEF who cannot tolerate ACE inhibitors/ARBs/ARNI. In the African-American Heart Failure Trial (A-HeFT), the beneficial effects of hydralazine–isosorbide dinitrate on event-free survival were consistent across multiple subgroups, including patients with diabetes (41% of total enrolled) [148]. Despite these benefits, long-term tolerability of combination therapy with hydralazine and nitrates (up to 6 or more pills daily, common side effects of headache and dizziness) is not high.

Ivabradine selectively inhibits the cardiac pacemaker current (If), which controls the spontaneous diastolic depolarization in the sinoatrial node. In the SHIFT trial (Systolic Heart failure treatment with the If inhibitor ivabradine Trial), ivabradine decreased the risk of cardiovascular death or HF hospitalization in patients with NYHA functional class II or III HF and left ventricular ejection fraction ≤35%. Ideally, patients should be in sinus rhythm with heart rates >70 bpm despite being on a beta-blocker at the maximum tolerated dose [149]. In SHIFT, the clinical benefits of ivabradine were similar in patients with and without diabetes, and there was no difference in the incidence of serious adverse events [150].

Digoxin could be considered in select patients with HFrEF to decrease HF hospitalization [151, 152], and may be used to control ventricular response in older patients with atrial fibrillation. Vericiguat, an oral guanylate cyclase stimulator, may be considered to reduce HF hospitalization and cardiovascular death in selected high-risk patients with HFrEF and recent worsening of HF already on GDMT [94, 153]. Intravenous iron infusion in the setting of iron deficiency anemia improves NYHA class, peak functional capacity, and 6-min walk test as shown in the FAIR-HF (Ferric Carboxymaltose Assessment in Patients With Iron Deficiency and Chronic Heart Failure) [154] and CONFIRM-HF (Ferric Carboxymaltose Evaluation on Performance in Patients With Iron Deficiency in Combination with Chronic Heart Failure) Trials [155]. In patients with iron deficiency stabilized after an episode of acute heart failure (42% of whom had diabetes), treatment with ferric carboxymaltose was safe and reduced the risk of HF hospitalizations [156]. Intravenous iron is indicated for treating HF patients with NYHA II–III symptoms and ferritin <100 or 100–300 ng/mL and transferrin saturation <20% [95, 157].

Heart Failure with Preserved Ejection Fraction

Targeted therapy for HFpEF emphasizes the management of comorbidities such as DM, atherosclerotic disease, hypertension, iron deficiency anemia, obesity, or metabolic syndrome. Although 45% of patients with HFpEF have DM [4], the optimal treatment strategy of the diabetic phenotype of HFpEF is unknown due to the

paucity of evidence-based treatments for HFpEF. Oral hypoglycemic agents such as SGLT2 inhibitors, GLP-1R agonists, and metformin have pleiotropic effects with cardiac benefits as discussed above. In the PARAGON-HF (Prospective Comparison of Angiotensin Receptor Neprilysin Inhibitor With Angiotensin Receptor Blocker Global Outcomes in Heart Failure and Preserved Left Ventricular Ejection Fraction) trial, the prevalence of diabetes was 43% [158], which is higher than other HFpEF trials. In patients with HF and mid-range EF, sacubitril–valsartan was associated with a 13% relative reduction in the primary composite endpoint of cardiovascular death and total HF hospitalizations, but this did not meet statistical significance [159] In patients with HF and mildly reduced or preserved EF, dapagliflozin reduced the combined risk of worsening HF or cardiovascular death by 18% [77].

Device Therapy

Implantable cardioverter-defibrillators (ICDs) are indicated in patients with LVEF < 35% with NYHA class II–III symptoms after 3 months of optimal medical therapy [160, 161]. ICDs can also be considered for carefully selected NYHA class I patients who are at least 40 days post MI with LVEF of 30% or less [162–164]. ICDs should be considered only in patients who are expected to survive for at least 1 year. Cardiac resynchronization therapy (CRT) is recommended for NYHA class II–IV patients with LVEF < 35% and LBBB with QRS duration >150 ms [165–167]. Studies in patients with HF and diabetes have confirmed the benefit of preventing sudden cardiac death and improving overall survival with ICD and CRT [168, 169]. Moreover, procedure-related complications and length of stay are similar in HF patients with and without DM. Patients with DM and HbA1c < 7.0% have better outcomes after CRT than do those with suboptimal glycemic control [170].

Revascularization

Revascularization (coronary artery bypass grafting [CABG] or percutaneous coronary intervention [PCI]) is typically integrated into the treatment of patients with HF and angina or coronary artery disease. The BARI 2D (The Bypass Angioplasty Revascularization Investigation 2 Diabetes) trial specifically compared revascularization to medical therapy in 2368 patients with type 2 diabetes and coronary artery disease [171]. Patients with NYHA functional class III or IV heart failure were excluded, but approximately 6.6% of the cohort had a history of heart failure at enrollment. There was no difference in new onset HF between the revascularization and intensive medical therapy arms (21.3% vs. 21.2%).

While no clear mortality benefit was observed in the study, a subgroup analysis demonstrated that CABG plus medical therapy reduced the rate of cardiovascular events compared to medical therapy alone. In the FREEDOM (The Future

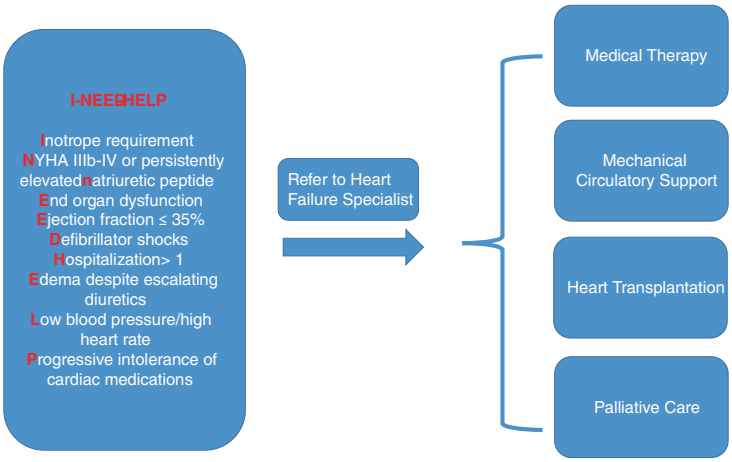

Fig. 27.8 Indications for referral for advanced cardiac therapies. I–NEED-HELP acronym is a guide for timely referral to a heart failure specialist. Spectrum of care includes medical therapy, mechanical circulatory support, heart transplant, and palliative care

Revascularization Evaluation in Patients With Diabetes Mellitus: Optimal Management of Multivessel Disease) trial, CABG reduced the rates of death and myocardial infarction compared to PCI among patients with DM and multivessel coronary artery disease [172].

Advanced Therapies

The acronym I-NEED-HELP (Fig. 27.8) should trigger consultation with a heart failure specialist to optimize medical therapy in tandem with the interdisciplinary team (see Fig. 27.3) and consider candidacy for, and timing of, advanced therapies. An estimated 30–40% of patients undergoing evaluation for left ventricular assist device implantation have diabetes [173, 174]. Post-operatively, diabetes increases the risk of all-cause mortality (hazard ratio 1.73; 95% confidence interval 1.18–2.53; $p = 0.005$) and non-fatal composite of stroke, pump thrombosis, and device infection (hazard ratio 2.1; 95% confidence interval 1.35–3.18; $p = 0.001$) [174]. In older data, diabetes was associated with a higher risk of mortality following Novacor LVAD placement (OR 1.76, 95% CI 1.05–2.94) [175], but did not increase mortality or rates of major adverse events in continuous flow LVAD support [173]. The improvement in outcomes could be related to improved risk profile of newer generation LVAD technology.

Diabetes with poor glycemic control (A1c > 7.5%) and end-organ damage (other than non-proliferative retinopathy) is a relative contraindication to heart transplant [176]. In carefully selected patients with advanced HF and diabetic nephropathy, combined heart–kidney transplant is an option. Following heart transplant, patients

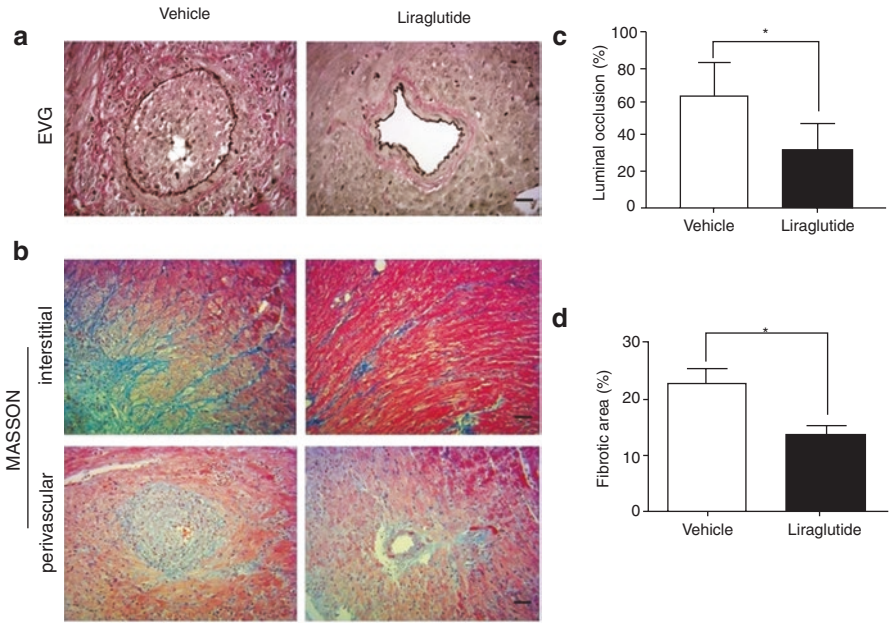

Fig. 27.9 Liraglutide inhibits cardiac allograft vasculopathy and fibrosis in a murine model. Cardiac allograft vasculopathy (CAV) and cardiac fibrosis were induced by heterotopic heart transplantation with a class II major histocompatibility difference between the donor and the recipient. The tissues were analyzed at 8 weeks after surgery. (**a**) elastic van Gieson (EVG) staining of cardiac allografts of vehicle-treated and liraglutide-treated mice. (**b**) Masson's trichrome staining (blue areas) of cardiac allografts of vehicle-treated and liraglutide-treated mice in the interstitial and perivascular regions. (**c**) Quantitative analysis of CAV by assessing luminal occlusion of at least 8 arteries per mouse in each group at 8 weeks after transplantation ($n = 6$ per group). Scale bars = 20 μm. (**d**) Quantitative analysis of the percentage of the fibrotic area in 10 given microscopic visual field areas (200×) per mouse from each group ($n = 6$ per group). Scale bars = 20 μm. Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$ versus vehicle. MASSON: Masson's trichrome staining. (Reprinted with permission from Wang Z et al. Liraglutide, a glucagon-like peptide-1 receptor agonist, attenuates the development of cardiac allograft vasculopathy in a murine heart transplant model. Transplantation. 2019 Mar;103(3):502–511)

are at increased risk of developing diabetes due to immunosuppressive agents such as steroids and calcineurin inhibitors. Treatment of post-transplant diabetes is similar to that discussed above (see "Pharmacological Management of Diabetes in Heart Failure"), although increasing insulin requirements are common in the setting of steroid use. Preliminary clinical data suggest that GLP-1R agonists may have salutary effects on weight loss and insulin requirements in heart transplant recipients [177], while preclinical studies show attenuation in cardiac allograft vasculopathy (Fig. 27.9) [178]. Future studies are needed to determine the safety and efficacy of SGLT2 inhibitor use in this setting [179, 180].

Conclusion and Future Directions

Diabetes and cardiovascular disease have a potent bidirectional relationship with a complex interplay of cellular, molecular, and metabolic interactions leading to diabetic cardiomyopathy and heart failure. Although heart failure therapies have been shown to reduce the risk of death in patients with and without diabetes, most antidiabetic agents have no effect on the prevention of cardiac dysfunction. GLP-1 receptor agonists have shown promising results in reducing the risk of myocardial infarction, cerebrovascular disease, or cardiovascular death. SGLT2 inhibitors have emerged as cardioprotective and renal protective therapy in patients with and without diabetes, with significant reductions in mortality and heart failure hospitalization in both HFrEF and HFpEF. Further studies are needed to better elucidate the complex interaction between diabetes and heart failure.

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Part VI
Treatment–Weight Loss Strategies

Chapter 28
Lifestyle and Nutrition Therapy

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Background/Introduction

Lifestyle Intervention and Diabetes Self-management Education and Support in Patients with Diabetes

Positive behavioral change through lifestyle intervention, diabetes self-management education and support (DSMES), medical nutrition therapy (MNT), weight management, and regular exercise together are the foundation for successful diabetes prevention and diabetes treatment. In this chapter we review the evidence supporting these key lifestyle interventions and how they are effective in both diabetes

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prevention and diabetes management. We discuss and review guideline-based dietary recommendations and the approach to weight management for patients with diabetes. We also review some of the new virtual tools and web-based applications such a continuous glucose monitoring (CGM) to help patients modify their behavior, institute positive change and thereby attain optimal blood glucose control, and improve their metabolic health. The guidelines for physical activity and exercise in patients with diabetes are also presented and the importance of smoking avoidance and cessation is emphasized.

Diabetes Prevention

Multiple studies and meta-analyses have illustrated the benefit of structured intensive lifestyle intervention programs in diabetes prevention [1–4]. The largest benefit occurs in the most intensive and structured lifestyle intervention programs [1]. Three landmark, large, randomized controlled diabetes prevention trials are the Diabetes Prevention Program (DPP) [5], the Finnish Diabetes Prevention Study [6] (DPS), and the Chinese Da Qing Diabetes Prevention Study [7] (Da Qing study). These trials clearly demonstrate the effectiveness of lifestyle intervention in preventing the development of diabetes.

The DPP, based in the USA, was the largest of these intensive trials and had an enrollment of over 3000 individuals with prediabetes (See Criteria for Prediabetes, Table 28.1) [5]. A particular strength of this trial was the diversity of participants in terms of their age, ethnicity, race, and geographic location. In this trial, individuals with prediabetes were randomized to an intensive lifestyle intervention arm, metformin (850 mg twice daily) or placebo and followed for 3 years with the primary endpoint being the development of diabetes. The lifestyle intervention arm in the DPP aimed at achieving and maintaining a 7% loss of initial body weight, 150 min of moderate aerobic activity weekly and completing a 16-week intensive education curriculum which included education on: a reduced calorie diet, exercise, and intensive lifestyle modification. The participants in the lifestyle arm were seen individually for education, dietary counseling, and coaching. The modest 7% weight loss goal was designated as this was deemed achievable by participants and would likely help prevent diabetes based on data from other studies [9–11]. The study participants were encouraged to try to attain this 7% weight loss within the first 6 months

Table 28.1 Prediabetes criteria [8]

FPG: 100–125 mg/dl (IFG)
OR
2-h PG 140–199 mg/dl after 75-g OGTT (IGT)
OR
A1C 5.7–6.4%

IFG impaired fasting glucose, *IGT* impaired glucose tolerance

of the trial by achieving a goal weight loss of 1–2 pounds/week. The caloric target was set by calculating the patients' daily caloric needs and then subtracting 500–1000 calories/day based on their initial weight. Participants were counseled to first restrict total dietary fat intake and then to concentrate on caloric reduction and limiting fat intake to 25% of total calories. The physical activity goal was derived to approximate 700–1000 kcal/week from physical activity which was consistent with the Surgeon General and Center for Disease Control Guidelines [12–14]. The protocol stressed "brisk walking" as a way to meet the exercise goal but alternative suggested aerobic exercise activities included biking, swimming, and dancing and up to 75 min of strength training was also permitted to attain the 150 min weekly goal [12]. The subjects were encouraged to allocate their exercise evenly over the course of the week and with a frequency of at least three times weekly with a minimum exercise duration of at least 10 min per session [12]. Participants who were sedentary were advised to gradually increase their activity in 30 min increments over 5 weeks.

The core elements of the DPP lifestyle protocol comprised: specific weight loss and exercise goals, individual case managers or coaches, consistent frequent follow-up with participants, a structured and standardized 16 session core curriculum followed by a more flexible maintenance program, supervised and guided exercise sessions, individualized coaching to overcome barriers to adherence by using a "tool box" of different techniques, materials relating to diverse ethnic populations as well as comprehensive local and national support to assist the lifestyle intervention teams [12].

The results of the DPP showed that intensive lifestyle intervention was significantly more effective than metformin in preventing the development of type 2 diabetes as the lifestyle intervention arm reduced the incidence of diabetes by 58% while metformin reduced the incidence by only 31% as compared with placebo [5]. The average weight loss in the DPP was 0.1, 2.1, and 5.6 kg in the placebo, metformin, and lifestyle intervention groups, respectively ($p < 0.001$) [5]. Therefore, a modest amount of weight loss translated into a significant reduction in the incidence of diabetes [5]. The results of the DPP were comparable to the Finnish study in that they both achieved a 58% reduction in the incidence of diabetes and were superior to the Chinese study which demonstrated a 42% reduction in diabetes onset through diet and exercise [5–7].

The long-term follow-up of these three landmark studies have also demonstrated a sustained reduction in the development of diabetes—39% at 30 years in the DaQing [15], 43% at 7 years in the Finnish Study [6], and 27% at 15 years in the Diabetes Prevention Program Outcome Study [16]. The 30 year Da Qing follow-up study showed a 35% lower risk of composite microvascular outcomes. However, the DPP and Finnish trials did not show a long-term benefit in terms of microvascular disease [6, 15].

Lifestyle intervention trials aimed at diabetes prevention have also been shown to improve cardiac and metabolic risk factors, specifically blood pressure, lipid levels, and inflammatory markers. In the original DPP, lifestyle intervention also demonstrated significant improvements in both systolic and diastolic blood pressure,

HDL cholesterol, and LDL particle size [17, 18]. In addition, positive changes in biomarkers of inflammation, coagulation, and endothelial dysfunction were seen [17, 18]. In a recent metanalysis of 17 studies, combined diet and physical intervention programs demonstrated an improvement both in blood pressure and lipid levels [1].

Diabetes Self-management Education and Support

Diabetes self-management education and support (DSMES) and medical nutrition therapy (MNT) are the cornerstones of successful diabetes management and are recommended as imperative for all patients with diabetes in the guidelines from the American Diabetes Association [19]. Diabetes self-management education and support (DSMES) refers to the recommended standard 10 h of diabetes self-management education (DSME) plus an additional program phase to provide further support for patients. This further support phase may be clinically focused but may also include psychological, educational or behavioral support. The over-arching objective of DSMES is to provide patients with the knowledge and skills they need so that they can optimally care for their diabetes as well as the ongoing support needed to manage their diabetes. The main goals of DSMES are to facilitate informed decision making, self-care behavior, and problem solving to improve clinical outcomes, health, and overall well-being [19].

The American Diabetes Association (ADA) certified DSMES program entails a patient-centered, structured curriculum with 10 h of either classroom or individual teaching sessions [20]. Traditionally DSME was offered in hospital clinic settings. However, with the evolution of health care delivery systems and greater demand for quality diabetes care, DSME sites have expanded to include outpatient clinic settings, medical homes, and cyberspace through virtual Telehealth technology [21, 22]. The comprehensive DSME core curriculum taught by certified diabetes educators [20] emphasizes self-care behaviors on the following topics: medications, physical activity, monitoring and using patient generated health data, prevention of complications, nutrition, risk reduction, coping strategies for stress reduction, and problem solving [23].

Numerous studies have demonstrated the multitude of benefits of DSME on health outcomes including improved: quality of diabetes care [24], knowledge of diabetes and self-care behaviors [25], glycemic control [26], psychological outcomes [27], and quality of life [28]. In addition, DSME has been shown to be cost effective with significant cost savings related to a lower rates of hospitalization, readmissions as well as long-term complications of diabetes [29–31]. Commercially insured patients and Medicare patients who participate in DSME cost, 5% ($p < 0.001$), and 14% less ($p < 0.001$), respectively, than those who do not participate [32]. Medicare and most commercial insurance providers usually reimburse for DSMES services.

Glycemic control clearly improves through DSME. Hemoglobin A1C (HbA1c) levels decline after completing a DSME program by 0.74–0.90% points [26, 33, 34]. This reduction is clinically significant as data from the U.K. Prospective Diabetes Study (UKPDS) demonstrate that each 1% reduction in HbA_{1c} over 10 years translates into risk reductions of: 21% for any end point related to diabetes, 21% for deaths related to diabetes, 14% for myocardial infarctions, and 37% for microvascular complications [35]. Studies consistently show a diminishing effect on A1C reduction as time elapses from the initial DSME intervention [26, 36, 37]. The largest reductions in A1C are seen when patients are engaged in more than 10 h of education and when they participate in both group and individual classes [34, 38]. In most studies, peak A1C reduction occurs at 3–6 months after the initial intervention with a waning effect over time [26, 33]. To rectify this attenuating effect on A1C reduction, the ADA advises ongoing DSMES and MNT follow-up annually and as needed [19]. According to the 2020 Standards of Medical Care in diabetes, DSMES as well as MNT are advised at four critical times: "at diagnosis, annually, when complicating factors arise and when transitions in care occur" [19].

DSMES remains an underutilized resource as data indicate that only 5–7% of eligible Medicare and commercially insured patients receive this very important service [39, 40]. To address this underuse of DSMES and improve care for patients with diabetes, a joint Consensus Conference convened which included the ADA, the Association of Diabetes Education Specialists, Academy of Nutrition and Dietetics among others and published recommendations highlighting the importance and value of DSMES as well as positive measures needed to facilitate, promote, and ensure utilization of DSMES [41].

Lifestyle Intervention Programs for Diabetes

Intensive lifestyle intervention (ILI) through a multicomponent behavioral approach has demonstrated success in achieving sustained weight loss [42], improved glycemic control [42], a reduction in requirement for antihyperglycemic medications [43], as well as remission from type 2 diabetes [44]. The DiRECT trial, an ILI trial in the UK, examined the durability of a primary care-based weight loss program in achieving diabetes remission which was able to sustain remission of diabetes in more than one-third of its participants with type 2 diabetes [44]. Both ILI and DSMES have been shown to have similar benefit in terms of glycemic control but ILI has been shown to be more effective in achieving weight loss and lowering body mass index [38]. Lifestyle and behavioral programs have been shown to benefit those who have suboptimal or poor glycemic control more than those who are in good glycemic control [38].

The Look AHEAD (Action for Health in Diabetes) trial was a large randomized controlled trial aimed at assessing the long-term cardiovascular effects of intensive lifestyle modification (ILI) in comparison with standard DSME delivered over 4

years in overweight or obese patients with type 2 diabetes [42]. Over 5000 patients, in 16 different national centers were randomized to either intensive lifestyle modification versus DSME with a follow-up plan.

The main objectives of the study were to achieve a 7% weight loss through calorie reduction and a structured exercise program [42]. The specific intervention strategies for the ILI included: a caloric goal of 1200–1800 kcal/day (with <30% from fat and >15% from protein), the use of meal replacement products and over 175 min weekly of moderate physical activity [42].

The intensive lifestyle intervention protocol was similar in design to the DPP [45]. The participants' characteristics included: obese or overweight individuals (mean BMI 36 kg/m^2) who were 45–75 years in age and had a mean baseline A1C of 7.25%. Those in the intensive lifestyle intervention arm received support by trained professionals such as lifestyle coaches who were either registered dietitians, psychologists, and/or exercise physiologists. They were seen for a total of four sessions monthly (three group and one individual) for the first 6 months and then three sessions (two group and one individual) monthly over the next 6 months. In years 2–4, participants were seen monthly in person and had an additional contact monthly via phone, email, standard mail or group class. The group sessions included the same 10–20 participants, lasted 60–75 min in duration and were comprised of: an individual private weigh in, self-reports of monitoring and personal goals, topic presentations, discussions on barriers to success as well as goal setting. The sessions were focused on weight reduction and emphasized weight setting goals, physical activity, and caloric intake. Strategies to reduce fat and calories were also highlighted. A toolbox method was used to help when patients had difficulty in attaining goals. For example, if patients were not meeting their weight loss targets, techniques such as motivational interviewing, problem solving, and behavioral contracts were employed. If these tools were not successful, additional advanced approaches were tried such as frozen meals, community classes or weight loss medication (orlistat).

In comparison, those in the DSME control group received three standard diabetes educational group sessions which concentrated on diet, exercise, and social support in years 1–4. Subsequently, they participated in one group diabetes education session yearly. The trial did not show a reduction in cardiovascular mortality or morbidity through ILI [42]. The overall cardiovascular events rates were low in both groups which is a point of controversy regarding the trial outcomes. Possible explanations for the low overall cardiovascular event rate include more aggressive treatment of risk factors in the control group as compared with the ILI group as more patients in the control group received statin therapy. Patients in the study were also likely more motivated and health conscious as they volunteered to participate and had to be able to meet certain fitness targets to qualify for participation. The mean A1C of both groups was just mildly elevated at 7.25% as compared with other ILI trials in which the A1C was higher at baseline [44, 46]. Meta-analyses demonstrate that patients in poor glycemic control derive more benefit from lifestyle intervention than those who are well controlled [38].

Despite the lack of difference in cardiovascular events in the Look AHEAD trial, multiple benefits were realized through ILI including superior weight loss (8.6 vs. 0.7% at 1 year and 6 vs. 3.5% at study end) as compared with the standard education group [42]. Participants in the ILI group also had greater reductions in waist circumference and improvement in fitness as compared with the standard intervention group (Fig. 28.2b, c) as well as greater reductions in A1C and all other cardiovascular risk factors other than LDL cholesterol [42]. Additional positive effects through ILI included: reductions in urinary incontinence, depression, sleep apnea and improvements in quality of life, mobility and physical functioning [42]. The improvement in quality of life was also associated with lower costs as there were fewer hospitalizations and medications [48].

Physical Exercise

"Physical activity is defined as all movement that increases energy use while exercise is defined as planned and structured physical activity" [49]. Exercise provides many benefits to patients with diabetes as it has been shown to improve glycemic control, decrease cardiovascular risk factors, promote weight loss, and improve quality of life [50]. Aerobic exercise entails physical activity of varying intensity that requires the large muscle groups and depends on the aerobic energy generating process [49]. Examples of aerobic exercise include walking, jogging, cycling, and dancing. In contrast, resistance exercise (or strength training), which is primarily anaerobic, is a form of physical activity that causes the muscles to contract against an outside force with an aim to increase strength. Resistance exercises include the use of free weights, resistance bands, or other exercise equipment [49]. Both aerobic and resistance exercises have been shown to improve glycemic control but the improvements are greatest with combined aerobic and resistance exercise [51, 52].

Mechanisms of Improved Glycemic Control

Regular, moderate aerobic exercise occurring over a prolonged period has been shown to have multiple beneficial effects on muscle function that promote a more efficient use of energy. These effects include an increase in the number of mitochondrial enzymes and "slow twitch" muscle fibers as well as the formation of new muscle capillaries [53]. In addition, there is an increase in the mobilization of insulin responsive glucose transporters (specifically, GLUT4) to the cell surface which promotes glucose uptake into skeletal muscle and thereby increases insulin sensitivity [54]. The addition of resistance exercise increases this positive effect on glucose disposal.

The benefits of exercise on glycemic control are strongest in patients with type 2 diabetes, thought to be due to the higher intrinsic level of insulin resistance in comparison to patients with type 1 diabetes. The A1C reduction is, on average, 0.5–0.7%points for those with type 2 diabetes engaging in regular exercise training [55–57].

Cardiovascular Risk Factor Reduction

Moderate to high amounts of regular aerobic exercise training in patients both type 1 and type 2 diabetes are associated with significantly lower cardiovascular and overall mortality [58]. In patients with type 1 diabetes, regular aerobic exercise has been shown to improve waist circumference, body mass index, cardiopulmonary fitness, insulin sensitivity, lipid levels as well as endothelial function [59, 60]. In patients with type 2 diabetes, regular aerobic exercise also improves triglycerides, blood pressure, body composition and insulin sensitivity [56].

Recommendations

Regular aerobic and resistance exercise should be prescribed for all patients with both type 1 and 2 diabetes to help improve their glycemic control and their overall health provided no contraindication exists such as moderate proliferative retinopathy or severe non-proliferative retinopathy [19]. The recommendations should be individualized according to each patient's age, health status, and presence of complications [19]. It is advised that most adults, particularly those who are sedentary, begin their exercise program gently and gradually increase to more vigorous activity. It is not recommended to screen asymptomatic individuals with an exercise stress test before they begin an exercise regimen. However, stress testing should be considered in those with a high cardiovascular risk (history of coronary artery disease, peripheral vascular disease, carotid artery disease or multiple complications from diabetes) [19].

The ADA, American Heart Association and the American College of Sports Medicine advise that most adults with Type 1 and Type 2 diabetes engage in a total of 150 min or more of moderate intense aerobic exercise weekly and that the activity should be spread over 3 days with no more than 2 successive days without activity [19, 61]. The aerobic exercise should be sustained for at least 10 min in duration to attain a goal of 30 min or more daily on most days of the week [19]. Patients should also be encouraged to engage in 2–3 sessions weekly of resistance exercise on nonconsecutive days [19]. In addition, all individuals should be advised to decrease the amount of time spent in prolonged sedentary activity (>30 min) by transiently standing, walking or performing other low intensity physical activities

for 3 min in duration [19]. Such transient activity was found to be associated with favorable effects on post-prandial glucose, C peptide, triglyceride levels, and insulin levels in adults with type 2 diabetes [62]. The ADA also advises older adults to engage in flexibility and balance training 2–3 times weekly and suggests participating in yoga or Tai Chi as both have been shown to increase flexibility, muscle strength and balance [19, 63, 64].

Weight Management and Behavior modification

Obesity is a complex, heterogenous disease, which is thought to arise from multiple etiologies including genetics and epigenetics as well as environmental, behavioral, developmental, and psychological factors. Obesity results from physiological dysfunction and not from simply energy imbalance as was formerly accepted. Obesity is also an independent and a major cardiovascular risk factor and is closely associated with other cardiac risk factors including diabetes mellitus, hypertension, and dyslipidemia. The prevalence of diabetes parallels the epidemic of obesity. Secular changes in our environment, diet quality, agricultural policies as well as lack of physical activity have contributed to the "diabesity" pandemic [65]. Some of the other modifiable cardiac risk factors include tobacco use, sedentary lifestyle, poor nutritional habits, and elevated blood pressure. Data from the Framingham Heart Study shows that individual risk factors act synergistically with one another increasing the risk of cardiovascular disease (CVD) by several fold. A multifaceted lifestyle approach is an effective means to reduce the risk factors that contribute to CVD [66].

Prevalence

The NHANES data show that the prevalence of obesity was 42.4% among U.S. adults in 2017–2018, with significant variation in racial incidence. The highest prevalence of obesity was among the non-Hispanic black adults compared with all other race and Hispanic-origin groups. The prevalence of obesity was lowest among non-Hispanic Asian adults (17.4%) compared with non-Hispanic white (42.2%) and Hispanic (44.8%) adults.

The age-adjusted prevalence of severe obesity among U.S. adults was 9.2% in 2017–2018. Women had a higher prevalence of severe obesity (11.5%) than men (6.9%) [67]. If these trends continue, it is estimated that by 2030 over half of American adults will have Obesity. The global economic impact of obesity is estimated at $2 trillion, which approximates 2.8% of the global domestic product [68].

Definition

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health as defined by the World Health Organization. Body mass index (BMI) is the most practical and inexpensive tool to assess body fat based on height and weight in an office setting. It is calculated using height and weight, BMI = body weight (kg)/height(m^2) [66]. The World Health Organization (WHO) and the International Obesity Task Force recommends the use of BMI to define and classify weight categories as shown in Table 28.2.

Quantifying total body fat is possible using specialized equipment like DEXA dual energy- X-ray absorptiometry and bioelectrical impedance but is difficult, costly, and not readily available. Distribution of fat is strongly and independently associated with CVD more than the BMI [69]. The assessment of abdominal obesity by waist circumference (men >40 in. and women >35 in.) confers an increased risk of cardiovascular disease. Excess bodyweight, in particular abdominal obesity, is a major contributor to other independent risk factors, making weight reduction the fundamental cornerstone in reducing the risk of CVD.

The linkage between obesity and CVD is not completely understood. In addition to the co-morbidities associated with obesity, adipose tissue remodeling results in metabolically active adipocytes releasing inflammatory markers that, in turn, increase oxidative stress and insulin resistance and lead to the development of metabolic syndrome, type 2 DM, and cardiovascular disease. Adiposopathy is defined as dysfunctional adipose tissue (or sick fat) in genetically and environmentally susceptible individuals predisposes patients to the development common of type 2 diabetes mellitus, high blood pressure, dyslipidemia, and increased CVD risk [70].

Table 28.2 Classification of overweight and obesity by BMI, waist circumference, and associated disease risks

			Disease risk[a] relative to normal weight and waist circumference	
	BMI (kg/m^2)	Obesity class	Men 102 cm (40 in.) or less Women 88 cm (35 in.) or less	Men > 102 cm (40 in.) Women > 88 cm (35 in.)
Underweight	<18.5		–	–
Normal	18.5–24.9		–	–
Overweight	25.0–29.9		Increased	High
Obesity	30.0–34.9	I	High	Very high
	35.0–39.9	II	Very high	Very high
Extreme obesity	40.0[b]	III	Extremely high	Extremely high

[a]Disease risk for type 2 diabetes, hypertension, and CVD

[b]Increased waist circumference also can be a marker for increased risk, even in persons of normal weight [5]

Abnormal and excessive accumulation of body fat results in increased risk of disease affecting multiple organ systems, impairing quality of life and resulting in premature mortality. Severe obesity can reduce life expectancy by an estimated 5–20 years [71].

Treatment of Obesity

Obesity is a complex, relapsing chronic disease that requires a personalized multidisciplinary approach. We now recognize that the long-held simple energy balance theory which prompted clinicians to advise patients to simply eat less and move more does not result in sustainable weight loss. Behavioral modification and/or lifestyle intervention has been an important part of weight loss programs for more than half a century [11, 72, 73]. Data from two large RCTs, the Look AHEAD trial and the DPP, support the efficacy of these approaches. There is strong evidence that a 5% weight loss is needed to achieve beneficial outcomes in glycemic control, lipids, and blood pressure in patients with diabetes who are overweight or obese [74]. According to guidelines released in 2013 by the American College of Cardiology (ACC), the American Heart Association (AHA), and The Obesity Society (TOS), clinically meaningful health improvements can even be seen with a 2–5% weight loss [73].

The causes and manifestations of obesity are very heterogeneous and this results in a wide variability in response to treatment. Four subtypes of obesity have been identified: diabetes with low HDL (Class I), disordered eating (Class II), mixed (Class III), and extreme obesity with early onset (Class IV) [75]. Based on patients' obesity subtype, individualized therapy including behavioral therapy, pharmacotherapy or bariatric surgery is recommended. Patients with disordered eating appear to have the best response to bariatric surgery [75].

Lifestyle modification, which includes physical activity, diet, and behavioral therapy, is the cornerstone of therapy. Modifiable negative lifestyle factors include chronic stress, poor nutrition, physical activity, and circadian disruption from sleep deprivation [76]. Research supports intensive lifestyle intervention as a means to achieving meaningful weight loss [11]. Additionally, in 2015, the Endocrine Society released new obesity treatment guidelines which stated that, "Diet, exercise, and behavioral modification should be included in all obesity management approaches for body mass index (BMI) of 25 kg/m^2 or higher. Other tools, such as pharmacotherapy for BMI of 27 kg/m^2 or higher with comorbidity or BMI over 30 kg/m2 and bariatric surgery for BMI of 35 kg/m^2 with comorbidity or BMI over 40 kg/m^2, should be used as adjuncts to behavioral modification to reduce food intake" [77].

Behavior Modification

Health behaviors such as eating patterns, physical activity, sleep hygiene, and effective stress management are under the complex influence of many psychological and social factors. Identification of common psychopathological diagnoses such as anxiety, depression, and disordered eating require consultation with mental health specialists for early treatment before patient engagement can be elicited. Using motivational interviewing skills, health care providers use intrinsic motivators to promote positive behavior change, improve confidence, and encourage achievable goals.

There are multiple behavioral change theories and models. Some of the key strategies in the behavioral treatment of obesity include encouraging patients to self-monitor, problem solve, set personal goals, and seek social support which all work synergistically to promote self-efficacy. Self-monitoring of physical activity and food intake is critical to successful behavioral therapy. Frequent self-weighing can be an important tool for weight regulation as it allows daily adjustment of caloric intake and energy expenditure to maintain balance [78]. Smart phone applications allow patients an easy way to monitor their caloric intake, exercise, and help with adherence to personalized goals. Some of the commonly used "apps" include Lose It, My Fitness Pal, Fitbit, Weight Watchers, and Cronometer. Similarly, wearable activity monitors show promising benefit in increasing physical activity and decreasing weight [79]. Social support is instrumental in pursuing desired personal health behavior goals. Goal setting is a process of identifying values and having goals that coincide with these values. One such technique is the SMART approach to ensure that goals are attainable, valued, and helpful to the individual. [SMART = *S*pecific, *M*easurable, *A*chievable, *R*ealistic, *T*imely]. Problem solving is helpful in brainstorming possible solutions when a barrier has been identified. Environmental restructuring involves identifying external cues that reinforce unhealthy behaviors [80]. The above-mentioned behavioral adaptations can only be implemented if there are no untreated psychological factors. It is well recognized that psychological distress and unmanaged stress can result in disordered eating.

In order to understand how these internal individual factors impact behavior, we must first review the physiology of how our brain is intricately involved in weight regulation. The hypothalamus is responsible for modulating hunger and energy metabolism. It receives signals from organs such as the pancreas, stomach, large and small intestines as well as adipose tissue via the vagus nerve which help modulate appetite and fat storage. In turn, individuals' behaviors are intricately regulated by the neuronal systems. Activation of the stress response can alter the hypothalamic regulatory systems. The so-called stress response represents an integrated reaction to stressors, broadly defined as real or perceived threats to homeostasis or well-being [81]. Prolonged psychological and environmental stress can result in changes within the brain via a cascade of immune, endocrine, and neural mediators which lead to a systemic effect on organ systems. Both major and minor stress occurring in daily life that can lead to health damaging behaviors. Over time, this

results in wear and tear on the body, termed the "allostatic load." This "allostatic load" is impacted by genetics, life experiences, as well as individual lifestyle habits and ultimately, through adaptation, regulates life-long patterns of behavior and physiological reactivity [82].

Chronic stress can induce changes in cells via inflammation, oxidative stress, and hypothalamic-pituitary -adrenal axis (HPA) dysfunction, and these key pathophysiologic processes link stress to negative health outcomes. Chronic stress can also alter health behaviors which can cause adverse health outcomes. This is demonstrated in a pilot study showing correlations of lifestyle factors with epigenetic aging as determined by DNA methylation. A higher BMI was associated with higher DNA methylation while physical activity was inversely associated with DNA methylation [83].

Stress impacts unhealthy eating behaviors as it can trigger body's reward system to seek high palatable foods containing large amounts of sugar and fat. Chronic stress has also been associated with higher levels of cortisol, which cause cravings of energy-dense palatable foods and contribute to abdominal obesity [84]. Chronic stress also has been associated with mental health disorders like depression and anxiety through dysfunction in HPA axis [85]. Obese adults have a 55% increased risk of developing depression over time. Conversely, depressed individuals have a 58% increased risk of becoming obese [86]. The binary relationship also exists with unhealthy eating and mood disorders. Unhealthy eating can result in diets that are high in saturated fats, which can contribute to dysphoria and intestinal wall leakiness, resulting in a change in the gut microbiome. It is thought that altered gut microbiome may contribute to symptoms of anxiety and depression. Mood disorders can lead to maladaptive changes in eating habits or "emotional eating" that may result in over-eating. As illustrated in Fig. 28.1, a complex interplay exists between mood disorders, neurotransmitters, hormones, disease states (such as cardiovascular disease and diabetes), and intra-abdominal fat.

Lastly, being overweight or obese is often stigmatized in American culture and this includes healthcare providers [87]. The notion that obesity is self-imposed and a behavioral condition has led to weight discrimination. Weight stigma is a common stressor that can lead to both disordered eating and a lack of motivation to adopt healthy behaviors. Fortunately, our stress response is modifiable, and an important target for health interventions. Prolonged activation of stress physiology impacts healthy behaviors, mood, and motivation. Inclusive of psychological support, are mind-body therapeutic strategies that evoke a "relaxation response." The relaxation response is the opposite of the stress response, which is a hypometabolic physiological state [88]. Mind body interventions activate the relaxation response by reducing sympathetic nervous system activation and increasing parasympathetic nervous system activity, and thereby restore homeostasis [89]. There are various mind-body therapies, like meditation, movement as well as adaptive coping strategies. The regular practice of such mind-body therapies can improve self-regulation skills. Regular exercise, adequate sleep, and healthy eating approaches (including mindful eating) help patients to manage stress and improve overall well-being.

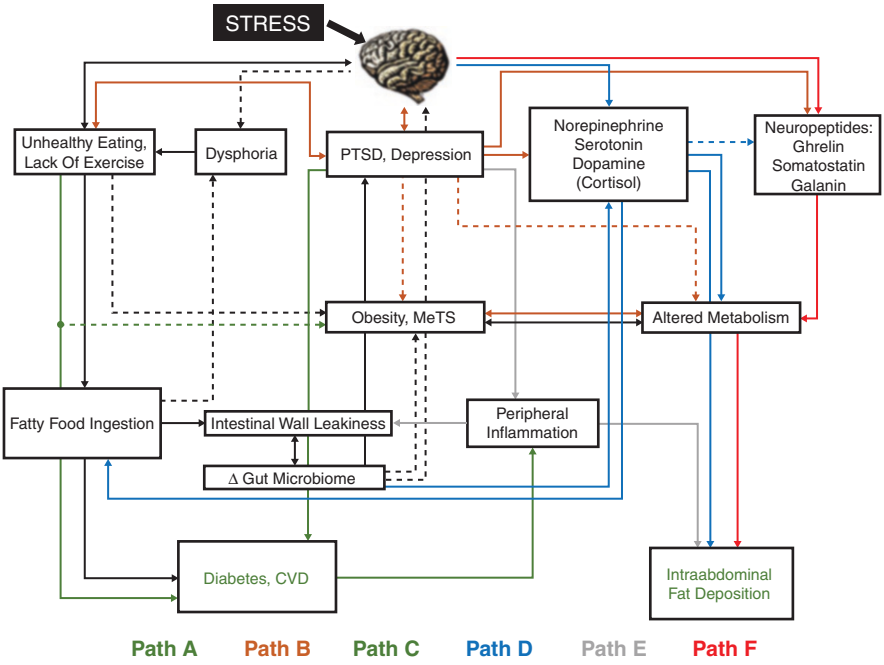

Fig. 28.1 Illustrates the intricate relationship between diet, obesity, and behavior. Stress acts through the brain to affect eating and exercise behaviors (Path A) and stress-related psychiatric disorders (Path B), both of which can lead to changes in metabolism, metabolic syndrome (MetS), and obesity (Paths A and B). Dual relationships also exist between unhealthy eating, PTSD/depression, and the brain. Diets high in saturated fat (Path A) negatively impact mood (dysphoria) and precipitate changes in the gut microbiome which thereby regulate obesity, MetS, and metabolism (Path A). Physical illness such as cardiovascular disease (CVD) and diabetes (Path C) as well as intra-abdominal fat (Path C) are affected by stress and cause inflammation. An elaborate system of neurotransmitters (norepinephrine, serotonin, dopamine) (Path D), inflammatory markers (Path E) and neuropeptides (ghrelin, somatostatin, galanin) (Path F) in the gut and brain are also affected by stress via the brain, influence gut microbiota and physical disease in a binary fashion and in turn modulate feeding behavior and psychiatric disorders. Within the figure, the line color indicates the path, with dashed lines indicating primary pathways and solid lines representing secondary pathways. *Nutrients* 2020, *12*(8), 2428; https://doi.org/10.3390/nu12082428

Positive psychology interventions have been used as effective tools to improve psychological well-being. These interventions are designed to enable patients to use their strengths to manage stress and emotions and thereby work toward healthy habits and lifestyle changes. Some of these interventions include an emphasis on positive emotions, optimism, self-compassion, and gratitude. A positive psychology-motivational interviewing Pilot for patients with type 2 DM with low physical activity demonstrated a moderate to large positive effect on both health behaviors and medical outcomes [90].

There are several online tools available (through use of smart phone applications or online websites) that can be helpful in screening patients for mood

disorders and unhealthy eating behaviors. Some of the questionnaires include Patient Health Questionnaire-9, Generalized Anxiety Disorder-7 as well as the Eating and Appraisal Due to Emotions and Stress questionnaire. Online group support is also available for patients seeking encouragement, accountability, and or motivation. Such groups include Weight Watchers, Overeaters Anonymous, and SparkPeople.com. There are several smart phone applications for mindfulness and meditation, including Insight Timer, Headspace, Calm, and Ten Percent Happier.

Medical Nutrition Therapy Guidelines for the Management of Type 2 Diabetes Mellitus and Cardiovascular Risk Factors

Background/Introduction Medical nutrition therapy (MNT) is an evidence-based application of the Nutrition Care Process and a key component of diabetes education and management. Medical Nutrition therapy for the management of diabetes in the clinical setting is delivered by a Registered Dietitian/Registered Dietitian Nutritionist (RD/RDN) [91]. MNT aims to prevent or slow complications of DM and manage comorbid cardiovascular risk factors. RDs/RDNs provide individualized, evidence-based nutrition and lifestyle counseling and recommendations to patients in an inpatient or outpatient setting through the practice of MNT.

MNT follows a structure defined by the Academy of Nutrition and Dietetics (AND) outlined in the Nutrition Care Process (NCP) [91]. The NCP steps include: Nutrition Assessment and Reassessment, Nutrition Diagnosis, Nutrition Intervention, and Nutrition Monitoring/Evaluation [91]. The Academy of Nutrition and Dietetics Position Paper: The Role of Medical Nutrition Therapy and Registered Dietitian Nutritionists in the Prevention and Treatment of Prediabetes and Type 2 Diabetes specifies assessment criteria, evidence-based nutrition interventions, coordination of care, and nutrition monitoring and evaluation considerations.

The Academy of Nutrition and Dietetics Evidence-Based Nutrition Practice Guidelines offer a structural outline on how to implement medical nutritional therapy in adults with diabetes. These guidelines suggest an initial series of 3–6 encounters with a RD/RDN lasting from 45 to 60 min within the first 6 months of diagnosis. There should be additional sessions as needed to be determined by the dietitian, and at least one annual follow-up encounter to reinforce lifestyle change and monitor and evaluate outcomes [91]. Despite the documented effectiveness of MNT and Diabetes Self-Management Education and Support (DSMES) intervention for diabetes management and typical coverage of care from Center of Medicare & Medicaid Services, national data suggests that only about half of patients with diabetes report having received some kind of diabetes education such as a DSMES program, and fewer are reported to see an RD for MNT [40].

Evidence for Medical Nutrition Therapy

Medical nutrition therapy for diabetes, alone or as part of a DSMES program, is shown to be effective in lowering A1c by up to 2% [91, 92]. Additionally, research supports that MNT may reduce saturated fat intake by 5–8%, reduce energy intake for weight loss between 232 and 710 kcal/day, and lower triglycerides by 11–31%, LDL Cholesterol 7–22%, and total cholesterol 7–21% [91].

The ADA Standards of Medical Care in Diabetes recommend all patients be assessed and referred for Nutrition Counseling, Diabetes Education, and emotional health counseling [91, 92].

Healthy Eating Patterns for Management of DM, ASVD, and Obesity

In 2019, The American Diabetes Association published, "Nutrition Therapy for Adults with Diabetes or Prediabetes: A Consensus Report" [92]. The ADA consensus report reviewed evidence-based dietary patterns and established guidelines for management of T2DM and ASCVD risk factors [93]. Eight eating patterns were evaluated for the report [92]. For a full description of these eating patterns and potential health benefits of each, we refer you to this report published by the American Diabetes Association [92]. Table 28.3 outlines the effects of different dietary patterns on cardiometabolic risk and cardiovascular disease. Factors common to several eating patterns align with the United States Department of Agriculture (USDA) Dietary Guidelines for Americans and include an emphasis on intake of non-starchy vegetables, consumption of whole foods rather than highly processed foods as well as replacement of sugar-sweetened beverages with water [93].

The USDA Dietary Guidelines for Americans, published every 5 years, promotes nutrient-dense foods and beverages. Nutrient-dense foods are defined as those which provide vitamins, minerals, and other health-promoting components and have little added sugars, saturated fat and sodium. Nutrition guidance, jointly issued from the American College of Cardiology (ACC) and the American Heart Association (AHA), mirror the USDA Dietary Guidelines for Americans. Additionally, lean animal or plant-based proteins are suggested by the ACC and AHA [95]. The Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE) 2020 consensus statement also recommends plant-based eating and support the Academy of Nutrition and Dietetics Position Paper on Vegetarian Diets which notes that vegetarians and vegans are at reduced risk of ischemic heart disease, type 2 diabetes, hypertension, obesity, and certain types of cancer [96].

Table 28.3 The effects of different dietary patterns on cardiometabolic risk, cardiovascular disease, cancer, and mortality [94]

Author	Diet	Year	MA (No of studies)	*N*	Results	Ref.
Nordmann	MedDiet	2011	Yes (6)[a]	2650	−2.2 kg weight; −0.6 kg/m^2 BMI; SBP—1.7 mmHg; DBP—1.5 mmHg; FPG—3.8 mg/dl; TC—7.4 mg/dl; hsCRP—1.0 mg/l compared with low-fat diets	Nordmann et al. (2011)
Qian	MedDiet	2016	Yes (24)[a]	2460	−1.56 kg weight; −0.57 mmol/l FPG; −0.31 mmol/l TG; +0.06 mmol/l HDL-C; −2.31 mmHg SBP compared with CHO-diets	Qian et al. (2016)
Liyanage	MedDiet	2016	Yes (6)[a]	10,950	A decrease of: 31% in CVD risk and 34% in IS risk	Liyanage et al. (2016)
Grosso	MedDiet	2017	Yes (29)[a]	–	A decrease of: 28% in CHD risk and 24% in IS risk	Grosso et al. (2017)
Dinu	MedDiet	2018	Yes (29)[a, b]	12,800,000	Significant ($p < 0.001$) risk reductions in all-cause mortality, CVD, CHD, MI, and T2D incidence. Significant ($p < 0.05$) risk reduction in certain site-specific cancers and inflammatory/metabolic parameters	Dinu et al. (2018)

(continued)

Table 28.3 (continued)

Author	Diet	Year	MA (No of studies)	*N*	Results	Ref.
Siervo	DASH	2015	Yes (20)[a]	1917	−0.2 mmol/l TC; −0.10 mml/l LDL-C; −5.2 mmHg SBP; −2.6 DBP mmHg	Siervo et al. (2015)
Maddock	DASH	2018	No	1409	High adherence led to lower BP ($p \leq 0.08$), higher HDL-C ($p < 0.001$) and lower TG ($p < 0.001$) levels, as well as reduced—0.28 PWV and—0.24 cIMT	Maddock et al. (2018)
Schwingshackl	DASH	2018	Yes (68)[b]	1,670,179	Association with a decrease of: 22% in CVD risk (incidence or mortality) and 22% in all-cause mortality	Schwingshackl, Bogensberger, and Hoffman (2018)
Chiavaroli	Portfolio	2018	Yes (7)[a]	439	A decrease of: 12% TC,17% LDL-C, 16% TG, 14% non-HDL-C; 15% apoB; 1% SBP; 2% DBP; 32% hsCRP and 10% 10-year CHD risk combined with a NCEP step-II dietary pattern	Chiavaroli et al. (2018)
Wang	Vegetarian	2015	Yes (11)[a]	832	−0.36 mmol/l TC; −0.34 l mmol/l LDL-C; −0.10 mmol/l HDL-C; −0.30 mmol/l non HDL-C	Wang et al. (2015)

Table 28.3 (continued)

Author	Diet	Year	MA (No of studies)	*N*	Results	Ref.
Dinu	Vegetarian	2017	Yes (10)[b]	–	Association with a decrease of: 25% in CHD mortality	Dinu et al. (2017)
Ramezani-Jolfaie	Nordic	2018	Yes (15)[a]	513	−0.38 mmol/l TC; −0.30 mmol/l LDL-C; −3.97 mmHg SBP; −2.08 mmHg DBP	Ramezani-Jolfaie, Mohammadi, and Salehi-Abargouei (2019)
Hu	Low-carbohydrate	2012	Yes (23)[a]	2788	−2.7 mg/dl TC; −3.7 mg/dl LDL-C; +3.3 mg/dl HDL-C; −14.0 mg/dl TG	Hu et al. (2012)
Gjuladin-Hellon	Low-carbohydrate	2019	Yes (8)[a]	1633	+0.08 mmol/l HDL-C; −0.13 mmol/l TG compared with low-fat diet	Gjuladin-Hellon et al. (2019)

Abbreviations: *apo A-I* apolipoprotein A1, *apoB* apolipoprotein B, *BMI* body mass index, *CHD* coronary heart disease, *CHO* carbohydrates, *cIMT* carotid intimamedia thickness, *CVD* cardiovascular disease, *DASH* dietary approaches to stop hypertension, *DBP* diastolic blood pressure, *FPG* plasma glucose, *HDL-C* high density lipoprotein cholesterol, *hsCRP* high sensitivity C reactive protein, *IS* ischemic stroke, *kg* kilograms, *LDL-C* low density lipoprotein cholesterol, *MA* meta-analysis; MedDiet, Mediterranean diet; *MI* myocardial infarction, *NCEP* national cholesterol education program, *PWV* pulse wave velocity, *Ref.* reference, *SBP* systolic blood pressure, *SFA* saturated fatty acid, *T2D* type 2 diabetes mellitus, *TC* total cholesterol, *TG* triglycerides
MA (No. of studies): This column shows if the evidence presented comes from a meta-analysis or not, with the number of studies included in each case in parentheses
[a]Meta-analysis of clinical trials
[b]Meta-analysis of observational studies

Evidence-Based Medical Nutrition Therapy Recommendations

Recommendations from the 2019 Nutrition Consensus Report and a summary of these are enumerated in Table 28.4 [97]. These recommendations were also integrated into the recent ADA Standards of Care in Diabetes [98].

Table 28.4 Summary of major nutrition practice guideline (NPG) recommendations from the Academy of Nutrition and Dietetics Nutrition Practice Guideline for Type 1 and Type 2 Diabetes in Adults [97]

Diabetes NPG recommendation		Rating
Fiber	Encourage fiber from foods such as fruits, vegetables, whole grains, legumes, as recommended by DRI[f] (21–25 g/day for adult women and 30–38 g/day for adult men) or USDA[g] (14 g/1000 kcal) due to overall health benefits	Fair, imperative
GI[h] and GL[i]	Advise that lowering GI or GL may or may not have a significant effect of glycemic control	Fair, conditional
Nutritive sweeteners	Educate that NS[j] when substituted isocalorically for other CHOs, will not have a significant effect on HbA1c[k] or insulin levels	Fair, imperative
	Advise against excessive intake of NS to avoid displacing nutrient-dense foods and to avoid excessive caloric and CHO intake	Fair, imperative
Nonnutritive sweeteners	Educate that intake of FDA[l]-approved NNS[m] (such as aspartame, sucralose, and stevia) within recommended intake will not have a significant effect on glycemic control	Weak, imperative
	Educate that substituting foods and beverages containing NNS can reduce overall calorie and CHO intake. However, other sources of calories and/or CHO in these foods and beverages need to be considered	Fair, imperative
Protein	Educate that adding protein to meals and snacks does not prevent or assist in the treatment of hypoglycemia. Ingested protein appears to increase insulin response without increasing glucose levels	Fair, imperative
	For adult with diabetic kidney disease, advise that a protein restriction is not needed. Protein intake (range = 0.7–2.0 g/day) had no significant influence on glomerular filtration rate	Strong, conditional
	For adult with diabetic kidney disease, advise that the type of protein (vegetable-based vs. animal-based) has no significant effect on glomerular filtration rate	Weak, conditional
Cardioprotective eating pattern	Encourage a cardioprotective eating pattern, within the recommended energy intake; decrease in saturated fat intake and increase in unsaturated fat shown to reduce total cholesterol and low-density lipoprotein cholesterol. Nonsignificant effect of differing amounts of saturated fat, unsaturated fat, and $n-3$ fatty acids on glycemia and insulin levels	Strong, imperative
Sodium	Individualized reduction in sodium intake. Recommendation to reduce to <2300 mg/day is appropriate. In context of hypertension, further reduction in sodium intake should be individualized	Fair, imperative

Table 28.4 (continued)

Diabetes NPG recommendation		Rating
Vitamin, mineral, and herbal supplements[n]	Advise that there is no clear evidence from benefit of supplementation in people who do not have underlying deficiencies; routine supplementation with antioxidants, other micronutrients (such as chromium, magnesium, and vitamin D), and herbal supplements (such as cinnamon) not advised	Fair, conditional
Alcohol[n]	When choosing to drink alcohol, advise moderation (1 drink per day or less for adult woman and 2 drinks per day or less for adult men). If using insulin or insulin secretagogues, alcohol can increase risk for delayed hypoglycemia	Weak, conditional

[f]FG fasting glucose
[g]To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.0555. To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0. Glucose of 108 mg/dL¼6.0 mmol/L
[h]TC total cholesterol
[i]NS nonsignificant
[j]HDL-C high-density lipoprotein cholesterol
[k]TG triglycerides
[l]Wt weight
[m]BDA British Diabetic Association
[n]CHO carbohydrate

Goals of MNT for DM and ASVD Risk

A summary of Goals of Nutrition Therapy from the American Diabetes Association Standards of Medical Care in Diabetes [98] are as follows:

- To promote and support healthful eating patterns which emphasize a variety of nutrient-dense foods in appropriate portion-sizes to improve overall health and to attain individualized glycemic, blood pressure, weight, and lipid goals.
- To address individual nutrition needs based on personal and cultural preferences, health literacy and numeracy, access to healthful foods, willingness and ability to make behavioral changes, and barriers to change.
- To maintain the pleasure of eating by providing nonjudgmental messages about food choices while limiting food choices only when indicated by scientific evidence.
- To provide an individual with diabetes the practical tools for developing healthy eating patterns rather than focusing on individual macronutrients, micronutrients, or single foods.

Personalized Nutrition Interventions

Carbohydrates

No consensus exists on the optimal amount of carbohydrate for people with diabetes [92]. Table 28.5 outlines recommendations from the American Heart Association (AHA), ADA and the Institute of Medicine on macronutrient intake [99]. Dietary strategies range from very-low carbohydrate to moderate or high carbohydrate recommendations [91, 92]. Evidence-based research and current nutrition and diabetes care standards advocate a reduced (low to moderate) carbohydrate dietary pattern [19]. However, carbohydrate targets and dietary patterns should be individualized [98]. Carbohydrate counting may be achieved by utilizing information from labels and nutrition databases or by using 15-g carbohydrate food lists (exchange system) or the plate method for carbohydrate estimation.

Food groups with appreciable carbohydrate include grains, vegetables (starchy and non-starchy), fruit, dairy products (excluding cheese), beans, and legumes [93]. Carbohydrates provide many essential nutrients and glucose for energy and are considered an essential part of a diet for managing patients with diabetes. The total amount and type of carbohydrate consumed at meals and snacks has an impact on glycemia, weight, and ASCVD risk factors [92]. Typically, dietitians discern between nutrient-dense carbohydrate foods and those with refined sugars which are of little nutritional value. Whole grains, fruits, vegetables, and legumes typically contain beneficial nutrients such as fiber, protein, and higher vitamin and mineral content and have less caloric and glycemic impact than refined sugars and processed foods. Added refined sugars are also known to negatively impact weight, triglyceride levels, and insulin action [91, 92].

Protein Protein is one of the three macronutrients which provide calories for the body and serves to enhance muscle strength and growth as well as influence satiety. Protein recommendations for DM and ASCVD revolve around balancing macronutrients after controlling for carbohydrate and fat. The recommended range of calories from protein are broad, and therefore should be individualized to the patients' lifestyle and health considerations. The Recommended Daily Allowance for protein is 0.8 g/kg, and the general protein values from the dietary reference intake (DRI) show a wide range of total calories from protein (from 10 to 35%) or 0.8–1.5 g/kg body weight [93]. Research indicates that diets on the higher-end of the DRI Protein range (1–1.5 g/kg body weight or 20–35% kcals) may have benefits in managing blood glucose and achieving and maintaining weight loss without negatively impacting renal function [100]. Other compelling research indicate that higher protein intakes up to 2.0 g/kg demonstrate positive changes in body composition and metabolism, attenuate muscle-mass loss during weight loss, and increase HDL cholesterol [101].

The ADA and AND affirm that protein has been shown to increase insulin response without increasing plasma glucose concentrations and that somewhat

Table 28.5 Dietary Guidelines to reduce chronic diseases[a] [99]

Nutrient	IOM	AHA	ADA	ACS
Carbohydrate	45–65% (≥130 g/day)	(1) Consume a diet rich in vegetables and fruit	45–65%[b, c] (≥130 g/day)	(1) Eat ≥5 servings of a variety of vegetable and fruit each day
		(2) Choose whole-grain, high-fiber foods		(2) Choose whole grains over refined grains
		(3) Minimize intake of beverages and foods with added sugars		
Protein	10–35% (0.8 g/kg)	(1) Use lean cuts of meat and remove skin from poultry	10–35% (≤20% if diabetic)	(1) Limit consumption of processed and red meats
		(2) Consume fish, especially oily fish, at least twice a week		(2) Choose fish, poultry, or beans as an alternative to beef, pork, and lamb
Fat	20–35%	25–35%[d]	20–35%[c]	NR
Linoleic acid	5–10%	NR	NR	NR
α-Linolenic acid	0.6–1.2%	NR	NR	NR
Saturated fat	As low as possible	<7%	Normolipidemic: <10%	NR
			Hyperlipidemic: <7%	
trans-Unsaturated fat	As low as possible	<1%	As low as possible	Consume as few *trans* fats as possible
Cholesterol	As low as possible	<300 mg/day	Normolipidemic: <300 mg/day	NR
			Hyperlipidemic: <200 mg/day	
Dietary fiber	Women: 25 g/day	Increase fiber intake by eating beans (legumes), whole-grain products, fruit, and vegetables	14 g/1000 kcal	Increase fiber intake by eating beans (legumes), whole-grain products, fruit, and vegetables
	Men: 38 g/day			

[a]*IOM* Institute of Medicine (1), *AHA* American Heart Association (2, 5), *ADA* American Diabetes Association (3, 6), *ACS* American Cancer Society (4), *NR* no specific recommendation
[b]Whole grains, fruit, vegetables, and low-fat milk as the primary sources
[c]Carbohydrate + monounsaturated fat should provide 60–70% of energy
[d]Use liquid vegetable oils in place of solid fats

higher intake levels (1–1.5 g/kg) are associated with a higher risk for renal impairment. Recommendations suggest individualization of protein intake directives for persons with diabetes within the DRI [91]. MNT recommendations focuses on the protein source and meal composition as important factors for rather than absolute protein quantity. The USDA and the Health and Human Services Dietary Guidelines

2015–2020 emphasize more nutrient-dense (lean) protein sources including foods from both animal and vegetable sources such as seafood, meat, poultry, eggs, nuts, soy, and high-protein mixed macronutrient foods like reduced-fat dairy, beans, and legumes. Lean proteins are defined as (<2–3 g fat/serving), such as skinless, white meat poultry, fish, beans/legumes, lean cuts of beef such as sirloin steak, >90% lean ground beef, or tenderloin cuts of beef and pork.

The American Association of Endocrinologists (AACE) and the American College of Endocrinology (ACE) 2020 Consensus Statement also recommends plant-based eating for T2DM [94]. The RDN may suggest gradual introduction of meatless meals once weekly and/or provide simple recipes for dishes that incorporate beans, lentils, chickpeas, tofu or other soy meat substitutes.

General guidance regarding protein intake for persons with diabetes includes consuming carbohydrate food sources alongside protein foods (or choosing high protein CHO mixed foods) which mitigates the post-prandial blood glucose response and helps persons with diabetes attain greater satiation, especially when pursuing a caloric deficit [92]. Recommendations also note that consuming lean protein and or high-quality fat food sources in place of carbohydrate foods, particularly in place of low-quality CHO, can improve weight loss and the glycemic response [100].

Dietary Fat

Fat plays many important roles within the body including serving as an efficient alternate energy source when glucose is limited [101]. Dietary fat intake influences DM and ASCVD in several ways. The food sources of fat (and carbohydrate) that we consume effect plasma lipids. LDL cholesterol is most influenced by saturated fatty acid (SFA) intake and is highly atherosclerotic; whereas HDL, from mono- and polyunsaturated fatty acids (MUFA/PUFA), has beneficial effects on blood cholesterol levels and is considered to be anti-atherosclerotic. Elevated triglycerides may be related to intake of refined sugars as well as dietary saturated and transfat intake and contribute to the condition of fatty liver which exacerbates insulin resistance. Finally, trans fats are a highly detrimental form of fat that can trigger inflammatory markers, contribute to the onset and progression of atherosclerosis, and interfere with other important metabolic and cellular functions [102]. Food sources of saturated fat include fatty meats, butter, cream/milk fat, cheeses made from whole milk, palm and coconut oil, and cocoa butter. Trans fat sources include hydrogenated oils found in commercial baked goods and deep-fried foods.

Mono and polyunsaturated fats positively influence amount of HDL vs. LDL cholesterol and are known to have anti-inflammatory properties for improved overall health and mitigation of disease risk when consumed in place of SFA [91]. Monounsaturated fats (MUFAs) contain one double-bond and are shown to reduce vascular inflammation and contribute to greater synthesis of HDL cholesterol particles vs. LDL. MUFA food sources include certain oils such as olive oil, canola, safflower/sunflower, as well as nuts and avocado [91].

Polyunsaturated fatty acids (PUFAs) contain two or more double bonds and have the same benefits of the anti-inflammatory properties as MUFA. Within PUFA, two subgroups exist, omega-3 and omega-6 fatty acids (FA). Omega-3/6 FA are recognized as being particularly pro-health, with omega-3 FA in particular having a substantial impact in reducing ASCVD and associated DM risk. Omega-3 FA include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic Acid (ALA). Food sources of omega-3 FA include flaxseed/oil, fatty fish such as salmon, sardines, mackerel and herring and soy foods (tofu/soybean oil), as well as walnuts, sunflower seeds, and oils with higher omega-3 PUFA content including flaxseed and cod liver oil. Physical activity from cardiovascular and resistance exercises can significantly increase HDL and reduce LDL cholesterol, while assisting with weight loss and glycemic control [19]. Additionally, the vitamin niacin has been shown to be effective in increasing HDL cholesterol.

Eating plans such as the Mediterranean approach which replace foods high in saturated and trans fats with those rich in monounsaturated and polyunsaturated oils should be recommended.

For optimal outcomes in the management of T2DM and ASCVD risk factors, it is desirable to have lower levels of LDL cholesterol (<100 mg/dl for primary prevention and <70 mg/dl in high risk patients), higher levels of HDL cholesterol (>40 mg/dl in men and >50 mg/dl in women), and moderate total cholesterol (<180 mg/dl) and triglyceride (TAGs) levels (<150 mg/dl). To achieve these targets, MNT aims to educate the person with diabetes on which food sources negatively impact blood lipids, weight, and overall health and which have a positive impact.

Fiber Fiber is a component in carbohydrate foods which provides many benefits when consumed in adequate amounts as a part of a healthy diet for management of DM and ASCVD. Fiber, due to its structure, passes through our digestive tract mostly undigested and therefore has little to no impact on blood glucose. There are two major types of fiber from food: soluble fiber and insoluble fiber. Soluble fiber absorbs water in the gut and promotes feelings of fullness and satiety; additionally, soluble fiber binds with some cholesterol (including LDL) in the gut during transit and thereby has a positive effect on lowering cholesterol [100]. Insoluble fiber is not soluble in water and provides bulk or "roughage," which during digestion can help increase bowel regularity and make stool easier to pass. Additionally, higher fiber content in complex CHO foods can delay the absorption of glucose into the blood stream and precipitate a more stable post-prandial glycemic response. These health properties are beneficial for weight loss and appetite control as well as managing blood glucose and ASCVD risk factors. As part of a diet for patients with Diabetes and ASCVD risk factors, 25–35 g of fiber are recommended from food sources containing soluble and insoluble Fiber [103]. MNT recommendations promote high-fiber food sources such as whole grains, vegetables, and fruits, nuts, and beans and legumes. These foods also contain other pro-health components such as vitamins, minerals, protein, and heart-healthy fats and are typically lower in calories than other food choices. Good sources of fiber have at least 3–5 g of fiber per serving.

Omega-3 Supplement/Fish Oil Studies have shown that an adequate intake of omega-3 FA (FA) has a favorable effect on hypertension and blood vessel dysfunction and at therapeutic doses may reduce TAGs and reduce inflammation [100]. The omega-3 FA EPA and DHA are found mostly in fish oils (marine sources) whereas ALA omega-3 acids come primarily from plant sources such as flax, nuts, and vegetable oils. Studies show that marine fish oils EPA/DHA may have more significant impact on CVD risk factors and that standards diets do not provide enough [104]. Fish oil supplements in pill form are typically a blend of EPA/DHA. Studies show that fish oil may be most effective at lowering triglycerides (TAGs) vs. lowering LDL or raising HDL cholesterol [104]. A summary of research from the AHA suggests that omega-3 FA supplementation from fish oil may be effective in lowering TAG in persons with levels above 500 mg/dl and that 4 g of prescription grade Omega-3 FA may lower TAG levels by 20–30% [104]. Medical Nutrition Therapy guidelines advise individuals to consume adequate omega-3 EPA and DHA from (fatty) fish, i.e. 2–3 servings per week and/or by following a Mediterranean diet pattern [92].

Weight Management in MNT for Diabetes and ASCVD Excess adiposity and obesity play a role in the etiology of Insulin resistance of as well as in ASCVD risk factors and may negatively impact ability to perform physical activity, which in turn influences weight and disease outcomes [100]. Weight loss via a reduced caloric intake and/or caloric expenditure while maintaining a healthy eating pattern is recommended for persons with diabetes who are overweight or obese. A modest weight loss of 5–7% has been shown to have significant clinical benefits including improved glycemia, blood pressure, and lipids [92]. Greater improvements in glycemia and cardiovascular health are seen when weight loss of 10% or greater is achieved in individuals with morbid obesity (BMI > 40) [100]. Weight management guidelines indicate that there is no one dietary strategy superior for weight loss, rather any dietary intervention which provides a caloric deficit initially and which the individual patient is likely to be able to follow long-term is best [91]. In MNT, RDs consider the physical and psychosocial status of the patient and collaborate with them to develop a dietary intervention which suits the patient's goals, personal preferences, and cultural background [19]. The RDN may advocate portion control, structured meal plans or meal replacements to aid with weight loss [100]. They also educate patients on healthy eating and personalized nutrition targets for weight loss, and advocate adherence and skill-building with self-monitoring strategies such food and physical tracking, goal setting, and meal-planning [19]. To achieve healthy weight loss which is generally defined as 0.5–2 lbs per week, it is appropriate to advise daily caloric deficits ranging between [−250–1000 kcals] [100].

These targets should be individualized based on patients' current anthropometrics, eating patterns, weight, health history, and personal preferences. General caloric estimates for nutritionally adequate weight loss are 1200–1500 kcals/day for women and 1500–1800 kcals/day for men [103]. The caloric deficit may be achieved through healthier food choices, portion control, diet patterns which restrict foods or

macronutrients, and/or an increase of caloric expenditure from physical activity [90, 91]. RDNs coordinate with an interdisciplinary care team regarding successful weight loss outcomes for patients. The key team members may include behavioral health therapists, endocrinologists, and bariatric surgeons if surgical intervention is warranted.

Summary MNT involves a systematic process of assessment, diagnosis, intervention, monitoring and evaluation according to the principles of the Academy of Nutrition and Dietetics' Nutrition Care Process [91, 92]. RDs lead patient-centered, individualized, MNT for patients with T2DM and associated ACVD risk factors independently or as part of DSME-S programs [92]. The goals of MNT in patients with DM include attaining and maintaining: glycemic and cardiovascular targets, a healthy diet, as well as the adoption of behaviors which support lasting positive lifestyle change [91].

The cardioprotective dietary plan is one such that encompasses multiple dietary strategies for improved health [100]. The advised pattern of healthy eating includes a greater intake of fresh, high-fiber foods, and MUFA/PUFA fat sources and limiting the intake of saturated fat, sodium, simple carbohydrates as well as high-fat meat and dairy products. Increased physical activity, smoking cessation, and calorie-control complete the advised plan. Ultimately, MNT led by RDs can help individuals with diabetes manage multiple DM and ASCVD risk factors through collaborative development of individualized nutrition and lifestyle plans which improve disease outcomes and lead to lasting health behavior change.

Diabetes Technology

Diabetes is a medical condition requiring a high degree of self-management and daily decision making. Technological advances have given people with diabetes tools to help achieve and maintain optimal glucose control while relieving some of the burden. The use of technology in the management of diabetes has increased significantly, prompting The Endocrine Society to form a task force to set evidenced-based practice guidelines [105]. Similarly, the American Diabetes Association introduced diabetes technology as a section in its yearly Standards of Care Medical Care in Diabetes Guidelines [106]. The latest innovations are in the areas of insulin delivery, glucose monitoring, and the development of mobile applications.

Insulin delivery devices have expanded from vials and syringes to insulin pens which allow for simpler, portable, and more user-friendly insulin administration [107]. Newer "smart" or "connected" insulin pens have been on the market since 2017, starting with Companion Medical's launch of the InPen™ [108]. This blue tooth enabled pen device can be programmed to deliver a precise dose of insulin based on individual carbohydrate ratios, insulin sensitivity factor, target blood glucose, and active insulin on board [108]. The inclusion of active insulin calculated

from a previous dose is particularly helpful to prevent stacking of insulin doses which can result in hypoglycemia. The device keeps track of all insulin and glucose data including timing of insulin in relationship to meals and insulin dose. It can be paired with a mobile application on a smartphone for convenient viewing of data [108]. Data is also transmitted to a personal cloud-based account allowing for review by the patient and, with permission, the provider. Reminders for meal and basal insulin doses can be set to improve adherence. This is an important feature as the hemoglobin A1C can rise by as much as 0.4% with only two missed doses in a 7 day period [109].

Continuous subcutaneous insulin infusion (CSII) pumps became available in the 1970s and have been transformed from large bulky devices to sleeker Bluetooth enabled insulin delivery systems [110].

A CSII pump delivers small doses of rapid acting insulin every few minutes and can be programmed to deliver variable amounts of basal insulin for different time blocks in a 24-h period, along with individualized carbohydrate ratios, sensitivity factor, and glucose targets while taking into account active insulin onboard.

Sensor augmented insulin pumps are newer version of insulin pumps [111], paired with a continuous glucose sensor and programmed to automatically reduce or suspend insulin delivery when a predetermined level of glucose is reached or predicted [110]. In 2013, Medtronic introduced the Minimed 530 G™ with the Enlite Sensor™ as the first sensor augmented pump on the market [110–112]. Tandem later came out with a sensor augmented pump called the T Slim X2 using Basal IQ™ software [113]. These pumps can be individualized by creating different programs for exercise or changes in workday and weekend schedules [110, 111].

Hybrid closed-loop systems are the latest version of insulin pumps, introduced as "artificial pancreas" systems [111]. These pumps are different from sensor augmented pumps in that they will both reduce and/or increase basal rates based on predicted glucose sensor data. Medtronic released 670G insulin pump in 2017 and next generation Bluetooth enabled 770 G in 2020- both using a program called Smartguard™ Automode™ [114]. An updated version 780G which can deliver automated correction boluses is expected to be available by mid-2021. Tandem's hybrid closed-loop systems, T Slim X2™ pump using Control IQ™ software was released in February 2020 [113]. This pump is further advanced in that in addition to adjusting basal rates it will also deliver an automatic insulin correction when glucose readings are predicted to be above 180 mg/dl [113]. The T Slim X2 insulin pump can be paired with the t:connect™ mobile app which allows viewing of real-time glucose and insulin data which is automatically uploaded hourly to a secure, cloud-based portal. Insulin delivery can be further individualized by setting an exercise or sleep mode. The exercise mode will change the target glucose from 112 to 140 mg/dl to reduce the risk of activity induced hypoglycemia. The sleep mode can be programmed by hour and day of the week with an algorithm that adjusts basal rates to keep glucose in the target range of 112 mg/dl [113]. The main advantage of this type of insulin delivery is to increase blood glucose time in target with fewer episodes of hypoglycemia [111].

Personal continuous glucose monitors (CGM) were first introduced in 1999 as an adjunct to fingerstick blood glucose readings [115]. In 2018 the FDA approved the DexCom G6 CGM as a stand-alone device with no requirement for a confirmatory fingerstick blood glucose reading to make insulin dosing decisions [116]. These sensors are placed on the arm, leg or abdomen and measure glucose in interstitial fluid every 5 min. Glucose readings can be viewed in real time on a receiver, mobile phone or smart watch. This information allows for timely adjustments in insulin based on activity or food intake. This is of particular importance in identifying hypoglycemia, a risk when intensifying treatment to achieve BG target or for those with hypoglycemic unawareness [116]. Wearing a CGM has been shown to improve glycemic control, reduce incidence of hypoglycemia, and decrease diabetes distress [111, 112, 117].

Two real-time CGM devices are the G6 DexCom sensor™ [118] and the Medtronic Guardian™ Sensor [107]. These CGMs can be programmed with audible alarms triggered by both low and high blood glucose levels to allow for proactive treatment of hypoglycemia or hyperglycemia. Both can be used with a mobile phone application which allows for real-time display of glucose readings [114, 118]. In 2017 Abbot introduced the Freestyle LibreFlash™ and in 2020 the LibreFlash 2™ [119]. These CGMs record glucose levels every 5 min but must be scanned using a reader or smart phone in order to view glucose level [119]. One scan will record the previous 8 h of glucose data so scanning every 8 h will provide 24 h of continuous glucose data. Both can also be paired with the FreeStyle LibreLink tm mobile application for convenient glucose access on a smart phone [119]. Continuous glucose monitors provide real-time glucose trends and have greatly reduced the need to perform fingerstick blood glucose readings.

The information obtained from continuous glucose sensors has transformed the understanding of glycemic control beyond the quarterly hemoglobin A1C. New glucose metrics include time in range (TIR) and the glucose management indicator [120]. In 2017 the concept of time in range (TIR) was proposed by the International Consensus on the Use of Continuous Glucose Monitoring [120]. It is defined as the percentage of time spent in the target glucose range of 70–180 mg/dl [120, 121]. ADA proposed that TIR should be adjusted based on diabetes type and duration, age groups and co-morbidities, hypoglycemic unawareness or pregnancy [120]. A person without co-morbidities should achieve TIR of greater than 70% as this translates to an A1C of approximately 7% [120].

The Glucose Management Indicator (GMI) is based on 10–14 days of sensor data and calculates an estimated A1C based on the mean plasma glucose [120]. It is part of the Ambulatory Glucose Profile and is reported in the DexCom, Freestyle Libre, and Guardian 3 sensor reports [120]. Unlike the A1C, the GMI does not rely on the hemoglobin molecule so is not altered by factors that may affect the red blood cell turnover or glucose binding affinity such as anemia or the presence of a hemoglobinopathy [120, 122, 123]. Using these metrics along with the traditional A1C can guide more timely changes in diet, activity, and/or medication regimen (see Fig. 28.2).

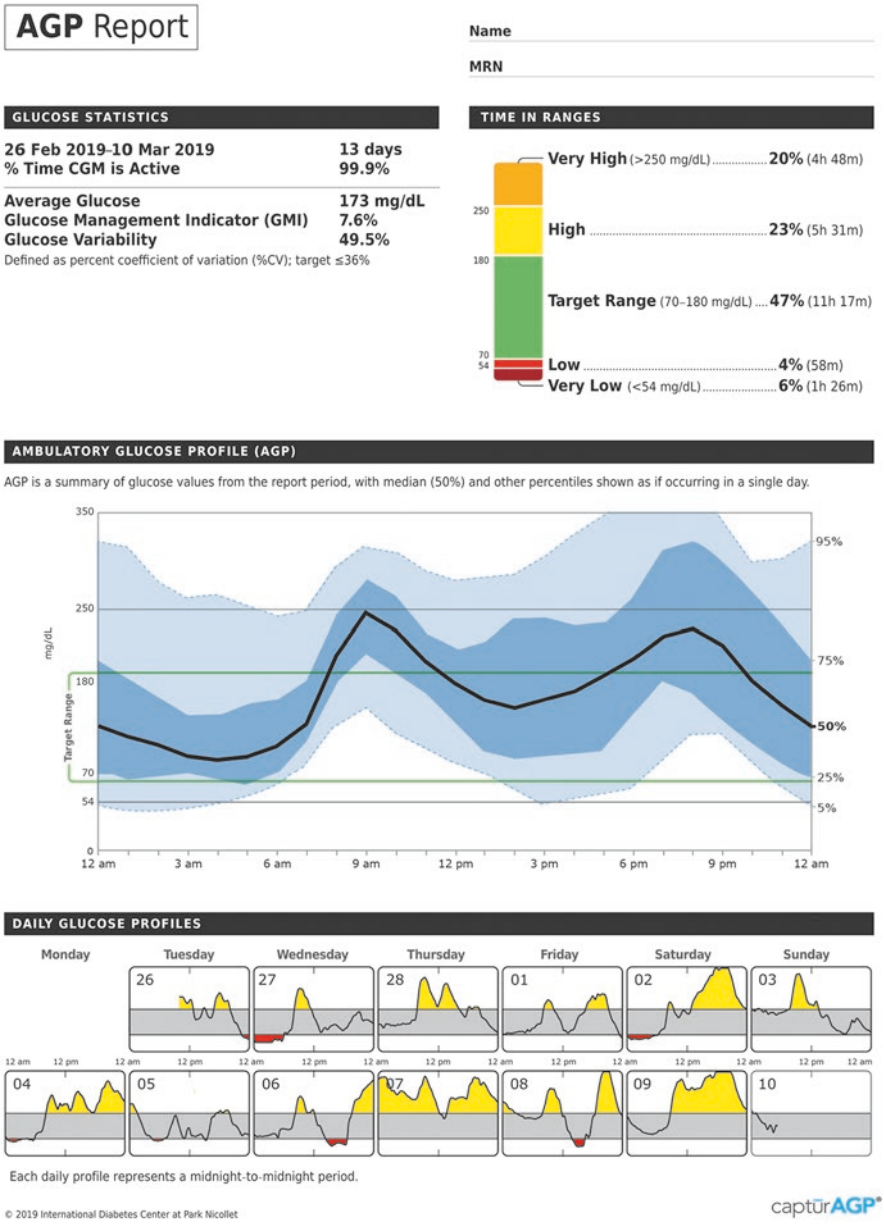

Fig. 28.2 Ambulatory Glucose Profile (AGP) [47]. Johnson ML, Martens TW, Criego A et al. Utilizing the Ambulatory Glucose Profile to Standardize and Implement Continuous Glucose Monitoring in Clinical Practice. Diabetes Technol Ther. 2019 Jun;21(S2):S217–S225. https://doi.org/10.1089/dia.2019.0034

Technology has revolutionized diabetes management and can reduce the associated physical and emotional burden. Automated insulin pumps, continuous glucose sensors, and the use of mobile applications can improve diabetes self-management and help people with diabetes achieve and maintain glycemic targets [124–126]. The future is promising for further developments that will assist patients and providers toward optimal diabetes control.

Smoking Avoidance and Cessation of Smoking

It is well established that active smokers with diabetes as well as individuals with diabetes exposed to passive smoke are at high risk for cardiovascular disease, premature death, microvascular complications as well as poor glycemic control [127, 128]. Though studies have clearly shown an increased risk of diabetes occurring after smoking cessation due to weight gain [129, 130], a recent large prospective cohort study demonstrated that when smoking cessation is accompanied by substantial weight gain, there is a short-term increased risk of developing type 2 diabetes but this does not mitigate the benefits of smoking cessation on reducing cardiovascular and all-cause mortality [131]. This study also demonstrated further evidence that improving diet quality and increasing physical activities assist those who are trying to stop smoking to achieve their weight maintenance goals. This finding of a reduction in cardiovascular mortality after smoking cessation is concordant with other studies [132, 133]. Most excess cardiovascular risk is known to be eradicated within the first few years after smoking cessation [134].

Multiple studies have also implicated smoking as a possible risk factor for the development of type 2 diabetes [129, 135–137]. Studies have shown that active smoking is associated with a 40% increased risk of incident type 2 diabetes [129, 137]. This association was found to be graded and independent of confounding factors. In a meta-analysis across 25 prospective cohort studies, the risk of developing diabetes was greatest for heavy smokers (>1 pack per day; relative risk 1.61) compared with lighter smokers (relative risk 1.29) or former smokers (relative risk 1.23) [138]. In one study, exposure to passive smoke (second-hand smoke) was also associated with an increased risk of diabetes [137].

Possible biological mechanisms for this strong association include studies that show smoking contributes to insulin resistance, impaired insulin secretion as well as an impaired response to an oral glucose load [138, 139]. Smoking has been associated with greater abdominal fat deposition and a higher waist to hip ratio and this may explain the impaired response to an oral glucose load [131]. Smoking has also been linked to chronic pancreatitis and pancreatic cancer which has been thought possibly related to a toxic effect of nicotine or other components of smoke on the pancreatic beta cells [140]. However, there are also plausible non-causal explanations for the association between smoking and incident diabetes as smoking is often associated with other unhealthy lifestyle behaviors that predispose patients toward

weight gain and diabetes such as inadequate physical activity, excessive alcohol intake, and a poor diet [141, 142].

Given the morbidity and excess mortality associated with smoking and diabetes, the ADA guidelines emphasize routine and comprehensive evaluation and assessment of tobacco use as critical to prevent smoking and promote smoking cessation [19]. If smoking or e-cigarette use is identified, smoking cessation counseling as well as other forms of treatment are strongly advised [19]. Pharmacologic treatment has been shown to be effective in helping to promote smoking cessation [143]. In motivated patients, combined counseling and pharmacologic therapy has been shown to be superior to either method alone [144]. Positive lifestyle measure such as an improvement in diet quality and an increase in physical activities help those who are trying to stop smoking minimize weight gain [131]. Long-term smoking cessation trials have demonstrated the benefits of increasing physical activities on minimizing weight gain after quitting smoking [145, 146].

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Chapter 29
Treatment: Lifestyle and Medication

Ahmed Khan and Osama Hamdy

Introduction

Type 2 Diabetes (T2D) is often associated with overweight or obesity. Exercise, dietary intervention, and behavior modifications are strongly recommended for patients with type 2 diabetes and have been associated with significant improvement in insulin sensitivity, endothelial function, and improvements in several markers of inflammation and coagulation.

Weight Management in Patients with Type 2 Diabetes and Obesity

Weight Management: A Clinical Approach

Obesity and T2D are two pathologic conditions that are strongly related. Management of both diseases requires a multidisciplinary approach in which the initial step is recommending lifestyle modifications. Many primary care physicians struggle to address or implement lifestyle modifications for their patients, ultimately leading to the initiation and then intensification of diabetes pharmacotherapy. An unfortunate side effect of some of the most commonly used antihyperglycemic agents is weight gain leaving patients in a detrimental cycle between regulating their body weight and controlling their diabetes.

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Conversely, a 7% reduction in body weight through lifestyle interventions showed a significant increase in insulin sensitivity. The Diabetes Prevention Program (DPP) demonstrated that weight loss over a period of 3 years helped individuals with pre-diabetes and obesity to reduce the risk of developing type 2 diabetes by 58%. Also, the Action for Health in Diabetes (Look AHEAD) study reported that lifestyle modification improved glycemic control in addition to lowering body weight among patients with type 2 diabetes. Participants in the lifestyle intervention arm of the look AHEAD study also showed that they were on fewer medications for diabetes, hypertension, and dyslipidemia, had fewer hospitalizations, and reduced risk for chronic kidney disease and depression when compared to the control arm of the study.

Multidisciplinary Approach to Weight Management

Multidisciplinary weight management is recommended by most medical societies for patients with obesity and type 2 diabetes. Data from the National Weight Control Registry showed that narrow approaches to weight management are rarely effective but that a broader, multifaceted approach is more sustainable.

In 2005, The Weight Achievement and Intensive Treatment Program (Why WAIT) of the Joslin Diabetes Center was created as an effective multidisciplinary model for weight management in real-world clinical practice for patients with diabetes and obesity. The program comprises 12 weeks of intensive lifestyle intervention during which participants are engaged in weekly group intervention followed by monthly follow-up sessions to help them maintain weight loss for the long term (Fig. 29.1).

Components of Multidisciplinary Approach

Medication Adjustment

Many of the currently used antihyperglycemic medications are known to cause weight gain (e.g., insulin, sulfonylureas, glinides, and thiazolidinediones). For optimal weight reduction through a multidisciplinary approach, healthcare providers should reduce, substitute, or even stop, whenever possible, these medications at the beginning of the weight management program. They may be substituted with medications that are weight neutral or by those that enhance weight loss (e.g., metformin, DPP-4 inhibitors, α-glucosidase inhibitors, GLP-1 analogs, SGLT-2 inhibitors, and pramlintide). Furthermore, approved anti-obesity medications (e.g., naltrexone/bupropion, topiramate/phentermine, liraglutide, or semaglutide) are encouraged for certain patients to control their appetite. Patients on insulin may be switched to long-acting insulins that induce less weight gain (e.g., insulin detemir, insulin

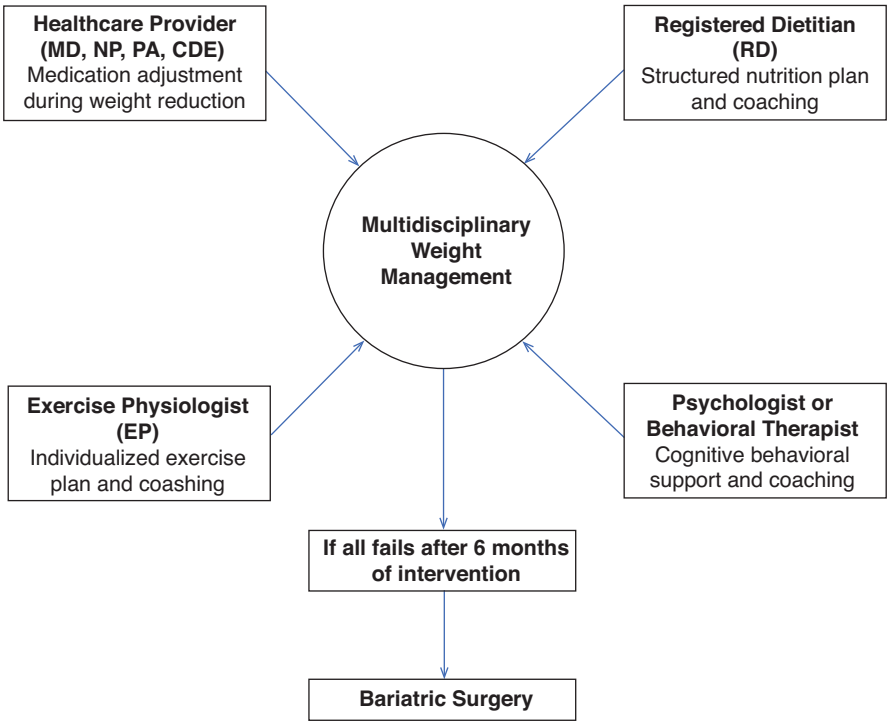

Fig. 29.1 The multidisciplinary approach to weight management in patients with type 2 diabetes and obesity

degludec, and insulin glargine U-300). To avoid unnecessary food consumption to match a presumed insulin dose, patients may be advised to administer short-acting insulin immediately after meals or within 20 min of starting their meal. Two insulins are FDA-approved for postprandial injection: insulin glulisine and fast-acting insulin aspart in a formulation with a form of vitamin B3 (niacinamide). In this perspective, patients are educated on injecting insulin based on what was eaten rather than on what they had assumed would be eaten. This technique may reduce mealtime insulin doses and consequently lessen the weight-gaining effect of insulin. Close monitoring of blood glucose levels is especially important during weight loss. Patients are advised to use a continuous glucose monitor or to check their blood glucose levels 5–8 times daily: before meals, before and after exercise, and at bedtime. Blood glucose logs should be reviewed weekly during the intensive period of weight management by healthcare providers including physicians, nurse practitioners, physician assistants, or certified diabetes nurse educators. Antihyperglycemic medications should be titrated accordingly to prevent hypoglycemia during weight reduction. The occurrence of hypoglycemia with weight loss as the result of improved insulin sensitivity can be a barrier to progressive weight loss and should be avoided even by preemptive medication reduction if blood glucose is within the target range (Table 29.1).

Table 29.1 Adjustments of diabetes medication during multidisciplinary weight management in patients with type 2 diabetes and obesity

	List A		List B	
	Weight gain			
	Significant	Modest	Weight neutral	Weight loss
	Pioglitazone	Sulfonylureas – Glimepride – Glipizide XL		GLP-1 analog – Exenatide – Exenatide ER – Liraglutide – Dulaglutide – Semaglutide – Oral-Semaglutide
	Sulfonylureas – Glyburide – Glipizide	Glinides – Repaglinide – Nateglide	DDP-4 Inhibitors – Sitagliptin – Saxagliptin – Linagliptin – Alogliptin	
	Insulin – NPH – Glargine – Regular – Aspart – Lispro – Glulisine	Insulin – Detemir – Degludec – Glargine U-300 – Glulisine (PP) – Aspart (PP)	α-Glucosidase inhibitor – Acarbose – Miglitol Colesevelam	Pramlintide SGLT-2 inhibitors – Canagliflozin – Dapagliflozin – Empagliflozin – Ertugliflozin
			Bromocriptine	
Adjustments	Stop, reduce, or switch		Continue	Add

Nutrition Therapy

Before insulin discovery, dietary intervention was the corner stone of diabetes management. At that time, clinicians relied solely on carbohydrate restriction within a hypocaloric diet to manage hyperglycemia. However, nutrition therapy was quickly sidelined after the introduction of insulin therapy in 1921. Over the last two decades, supervised nutrition therapy became one of the most effective methods of diabetes and weight management. We recently tested, in a randomized clinical trial, the effects of different models of nutrition therapy on A1C and body weight in patients with T2D and obesity. Our study showed that a structured dietary plan delivered by a registered dietitian (RD) was superior to the currently recommended personalized dietary plan in improving glycemia and body weight. Structured nutrition plans include menus, snack lists, and diabetes-specific formulas. Over 16 weeks of intervention, the use of a structured meal plan, either alone or in combination with weekly phone support by RD, resulted in reduction of A1C by −0.66% (95% CI −1.03 to −0.30) and −0.61% (95% CI −1.0 to −0.23), respectively. It also reduced body weight by −3.49 kg (95% CI −4.93 to −2.05) and −2.93 kg (95% CI −4.45 to −1.42), respectively. In contrast, patients given an individualized meal plan did not show significant change in A1C or body weight from baseline.

Proper nutrition therapy usually starts by an RD evaluating potential participants. This evaluation includes review of dietary history and/or 24-h dietary recall and review of adherence to dietary recommendations during previous attempts of weight management. It also includes identification of potential barriers for following a nutrition plan. Each participant should receive a hypocaloric meal plan rounded to the nearest 1200, 1500, or 1800 kcal level for ease of application based on their gender, height, and previous energy intake. In the Look AHEAD study, participants whose weight was over 250 lbs at baseline were put on a 1500–1800 cal diet plan, and those whose weight was less than 250 lbs were put on a 1200–1500 cal diet plan. In Why WAIT, men are put on 1800 cal diet plans and women on 1500 cal diet plans. If target weight loss is not achieved within 6 weeks, the dietary plan is advanced to 1500 and 1200 cal, respectively. Women who are shorter than 150 cm are initially put on 1200 cal diet plans. Structured meal plans provide approximately 40–45% of daily energy intake from carbohydrates with 14 g of fiber per 1000 cal, <35% from fat with <10% from saturated fat, and 1–1.5 g/kg of adjusted body weight from protein. Effective dietary plans do not calculate protein intake as a percentage of the total calories consumed to avoid unintended reduction in absolute protein intake in a hypocaloric diet. Reduction in absolute protein intake may accelerate lean muscle loss during weight reduction. Minimizing loss in lean muscle mass during weight management is essential for long-term maintenance of weight loss.

Exercise Therapy

For better long-term results, an exercise physiologist (EP) develops a personalized exercise plan for each patient based on the individual's age, gender, health status, and exercise capacity. In clinical practice, exercise capacity may be tested by a simple method such as the 6-min walk test. The typical 150 min/week of aerobic exercise or 10,000 steps per day improves fitness but it is not enough for weight reduction or for maintenance of weight loss. Effective exercise intervention for weight management should include a balanced mix of aerobic (endurance) exercise to promote cardiovascular health, resistance (strength) exercise to maintain muscle mass, and flexibility (stretch) exercise to enhance functional capabilities and reduce risk of injury. Exercise plans may progress gradually over 12–24 weeks from 20 min/day for 4 days/week to 60 min/day for 5–6 days/week. After completing the initial intensive phase, participants are usually encouraged to continue to exercise for 60 min/day, 5–6 days/week and maintain ≥300 min per week with an emphasis on resistance training to maintain muscle mass. Resistance training is especially important since diabetes is known to worsen sarcopenia (muscle loss that frequently occurs with aging). Short bouts of exercise of 10 min each distributed during the day were shown to be more sustainable and were associated with similar benefits seen with longer exercise sessions. Use of different exercise methods like circuit and interval training reduce boredom and increase the duration of exercises. Exercise is

particularly important after the intensive phase of weight management as it helps to maintain the weight loss achieved during the intensive period.

Cognitive Behavioral Support

The ability to maintain long-term dietary and exercise modifications relies heavily on patients' mental and motivational status, which should be addressed through cognitive-behavioral therapy (CBS). Clinical psychologists, behavioral therapists, or social workers are ideal coaches in leading behavioral support sessions, which can be individual or within group settings. Sessions incorporate typical components of CBS, which include behavioral goal setting, self-monitoring of eating and exercise, stimulus control techniques, cognitive restructuring, assertive communication skills, and prevention of relapse. This model was used in the DPP, Look AHEAD study, and Why WAIT program, where it was described in detail.

Use of Digital Health for Scalable Application of Lifestyle Intervention in Patients with Diabetes

Because of the comprehensive nature of the multidisciplinary approach to weight management, access to such programs may be limited to few patients due to cost or lack of specialized healthcare providers. Mobile phone applications can deliver a diabetes-specific multidisciplinary weight management program at lower cost and with greater accessibility to patients. Currently, over 28,000 smartphone applications focusing on weight management through diet and exercise tracking exist, many of which have already demonstrated their capacity to improve body weight and health outcomes in people with diabetes. However, current mobile phone applications include, on average, less than 19% of behavioral strategies used in evidence-based lifestyle intervention programs. In particular, strategies for educating patients during their weight management, providing motivational support, reducing stress, and assisting patients with health decision-making have been overlooked. With the introduction of evidence-based design and the integration of blood glucose monitoring systems, mobile health applications present an opportunity for improved accessibility and scalability of weight management interventions.

Bariatric Surgery

Bariatric surgery is increasingly used for obesity management in patients with diabetes. It should be considered as a valid option for patients with T2D and class 2 and 3 obesity who are unable to reduce their body weight after 6 months of intensive lifestyle intervention. Bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), is a drastic procedure but frequently results in long-term weight loss. Studies have shown that among patients with T2D, bariatric surgery improves

glycemic control and reduces requirements of antihyperglycemic medications. Bariatric surgery may induce partial or complete remission from T2D for several years. The recent and more popular sleeve gastrectomy procedure carries fewer complications than RYGB surgery. A recent study demonstrated a synergistic effect of completing a multidisciplinary weight management program before receiving RYGB compared to receiving RYGB alone. Serious side events like severe hypoglycemia and severe postural hypotension are not uncommon after RYGB and may require revision of surgery. A similar procedure called endoscopic sleeve gastroplasty can be done through a gastric endoscopy, eliminating the need for laparoscopic approach. The least effective bariatric surgery for long-term results in patients with T2D is laparoscopic adjustable gastric banding (LAGB). Over the last few years, US bariatric surgeons started to prefer sleeve gastrectomy over other types of bariatric surgery for its better results and limited complications. Comparison between intensive medical and surgical interventions favored surgery for the magnitude of weight reduction, but the overall quality-of-life measures improved more significantly with non-surgical intervention. Changes in A1C were similar after 1 year between the Why WAIT medical intervention method and LAGB.

Weight Management in Patients with Type 1 Diabetes and Obesity

Introduction

In the past 20 years, the prevalence of obesity has tripled worldwide, to the extent that it is now being considered an epidemic. Although patients with type 1 diabetes (T1D) have traditionally been thought to have lower BMI, current research has shown otherwise. The trend of increasing obesity prevalence has increased at a faster rate in patients with T1D compared to the general population. Currently, around 50% of patients with T1D are either overweight or obese. They also have higher waist and hip circumferences when compared to healthy controls. In the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, which followed adult patients with T1D for an average of 18 years, prevalence of overweight increased from 29 to 42% and prevalence of obesity increased sevenfold from 3 to 23%. Weight gain appeared to be unrelated to aging and instead related to clinical factors such as insulin therapy. Comorbidities, often associated with excess body weight, reduce the benefits of good metabolic control. Thus, controlling body weight in patients with T1D is necessary due to the well-known relationship between obesity and cardiovascular disease (CVD). Metabolic abnormalities related to obesity, such as the pro-inflammatory state, are likely to modify CVD risk in this population. So far, complications related to CVD have been the leading cause of mortality in patients with T1D.

Mechanisms of Weight Gain

Insulin Therapy

Insulin is anabolic hormone that plays a role in inhibiting protein catabolism, stimulating lipogenesis, and slowing basal metabolism, resulting in increased fat accumulation. Inhibiting protein catabolism is another anabolic process, in which weight gain may also occur through an increase in lean body mass. These effects are enhanced by exogenous insulin administration, since exogenous insulin imperfectly mimics endogenous secretion. While endogenous insulin has its first pass to the liver through the portal vein to suppress gluconeogenesis, exogenous insulin circulates systemically first and disproportionately affects muscle and adipose in comparison to the liver.

Intensity of Insulin Therapy

Intensity of insulin treatment influences weight gain as shown in the Diabetes Control and Complications Trial (DCCT), where patients on intensive insulin therapy gained an average of 4.6 kg over 5 years, which is significantly more than patients in the study's conventional arm. In that study, participants treated with intensive insulin therapy administered insulin either by multiple daily injections (MDI) or through continuous subcutaneous insulin infusion (CSII) by insulin pumps. Participants on conventional therapy administered one to two daily injections of intermediate and rapid-acting insulin, usually with no daily adjustments. Weight gain was observed in the intensive insulin therapy cohort as a whole, regardless of MDI or CSII administration. Similarly, a meta-analysis comparing multiple outcomes in adults with T1D using either MDI or CSII found no difference in weight gain. Despite weight gain, intensive insulin treatment is the standard of care because of its strong clinical benefits such as reduction of glycated hemoglobin (A1C) and reduction of long-term microvascular complications. Part of weight gain in association with intensive insulin therapy in T1D has traditionally been seen as normalization of weight by correcting for glycosuria, diuresis, and catabolism. It was also noted that moderate weight gain did not negatively affect cardiovascular risk profile when associated with improved glycemic control. This furthers the point that weight gain in patients with T1D on intensive insulin therapy is complex and multifaceted, but thus far a healthy balance must be reached.

Double Diabetes

Double diabetes is a new term used to describe patients with T1D who also show clinical signs of type 2 diabetes (T2D) such as obesity and insulin resistance (IR). With the rising rates of overweight and obesity among patients with T1D, there are

no longer clear divisions between the two major diabetes subtypes except at time of diagnosis, as the disease appears to behave as a continuum with the two components of its etiology, insulin deficiency, and IR. Double diabetes tends to occur when the pro-inflammatory state associated with metabolic syndrome leads to reduced glycemic control, eventually requiring higher daily doses of insulin. Increasing insulin dosage due to IR can lead to further weight gain, thus exacerbating the weight problem. Patients with T1D who are overweight or obese are at a greater risk of developing double diabetes due to their significantly elevated levels of osteopontin (OPN). OPN is a sialoprotein associated with normal physiological processes as well as autoimmune disease and has been demonstrated to induce adipose tissue inflammation, increase pro-inflammatory cytokine release, and promote development of IR. Fortunately, weight loss reduces circulating OPN concentrations. Double diabetes is a cyclical mechanism of weight gain and IR that should be recognized and treated early.

Physical Inactivity

Increased physical activity to enhance weight loss is widely accepted, but adults with T1D tend to partake in less physical activity than adults without diabetes. The main barrier to physical activity reported is fear of severe hypoglycemia. Although this is a clear psychological barrier, it is also a valid concern since hypoglycemia is the most common adverse event of physical activity in patients with T1D. Hypoglycemia may occur during or up to 24 h after activity. To prevent hypoglycemia, patients usually reduce their insulin dose before exercise, but this strategy can only be used when exercise is planned in advance. An additional drawback is that patients try to keep their blood glucose higher before exercise in order to maintain proper glycemic profile during and after exercise. They do that by increasing consumption of carbohydrates before and during exercise, which results in increased energy intake and consequent weight gain.

Weight Management in Type 1 Diabetes

Nutrition Therapy

The American Diabetes Association recommends weight loss for all overweight or obese individuals with diabetes or at risk for diabetes. Many nutrition-based approaches for weight loss have been studied in individuals with or without diabetes, but very few studies were specific to patients with T1D. For patients with T2D, certain macronutrient compositions such as low-carbohydrate or low-fat calorie-restricted diets and different eating patterns including Mediterranean and vegetarian dietary plans were shown to be successful for up to 2 years. In a 2-year study comparing low-carbohydrate, low-fat, and Mediterranean dietary plans in obese

participants, mean weight loss was 2.9 kg in the low-fat group, 4.4 kg in the Mediterranean diet group, and 4.7 kg in the low-carbohydrate group. Among the 36 participants with T2D in the study, the Mediterranean diet, which is rich in vegetables and healthy fats and low in red meat, was the most favorable for changes in fasting plasma glucose and insulin levels. The low-carbohydrate diet resulted in the greatest A1C reduction of 0.9% over 2 years. Plant-based vegetarian or vegan diets and the Dietary Approaches to Stop Hypertension (DASH) diet have also been shown to induce weight loss and modest improvements in diabetes management. The low-fat vegan diet, devoid of all animal products, was associated not only with sustained weight reduction but also with reductions in total cholesterol and LDL-cholesterol in comparison to a cohort following the American Diabetes Association guidelines. In a similar study, participants on a vegan diet had a decrease in A1C, attributed to loss of visceral fat. Less restrictive vegetarian diets also promoted weight loss and reduced A1C. The DASH diet, emphasizing vegetables, fruit, low-fat dairy, nuts, seeds, and whole grains while limiting meat, poultry, eggs, and oils, has shown beneficial effects on body weight, total and LDL-cholesterol, and insulin sensitivity.

Although these dietary plans, with different macronutrient compositions, have been shown to induce significant weight loss, the American Diabetes Association has determined in its position statement that there is no ideal macronutrient composition for meal plans. Current recommendations state that patients with diabetes should work with nutritionists to develop individualized eating plans based on the patient's metabolic status, life circumstances, and food preferences.

Regardless of macronutrient breakdown, total energy intake must be appropriate to the weight management goal. However, there are distinctions to be made in the quality of macronutrients and how they affect CVD risk factors and glycemic parameters. For carbohydrate consumption, intake of dietary fiber has been inversely associated with all-cause mortality in diabetes, while high glycemic load and sugar intake were associated with increased mortality. In patients with T1D, meals with the same carbohydrate content but different glycemic indices produced significant differences in postprandial blood glucose, with low GI meals producing a 20% lower glycemic response than high GI meals. For protein consumption, diets containing leaner sources of protein such as chicken and soy result in more favorable lipid profiles than diets containing red meat. For fat consumption, type and source of fat are more important than the percentage or total amount of fat. Diets containing foods high in monounsaturated fatty acids, such as extra-virgin olive oil and nuts, decreased CVD risk and should therefore replace saturated and trans fatty acids.

Increased Physical Activity and Exercise

Although weight loss can be achieved with only restriction of energy intake, increasing physical activity and incorporating exercise training into a weight loss plan lead to greater loss of fat mass and preservation of lean muscle mass compared to energy restriction alone. Additionally, there are metabolic benefits to partaking in physical

activity for weight loss. In patients with T1D, physical activity has been shown to decrease cardiovascular risk and mortality, in addition to improving lipid profile and endothelial function. In patients with T2D, physical activity improves insulin sensitivity. As explained earlier, IR is not unique to those with T2D, as patients with T1D tend to be more insulin resistant than their counterparts without diabetes. Therefore, the benefits of exercise on insulin sensitivity are pertinent to this population, especially in those who are overweight or obese.

Highly variable data exists as to what type of physical activity is best suited for weight reduction. Resistance training alone is associated with fat loss but has a minimal effect on the overall weight loss. Even when resistance therapy is combined with aerobic training, this seems to lead to a similar amount of weight loss as aerobic training alone. One study showed that aerobic exercise was shown to lower visceral adipose tissue to a greater extent than progressive resistance training when compared to control groups. However, the major benefit of resistance exercise is to preserve lean muscle mass during weight loss. This is especially important since patients with diabetes have progressive lean muscle loss as they age.

In terms of exercise intensity, some studies have shown that high intensity interval training (HIIT), consisting of repeated bursts of rigorous exercise immediately followed by low intensity recovery, can lead to significant reductions in abdominal fat. However, other evidence showed that while this approach is time efficient, it is no more effective than continuous moderate aerobic exercise in promoting fat loss. This supports the observation that rigorous and moderate intensity aerobic training results in similar amounts of weight loss when intensities of physical activity are matched in energy expenditure. Patients can partake in the type of physical activity they find most suitable as long as their energy expenditure is in line with their weight loss goals. Risk of hypoglycemia during or after exercise can be minimized if blood glucose is closely monitored before, during, and after exercise, and individual adjustments in insulin or food intake are made. Patients with T1D should be safely able to participate in aerobic or weight-based physical activities if appropriate pre-exercise measures are taken.

Medications

Insulin

Adjustment of insulin treatment to facilitate weight reduction has been suggested. Long-acting insulin creates a pattern of 24-h hyperinsulinemia, which stimulates lipogenesis and inhibits lipolysis. Long-acting insulin such as NPH and glargine induce weight gain in patients with T1D. If long-acting insulin is indicated, insulin detemir, insulin degludec, and insulin glargine U300 are preferred as they cause less weight gain compared to NPH or insulin glargine U100. To minimize the hypoglycemic risk and the unnecessary consumption of extra calories, it is better to administer short-acting insulin immediately after meals or within 20 min from the start of

the meal. This gives patients the ability to calculate the short-acting insulin dosage based on the food that they actually consumed and not on what they presumed to eat. In patients with T1D, insulin glulisine is preferred in such scenarios due to its faster onset of action.

Metformin

Metformin is a potent anti-hyperglycemic agent used to treat T2D; however, several studies used metformin alongside intensive insulin therapy to treat patients with T1D and obesity. In a recent randomized control trial, patients with T1D using metformin had significant improvements in body weight and lipid profile over 3 years. While there was an initial reduction in A1C over the first 3 months of using metformin, this improvement was not maintained for over the next 33 months. However, these patients had a significant reduction in insulin dose requirements which is explained by metformin's action as an insulin sensitizer. So far, US Food and Drug Administration (FDA) has not approved metformin for use in patients with TID.

Glucagon-Like Peptide-1 (GLP-1) Analogs

GLP-1 is an incretin hormone that is involved in both peripheral and central pathways mediating satiation. GLP-1 analogs are currently used to treat T2D and obesity. They reduce appetite and slow gastric emptying and thus reduce body weight and body fat by lowering energy intake. Their use in patients with T1D resulted in significant weight reductions in overweight and obese patients. However, improvement in glycemic control did not reach statistical significance in trials using active comparators. Liraglutide, a GLP-1 analog, in conjunction with insulin has been shown to improve glycemic control and induce weight loss in patients with T1D. It was also found to reduce insulin dose. While it is not approved for patients with T1D, its higher doses (2.4 and 3 mg/day) can be used to treat obesity. In a crossover study, exenatide treatment reduced postprandial plasma glucose but did not change A1C in patients with T1D. Another study showed that adding once weekly exenatide to insulin therapy significantly improved A1C, body weight, BMI, and reduced insulin doses. Currently, all GLP-1 analogs are not FDA-approved for use in patients with T1D.

Amylin Analog

Pramlintide is an injectable, synthetic form of human amylin. Amylin is a beta-cell hormone co-secreted with insulin and is nearly absent in patients with T1D. Amylin regulates blood glucose by slowing gastric emptying, suppressing glucagon secretion, and suppressing appetite to decrease food intake. Injecting pramlintide before meals in patients with T1D improves A1C, decreases postprandial blood glucose level, reduces insulin need, and induces weight loss.

Sodium–Glucose Transporter-2 (SGLT-2) Inhibitors

This new class of medications reduces blood glucose by inhibiting glucose reabsorption in the proximal convoluted tubules of the nephrons. Excretion of glucose in urine reduces body weight in addition to reducing A1C. Recent studies showed cardiovascular benefits of three medications from this class; empagliflozin, dapagliflozin, and canagliflozin. Several studies were performed in patients with T1D showing reduction in plasma glucose and body weight but with increased incidence of ketoacidosis. Currently, this drug class is only FDA-approved for use in patients with T2D. Dual SGLT-1 and SGLT-2, sotagliflozin, was investigated for use in patients with T1D but was not granted FDA approval.

Anti-obesity Medications

There are four new anti-obesity medications approved recently by the US FDA (topiramate/phentermine, naltrexone/bupropion, liraglutide, and semaglutide). All of them plus the older medications like Orlistat and Phentermine are effective for weight loss with variable efficacy and side event profiles. No studies using these medications were specifically conducted in patients with T1D. However, these medications showed reduction in A1C and number or doses of diabetes medications in patients with T2D. It is not clear if this effect is related to weight loss or it is specific to the mechanisms of action of these medications.

Bariatric Surgery

Several case series have been reported in obese patients with T1D showing reductions in body weight and insulin doses as well as a modest reduction in A1C.

A study that compared the effects of bariatric surgery in patients with T2D and T1D diabetes found that surgery could benefit T1D patients in terms of weight loss and improved glycemic control. It was noted that after 1 year, the decrease in median A1C in patients with T1D was much less than in those with T2D. In contrast, a few studies suggest that improved glycemic control may not be a probable outcome of bariatric surgery.

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Chapter 30
Surgical Treatment for Obesity and Diabetes Mellitus

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American Statistics

According to the CDC, in 2017–2018, the prevalence of obesity in the US was 42.4% [1]. The estimated annual cost of obesity in 2008 was estimated at $147 billion. Currently, two-thirds of the US population is overweight and of those, half are obese. In 2017, two hundred and twenty eight thousand bariatric procedures were performed in the United States. This number represents only 1% of those actually eligible for bariatric surgery in the US [1].

Obesity

It is no hidden fact that obesity has been linked to significant increased cardiovascular disease among populations. According to the WHO, most of the world's populations live in countries where overweight and obesity kill more people than underweight. Obesity is defined by body mass index (BMI) which is calculated as weight in kilograms divided by height in meters squared. The resultant number is then used to stratify the degree of excess weight. *Obese* is the official term given when your BMI reaches 30. *Morbid obesity* is defined as a BMI over 40. Normal human BMI is considered 18–25 kg/m^2.

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History of Bariatric Surgery

The first surgical procedures for weight loss were performed in the 1950s and started at the University of Minnesota [2]. The procedure carried out at the time was the *jejunoileal bypass* (Fig. 30.1).

In the jejunoileal bypass, the proximal jejunum was transected and anastomosed to the distal ileum, bypassing a large amount of the small intestine which leads to significant malabsorption. This procedure had great weight loss due to the malabsorption, but had significant side effects including night blindness from lack of fat-soluble vitamins like vitamin A. Bacterial overgrowth of the bypassed intestine was also a problem that could lead to liver damage and cirrhosis. Many patients as a result had to have a reversal of this surgery [2].

In the 1960s, the *gastric bypass* (Fig. 30.2) was performed by Dr. Mason and Dr. Ito [3]. Since then a number of changes have occurred in technique ultimately leading to the current performance of laparoscopic gastric bypass today.

In this surgery, a small proximal pouch of stomach is separated from the larger stomach. The proximal jejunum is then transected and the distal end is brought up and connected to the small gastric pouch. The small pouch leads to satiety after a smaller volume of food is able to be taken in that then passes into the intestine. There is an element of malabsorption but not as significant as in the jejunoileal bypass. This is evidenced by fewer nutritional deficiencies particularly of fat-soluble vitamins with the gastric bypass as compared to the jejunoileal bypass.

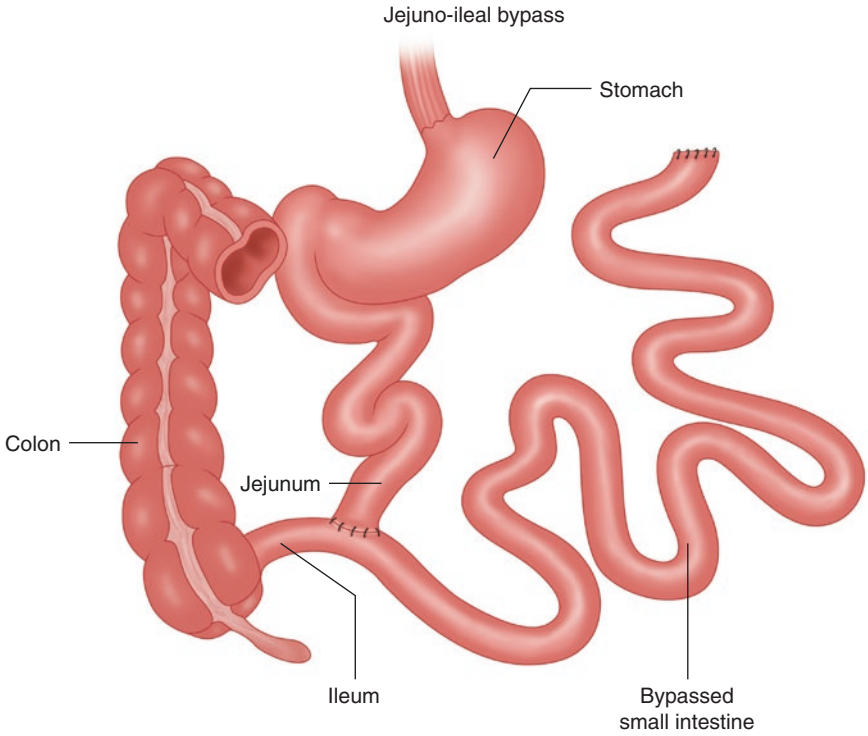

Fig. 30.1 Jejunoileal bypass

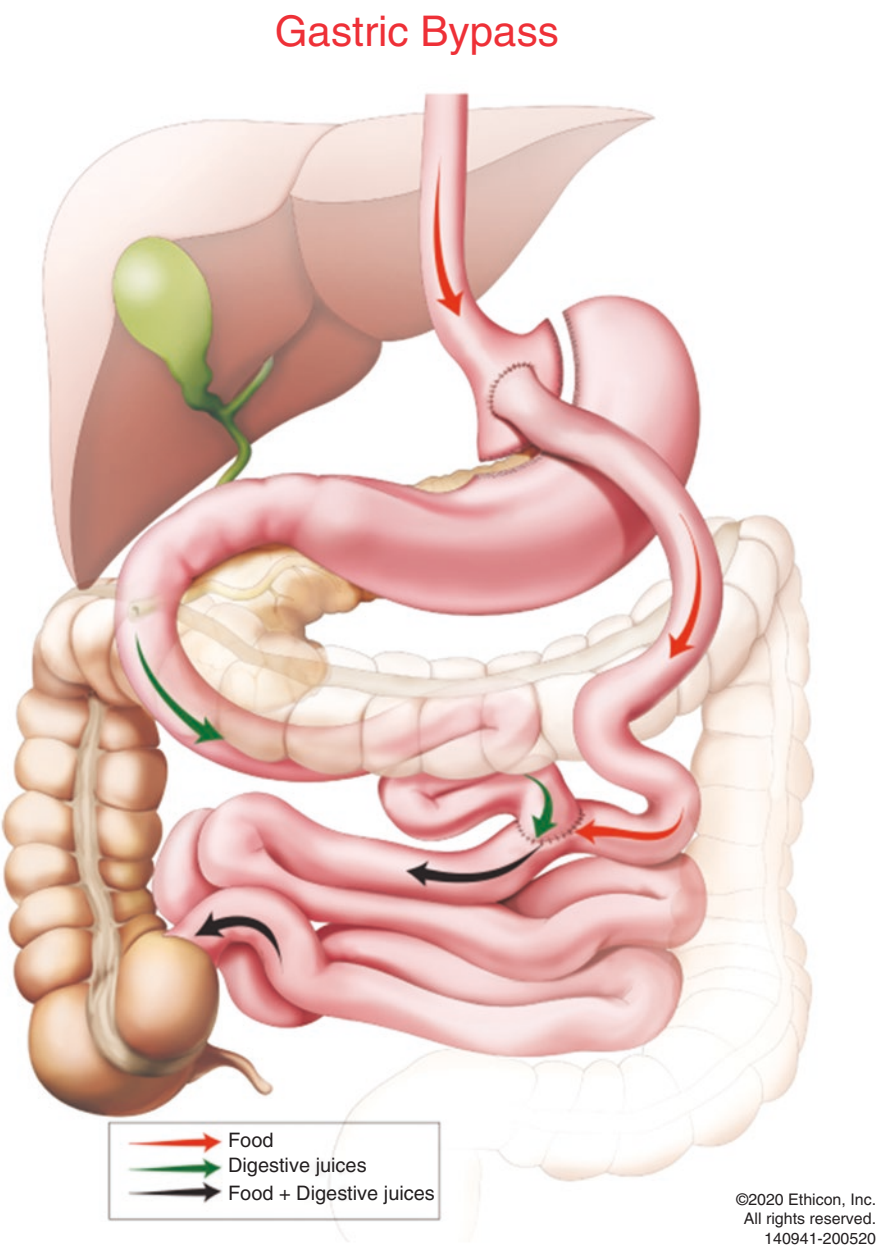

Fig. 30.2 Gastric bypass

The patients who have the Roux-en-Y gastric bypass lose approximately 61.6% of their excess weight [4]. After a gastric bypass, however, it is recommended that patients no longer have oral NSAIDS or smoke due to a high marginal ulcer risk. Marginal ulcers are ulcers that form on the jejunal side of the gastrojejunostomy. These ulcers if deep enough can be the cause of significant GI bleeding or perforation requiring emergency surgery to repair them.

The bypass also carries with it the risk of internal hernias, iron deficiency, as well as other vitamin deficiencies. Due to these potential complications, the sleeve has emerged as the most commonly performed bariatric procedure [5]. Fifty-nine percent of all bariatric procedures carried out in 2017 were sleeves.

The *sleeve gastrectomy* (Fig. 30.3) is a two-thirds gastric resection of the greater curvature.

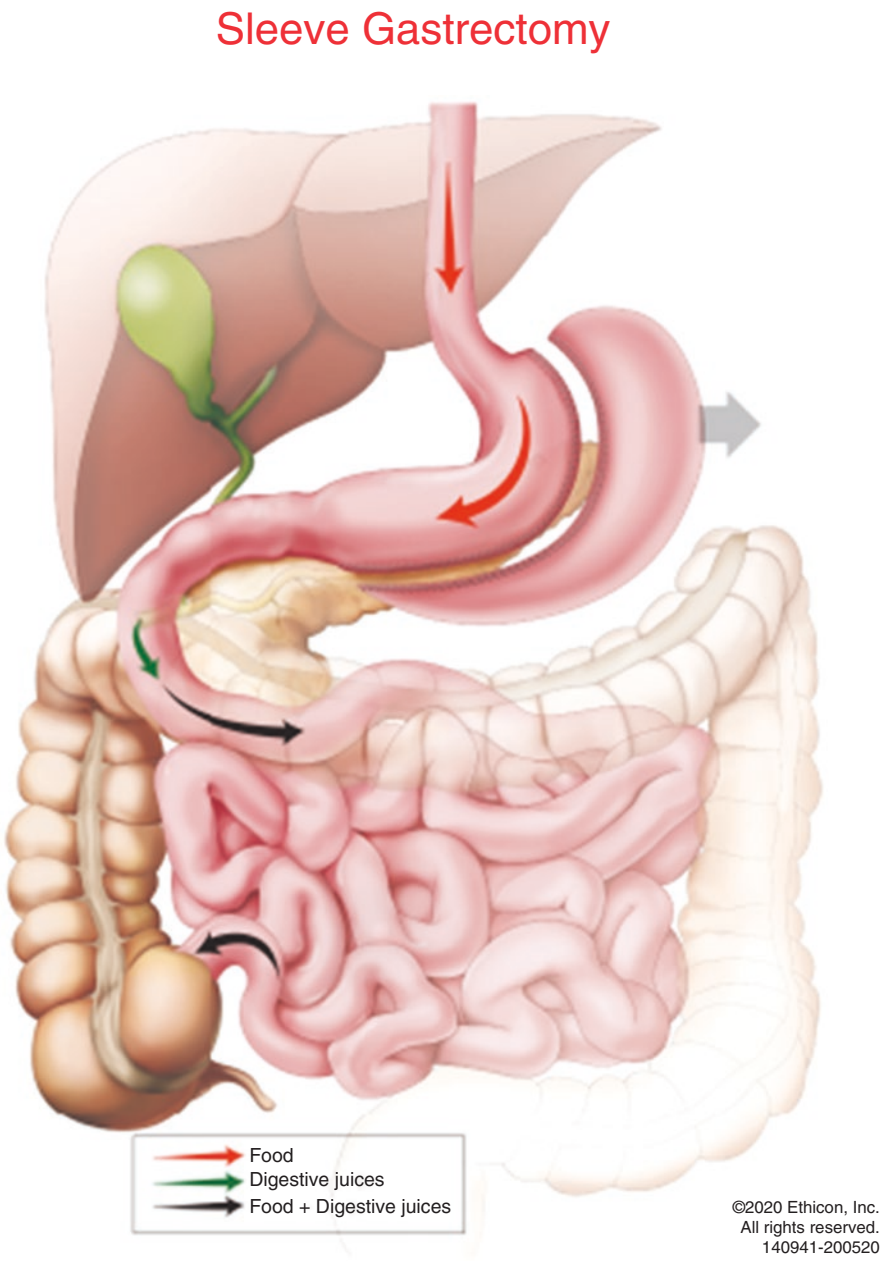

Fig. 30.3 Sleeve gastrectomy

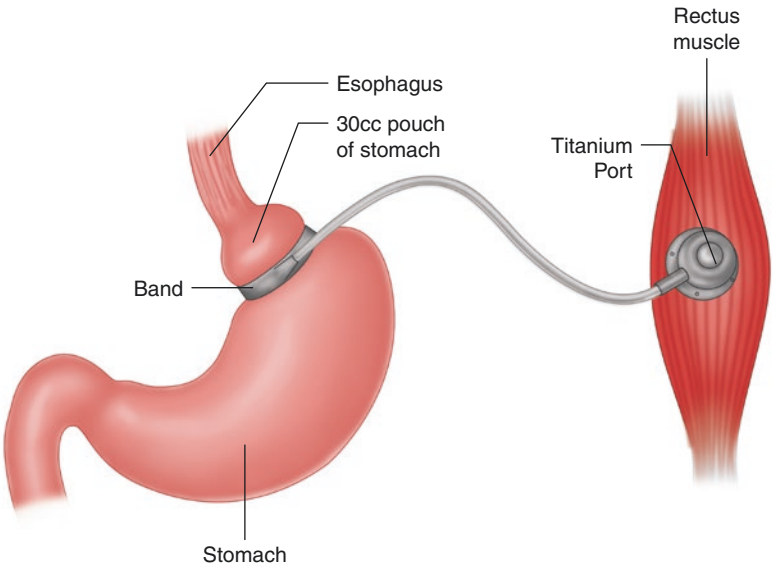

Fig. 30.4 Lap band

In the sleeve gastrectomy procedure, a bougie is inserted into the stomach which serves as a guide as to how wide to make the sleeve. Serial staplers are then used to the side of that bougie, removing the excess fundus. The excess fundus, labeled as resected stomach in Fig. 30.3, is completely removed from the abdomen.

This procedure results in an approximately 60–80% excess weight loss at 3 years for lower BMI patients [6]. The sleeve also allows patients to have oral NSAIDS after the staple line has healed. This is often an attractive option for patients who have chronic orthopedic issues or cardiac issues that require lifelong treatment with NSAIDS. There is also less of a risk of iron deficiency and no risk of internal hernia, since the intestine is not being rerouted. This has made the sleeve a very attractive option to many patients as either a primary procedure or staged procedure for higher risk BMIs.

The *lap band* (Fig. 30.4) came into favor in 1990s and was highly attractive to females who were still in their reproductive years.

There was no cutting or rerouting of the gastrointestinal track; hence, many felt more comfortable having this surgery. A silastic band was wrapped around the proximal stomach creating a 30cc pouch of stomach above the band. The band has an inner balloon that is filled with saline. That balloon can be adjusted by injecting or removing saline from a port buried on the anterior abdominal wall that connects via tubing to the band. The excess weight loss is approximately 49% at 5 years [7]. Complications with the lap band included erosion, slippage, and dysphagia. It has since been mostly replaced with the vertical sleeve gastrectomy which shows a better excess weight loss and does not have the issue of slippage or erosion.

Table 30.1 Patient criteria for candidacy for bariatric surgery

Unsuccessful weight loss with dietary and exercise interventions, and one of the following
BMI ≥ 40 kg/m^2
BMI 35–39.9 kg/m^2 with ≥1 comorbidities such as type 2 diabetes mellitus, hypertension, or obstructive sleep apnea
Per International Diabetes Federation criteria, BMI 30–34.9 kg/m^2 with type 2 diabetes mellitus and failure to achieve glycemic treatment targets with an optimal medical regimen
Acceptable operative risk
Psychosocially stable with no active depression, psychosis, or substance abuse
Well-motivated patient, able to adhere to postoperative dietary restrictions

BMI indicates body mass index
Sources: National Institute of Health and International Diabetes Federation

Indications for Surgery

The indications to be eligible for consideration for bariatric surgery are straightforward (Table 30.1). Unsuccessful weight loss with dietary and exercise changes along with one of the following: Class III obesity defined as BMI > 40 kg/m^2, Class II obesity defined as BMI 35–39.9 but with one or more comorbidities such as hypertension, obstructive sleep apnea, or type 2 diabetes mellitus. However, to focus on the proven treatment of diabetes, you are eligible if you have a BMI of 30–34.9 k/m^2, type 2 diabetes mellitus, and failure to achieve glycemic control with optimal medical treatments. You must also pass a psychological evaluation and be free from active depression, psychosis, or substance abuse. The patient must have an acceptable operative risk and be well motivated in order to adhere to postoperative dietary restrictions.

Comorbidity Resolution

Bariatric surgery results in significant weight loss which leads to resolution or significant improvement in many comorbid conditions such as sleep apnea, GERD, DM, HTN, and cholesterol [8, 9]. This chapter will focus on the cardiovascular benefits of weight reduction due to bariatric surgery.

Metabolic Syndrome

Obesity, hypertension, insulin resistance, and dyslipidemia together form a cluster of risk factors labeled metabolic syndrome. Due to the significant and lasting weight loss achieved by bariatric surgery, all of these factors are favorably reduced. This results in a significant decrease in overall cardiovascular risk factors [10].

Hypertension

In the United States, 50% of hypertensive patients have obesity. Thirty-three percent of obese patients have high blood pressure compared to 20% of normal weight patients [11]. The effect bariatric surgery has on Hypertension (HTN) remission has been looked at in various trials [12–16]. These studies have shown remission rates of 60–70% for HTN 1 year out after bariatric surgery. Another study by Jakobsen looked at medically versus surgically treated patients out for 6.5 years. Remission rates for HTN were 32% in the surgical group versus 12% in the medical group. In addition, it has also been postulated that due to a decrease in inflammation as well as decreased insulin resistance, which could decrease arterial stiffness and affect sodium reabsorption, ultimately leading to decreased blood pressure [17]. Long-term effects of the reduction/resolution of hypertension after bariatric surgery were proven in the GAEWAY trial. More than 50% of the patients who underwent surgery had complete remission of HTN, whereas no patients were free from antihypertensive therapy at 12 months [18].

Insulin Resistance/Type 2 Diabetes Mellitus

Perhaps the most impressive effect of bariatric surgery is its modulation of insulin sensitivity. In a ground breaking meta-analysis, it was reported that 76.8% of surgical patients experienced complete resolution of diabetes [4]. It was postulated that this occurred through intrinsic gut hormones in an entero-insular axis regardless of weight loss. Long-term studies quote a reduction of 13% of macrovascular complications, and reduction of 21% in microvascular complications [19].

When compared to medical therapy alone, bariatric surgery, specifically gastric bypass surgery had the greatest impact on reduction of glycated hemoglobin levels; specifically, a reduction of 2.9 percentage points versus a 1.4 reduction in the intensive medical therapy alone [20].

Heart Failure

Heart failure affects 6.2 million adults in the US and causes approximately 1 million hospitalizations per year. [21, 22]. Up to 40% of hospitalized patients for heart failure suffer from obesity [23]. In the Framingham heart study, it was found that obese patients have doubled the risk of developing heart failure compared with subjects with a normal BMI and identified weight as the third most important predictor of heart disease after age and dyslipidemia [24]. In the same study, 11% of male and 14% of female cases of heart failure were directly correlated to obesity, and each incremental BMI rise of 1 kg/m^2 increased the risk of heart failure by 5% for male subjects and by 7% for female subjects.

Obese patients have larger left ventricular mass and wall thickness which lead to higher left ventricular diastolic filling pressure [25, 26]. It has been postulated that epicardial fat and intramyocardial triglyceride content are related. [27] Excess epicardial fat may be directly cardiotoxic which might explain heart failure in obese patients [28, 29].

After bariatric surgery, it has been shown that there is an overall 22% reduced risk of mortality in surgical patients [30]. It has also been shown that the overall incidence of heart failure is five times higher in nonsurgical obese patients compared to obese subjects who had surgery [30]. Another study looked at gastric bypass patients up to 12 years out, and found a reduced risk of heart failure and a lower risk of death from heart failure. This study revealed a statistically significant reduction in congestive heart failure ($p = 0.0077$) for the gastric bypass cohort [31]. Roux-en-Y gastric bypass surgery has also been shown to decrease the incidence of new heart failure development compared to intensive lifestyle modification by almost 50% [32].

LV Structure and Function

Some studies have shown that the weight loss with bariatric surgery can undo left ventricular remodeling.

It has been thought that weight loss can lead to decreased thickness of the left ventricular wall. In a small study by Peterson et al., wall thickness did decrease and fewer patients had heart failure symptoms [33].

In one study in bariatric surgery patients, LV mass declined by 32% and RV mass by 16% over 17 months post operatively. There was also found to be a linear decrease in LV mass and body mass index up to 17 months post-surgery [34].

Another study found that weight loss in patients undergoing gastric bypass was associated with reverse cardiac remodeling and improved LV and RV functions [35].

In a group of 52 bariatric surgery patients followed by echo at 6 months after surgery, there was a significant increase of left ventricular end systolic volume (LVESV) and left ventricular end diastolic volume [36].

A recent study looked at sleeve and bypass patients free of cardiac disease pre- and 6 months post-surgery using the new imaging modality of three-dimensional (3D) strain echocardiography. They found that bariatric surgery has an important effect in reverse LV and RV remodeling and it substantially improves RV longitudinal strain [37].

Angina Pectoris

Stable angina pectoris (SAP) affects about 8.2 million adults in the United States [38]. Approximately, 22,000 patients are hospitalized each year for SAP [39]. In a case series looking at Stable Angina Pectoris patients who had bariatric surgery,

they found that the rate of hospitalizations for SAP was lower by two-thirds after bariatric surgery [40]. This decrease was seen in the first year and continued up to year two post-surgery.

Summary

It is still early on in the history of Bariatric surgery but multiple studies have proven the astronomical effects of surgically induced weight loss on cardiovascular health. It is effective in not only improving, but in fact, reversing, hypertension, diabetes, cholesterol, and sleep apnea, but also angina, CHF, LV structure, and function. More studies will be needed to see the effects of each different surgery on specific cardiac comorbidities. However, the trend of significant weight loss truly improves many factors that decrease the risk for cardiac disease and metabolic syndrome.

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Part VII
Medical Therapy of Type 2 Diabetes Mellitus

Chapter 31
Renin-Angiotensin-Aldosterone System in Diabetic Cardiovascular Complications

Vaidyanathapuram S. Balakrishnan

Well over half the mortality that is seen in the diabetic population can be ascribed to cardiovascular disease (CVD), which includes myocardial infarction due to accelerated and premature atherosclerosis as well as diabetic cardiomyopathy. Results from multiple clinical trials have shown that angiotensin-converting enzyme (ACE) inhibitor [1–7] and angiotensin AT1 receptor blockers (ARBs) [8, 9] have a favorable impact on cardiovascular outcomes in patients with diabetes. This illustrates the key role that the renin angiotensin system (RAAS) plays in the development and progression of diabetic CVD. In this chapter, we will consider the role of RAAS and how it is a critical driver of most of the pathophysiologic mechanisms behind diabetic CVD and why targeting this system has emerged as a critical therapeutic option.

Effect of Diabetes on the Renin Angiotensin Aldosterone System (RAAS)

Overview of the RAAS: Angiotensinases, Peptides, and Receptors

The renin-angiotensin-aldosterone system consists of a group of enzymes (angiotensinases), peptides, and their downstream cellular receptors whose main function is to control blood pressure by regulating vasoconstriction, sodium reabsorption,

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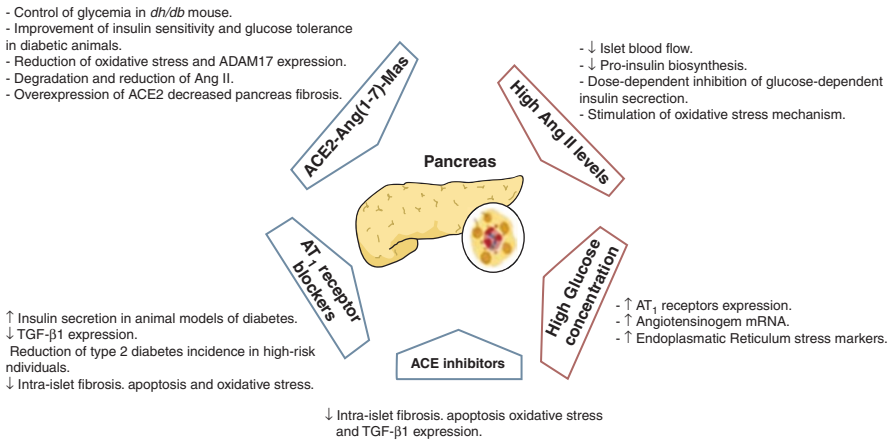

Fig. 31.1 Overview of the Renin–Angiotensin System. Ang II mediates most of its effects via the AT_1 receptor. In addition, Ang II can also be further processed to generate additional biologically active peptides or inactive peptide fragments. Abbreviations: *Ang* angiotensin, *ACE* Angiotensin-converting enzyme, *Ang III* Angiotensin Arg^2-Phe^8, *Ang IV* Angiotensin Val^3-Phe^8, *Ang-(1–7)* Angiotensin Asp^1-Pro^7, *AT-R* Ang II receptor, *DCP1* dipeptidyl carboxypeptidase 1, *AMP* aminopeptidase, *IRAP* insulin-regulated aminopeptidase, *PEP* prolyl-endopeptidase, *NEP* neutral endopeptidase

and body fluid homeostasis. Angiotensin peptides are derived from circulating predominantly hepatic-derived angiotensinogen (AGT), a 452 amino acid protein in the serpin family that undergoes N-terminal proteolysis by renin, to generate the relatively inactive decapeptide angiotensin I (Ang I) (Fig. 31.1). Renin is an aspartyl protease enzyme secreted as a prohormone by the juxtaglomerular apparatus of the kidney and activated to release active renin which is the rate-limiting enzyme of the RAS. Prorenin is produced by the juxtaglomerular cells in response to a variety of stimuli, including reduced renal perfusion pressure, sympathetic activation, and decreased sodium delivery to the macula densa. Recent studies have also shown that macrophages are an additional source of renin expression, which may have a critical role in atherosclerosis [2]. Cathepsin G and cathepsin D also have renin-like activities, which may substantially contribute to Ang I production by vascular smooth muscle cells (VSMCs) [10]. Once formed, Ang I is cleaved by angiotensin-converting enzyme-1 (ACE1) to produce the octapeptide angiotensin II (Ang II), which activates both angiotensin AT_1 and AT_2 receptor isotypes. There are other Ang 1 processing pathways, including dipeptidyl carboxypeptidase 1, chymase, or cathepsin G. The relative contributions of ACE1 and these alternative Ang I-processing pathways to Ang II generation appears to vary among specific tissues [11–13]. In the human heart *in vivo*, ACE1 appears to account for the majority of Ang II production [14]. Atherosclerotic plaques contain both ACE1 and chymase activity [15–17], suggesting that both pathways contribute to local Ang II generation within vascular lesions.

In earlier studies, Ang I was also shown to be cleaved by the carboxypeptidase angiotensin-converting enzyme-2 (ACE2) to generate Ang (1–9) [18], which results in decreased Ang II production. However, this observation was countered by the demonstration that the catalytic efficiency of ACE2 against Ang II is 400-fold higher than for Ang I and leads to angiotensin Asp^1-Pro^7, Ang-(1–7) generation in many tissues [19]. In addition, it has been shown that the Ang-(1–7) G-protein coupled receptor Mas can hetero-oligomerize with AT_1 receptor and by doing so, inhibit the actions of Ang II [20]. Targeted disruption of ACE2 in mice results in elevated levels of Ang II and severe cardiac contractile dysfunction [21]. Taking into account the enzymatic properties of the two ACEs and of the two main RAS mediators, Ang II and Ang-(1–7), RAAS appears to be a dual functional system in which the vasoconstrictor/proliferative or vasodilator/antiproliferative actions are driven by ACE1/ACE2 balance [22]. In addition, a direct conversion of Ang I to Ang-(I–7) was shown to be mediated by prolyl endopeptidase in vascular endothelial cells [23] and neutral endopeptidase in the circulation [24].

Ang II can undergo further proteolytic processing to generate additional biologically active peptides (Fig. 31.1). Conversion of Ang II to angiotensin Arg^2-Phe^8 (Ang III) occurs primarily via aminopeptidase A with Ang III retaining its ability to activate the AT_1 receptor [25, 26]. Ang III is a major effector peptide of the RAAS in the brain where it mediates neuronal effects on blood pressure control [27]. Aminopeptidase or endopeptidase cleavage of Ang II can also generate angiotensin Val^3-Phe^8 (Ang IV), which appears to activate endothelial nitric oxide synthase activity and thereby increases blood flow [28, 29]. Ang IV has been reported to bind the insulin-regulated aminopeptidase (IRAP), which may indirectly affect neuropeptide half-life [30]. However, a role of the AT_1 receptor in mediating Ang IV action has also been reported [31].

Ang II has two main receptors: Ang II type 1 (AT_1) and Ang II type 2 (AT_2) receptors. The AT_1 receptor appears to mediate most of the growth promoting metabolic and gene regulatory actions of Ang II [32, 33]. The pressor response to Ang II infusion is abolished in AT_1 receptor null mice [34]. However, while Ang II is the major agonist for the AT_1 receptor, there is evidence that Ang III, Ang IV, and mechanical stress can also utilize this receptor pathway [26, 31, 35]. On the other hand, AT_2 receptor is highly expressed in differentiated fetal mesenchymal tissue and appears to have a regulatory role in fetal development [36]. AT_2 receptor is generally reported to mediate effects opposing and counterbalancing those mediated by AT_1 receptor (Fig. 31.1), such as vasodilatation and antiproliferation [37]. For instance, AT_2 receptor-knockout mice exhibit high blood pressure and increased vascular sensitivity to Ang II and AT_2 receptor deficiency in mice has been shown to exacerbate atherosclerosis [38]. However, although treatment with AT_1 receptor blockers presumably causes increased stimulation of AT_2 receptors, the potential contributions of the AT_2 receptor in clinical outcomes or complications of diabetes remains to be determined.

Ang II is also a powerful stimulus for aldosterone secretion by the adrenal gland, mediated by its interaction with AT_1 receptor in the adrenal cortex. In addition to

circulating Ang II, local production of Ang II occurs in the zona glomerulosa and may play an important role in aldosterone release [39]. Aldosterone binds to the mineralocorticoid receptor in various tissues and induces multiple effects. Its major action is in the kidney, where it increases expression of epithelial sodium channels in the cortical collecting tubular epithelial cells leading to enhanced sodium and water reabsorption and potassium secretion. This leads to an increase in effective circulating volume, increased blood pressure, and decrease in serum potassium [40]. In addition to the distal nephron, aldosterone effects change binding to its mineralocorticoid receptor in other tissues, including the heart, vascular smooth muscle cells, and adipocytes. Clinical studies have confirmed associations between high circulating aldosterone levels and hypertension, central obesity, glucose intolerance, left ventricular hypertrophy, and heart failure [41–43].

Activation and Imbalance of the RAAS in Diabetes

There is significant evidence for upregulation of the RAAS in diabetes. Miller and colleagues observed an increase in plasma renin activity, mean arterial pressure, and renal vascular resistance in the early stages of diabetes [44]. It was also shown that losartan lowered blood pressure more under hyperglycemic than euglycemic conditions and captopril and eprosartan caused a greater renal vasodilator response during hyperglycemia, suggesting that glucose levels were associated with an activation of RAAS, increasing the sensitivity to RAAS antagonism [45]. Consistent with these observations, direct renin inhibition with aliskiren led to significant improvement in left ventricular hypertrophy and end-systolic volume only in patients with diabetes [46].

There are several mechanisms underlying activation of the RAAS in diabetes. Hyperglycemia directly stimulates local Ang II production in cardiomyocytes [47], cardiac fibroblasts [48], and endothelial cells [49]. Studies using cardiac myocytes suggest that the mechanism by which hyperglycemia increases Ang II activity in the heart is by the generation of intracellular Ang II by intracellular chymase [50].

Hyperglycemia leads to p53 glycosylation which has been linked to the transcription of angiotensinogen and subsequent production of Ang II from the local RAAS [51, 52]. Fiordaliso and colleagues demonstrated a direct correlation between the degree of hyperglycemia, p53 expression, and the quantity of Ang II. Ang II synthesis increased with the degree of hyperglycemia and this was attenuated by inhibition of p53 glycosylation [53].

Several metabolic abnormalities associated with hyperglycemia including advanced glycation end products, which form after prolonged hyperglycemia and oxidative stress, dyslipidemia, and low-grade inflammation can stimulate the Ang II/ AT_1 pathway by upregulating AT_1 expression [54–56].

Another mechanism that leads to enhancement of Ang II/ AT_1 activity is the downregulation of ACE2 which not only promotes Ang II activity but also reduces local Ang-(1–7) leading to an imbalance of the RAAS [36]. Tikellis and

colleagues showed that the induction of diabetes was associated with a significant reduction of ACE2 expression and activity in the heart and vasculature together with a significant increase in circulating Ang II and reduction of Ang–(1–7) levels [57].

Additional factors, including parasympathetic nervous activation, hypovolemia, and sodium reabsorption, may affect the regulation of the RAAS in diabetes. While changes in individual components of this system may affect overall RAAS activity, interpretation of these changes is limited by the potential of downstream modulation of Ang II action or stability. Moreover, since the RAAS appears to be locally regulated, it may not be appropriate to extrapolate changes in RAAS component levels beyond the specific tissues and conditions studied.

Ang II Sensitivity in Diabetes

Diabetes may increase RAAS action in the vasculature by increasing its sensitivity to the effects of Ang II. Both increased systemic and renal sensitivity to the pressor effects of Ang II have been reported in diabetes [58, 59], as well as in diabetic patients with microvascular disease [60, 61]. In cultured VSMCs, elevating extracellular glucose from 5 to 25 mM has been shown to exert additive and/or potentiating effects on Ang II-induced activation of the ERK/MAP Kinase and the JAK/STAT pathway [62, 63]. The effects of diabetes on enhancing Ang II action could be mediated by increases in AT_1 receptor expression, changes in post-receptor signaling mechanisms, and/or a reduction in cellular signals that suppress AT_1 responses. STZ-induced diabetes upregulates AT_1 receptor levels in the heart of rats [64, 65] and within atherosclerotic lesions in apolipoprotein E (apoE)-deficient mice [66]. Elevated concentrations of extracellular glucose increase AT_1 receptor expression in cultured VSMC [67]. While these increases in AT_1 expression may affect Ang II sensitivity and/or maximal effect in these vascular target tissues, physiological relevance of these changes in receptor levels as a rate-limiting determinant in Ang II action has not yet been demonstrated. In addition, the synergistic effects of Ang II and high glucose could be mediated by the convergence of these agonists on signaling pathways, such as protein kinase C and NADPH oxidase [68].

A number of factors have been shown to attenuate AT_1 signaling and action in the vasculature. The angiotensin AT_2 receptor has been shown to inhibit or counteract many of the trophic effects of AT_1 [32]. Thus, the relative expressions of AT_1 and AT_2 receptors subtypes may be important determinants in modulating the actions of the Ang II/AT_1 signaling pathway. In addition, activation of other vascular hormones systems induces signals that oppose or interfere with AT_1 signaling. For instance, nitric oxide donors have been shown to reduce Ang II-stimulated growth, migration, and gene expression in a variety of cultured vascular cells [69, 70]. A role of nitric oxide in suppressing AT_1 action is particularly intriguing since impaired nitric oxide actions are components of endothelial dysfunction in diabetes [71, 72]. Thus, nitric

oxide release by endothelial cells may normally suppress or oppose AT_1 action, and the impairment of this endothelial function in diabetes may lead to enhanced activity of the Ang II/AT_1 pathway.

Role of the RAAS in Cardiovascular Disease in Diabetes

Role of the RAAS in Atherogenesis in Diabetic Animal Models

Patients with diabetes are at much higher risk of developing atherosclerosis than non-diabetic subjects. Hypertension, dyslipidemia, obesity, and hyperglycemia only partly explain this increased incidence of microangiopathy and macroangiopathy. Although there is considerable evidence that the RAAS has a role in vascular remodeling, inflammation, thrombosis, and atherogenesis [73–75], the role of this system in atherosclerosis in the context of the other diabetes-associated cardiovascular risk factors is not fully understood. A growing body of evidence from both clinical studies and experiments in diabetic rodent models suggested that the RAAS contributes to CVD in both type 1 and type 2 diabetes.

The apolipoprotein E (apoE)-deficient mouse has increasingly been used as an experimental model of atherosclerosis, developing lesions ranging from lipid-laden fatty streaks to advanced fibroproliferative lesions by the age of 30 weeks [76]. Further, the induction of diabetes in these mice for a relatively short period of 6 weeks was associated with accelerated atherosclerosis in the aortic arch [77]. Induction of diabetes by injection of streptozotocin (STZ) in 6-week-old apoE-deficient mice was associated with a fourfold increase in atherosclerotic plaque area compared with non-diabetic animals [78]. This accelerated atherosclerosis was associated with a significant increase in aortic ACE expression and activity and connective tissue growth factor and vascular adhesion molecule-1 expression [78]. Treatment of STZ-induced diabetic apoE-deficient mice with the ACE inhibitor, perindopril, reduced lesion area, macrophage infiltration, and collagen content [78]. A similar reduction in aortic plaque area was observed in STZ-induced diabetic apoE-deficient mice treated with the AT_1 receptor antagonist, irbesartan [66]. Both ACE and AT_1 receptor expressions were increased in aortic lesions in the diabetic apoE-deficient mice, suggesting that the Ang II/AT_1 pathway was upregulated within the atherosclerotic plaque and contributed to the accelerated lesion formation in this model. Multiple factors may contribute to the increased expressions of ACE and the AT_1 receptor in atherosclerotic lesions in diabetes. As previously mentioned, hyperglycemia can increase both Ang II production and AT_1 expression [67, 79]. Alternatively, the upregulation of AT_1 receptor expression could be mediated by diabetes-induced inflammation. Elevated levels of C-reactive protein (CRP) have been associated with atherosclerosis in diabetic patients [80], and transgenic overexpression of CRP in apoE-deficient mice induces a sixfold increase of AT_1 receptor expression in atherosclerotic lesions [81]. There is also increasing recognition of the

role of the receptor for advanced glycation end products (RAGE) in the potentiation of diabetes-associated atherosclerosis. Diabetic RAGE/apoE double-knockout mice (RAGE/apoE DKO) showed significantly less plaque area than diabetic apoE knockout mice [82]. Further, treatment with quinapril for 20 weeks almost completely abolished plaque deposition in the diabetic RAGE/apoE DKO mice, with significant attenuation of vascular collagen deposition and reduced macrophage infiltration [82].

Proximal blockade of RAS by renin inhibition has also emerged as a potential therapeutic strategy to inhibit RAAS and lower blood pressure. Treatment with the novel renin inhibitor aliskiren over a broad dose range to fat-fed LDL receptor-deficient ($Ldlr^{-/-}$) mice markedly reduced the size of atherosclerotic lesions in both aortic arch and the root [83]. Although the evidence for beneficial effects of aliskiren on CVD is still lacking, clinical studies have demonstrated its blood pressure-lowering and renoprotective effects in diabetes [84, 85].

Effects of RAAS Inhibition on CVD Outcomes in Diabetic Patients

Several large randomized clinical trials have provided compelling evidence that RAAS inhibitors reduce cardiovascular events and mortality related to acute myocardial infarction (MI) and heart failure [86, 87]. Many of these trials involved subgroups of patients with diabetes, and comparison of the relative effects of RAS inhibition in diabetic and non-diabetic subgroups in these studies provides important insight into the role of RAAS in CVD in diabetes.

In a substudy of the EUROPA study (PERSUADE), a total of 1502 diabetic patients with known coronary artery disease were randomized to perindopril or placebo. Perindopril treatment was associated with a reduction in major cardiovascular events compared to placebo [6]. In the ADVANCE trial, patients with type 2 diabetes were randomized to treatment with a fixed dose combination of perindopril and indapamide or matching placebo. The perindopril and indapamide combination resulted in a significant reduction in major vascular events, including death [3]. In the UK prospective diabetes study (UKPDS), the effects of tight and less tight blood pressure control by the ACE inhibitor, captopril, or the beta-blocker, atenolol, were compared in patients with both hypertension and type 2 diabetes. This study demonstrated that tight blood pressure control was more effective than less tight control in reducing macrovascular endpoints, including stroke and deaths related to diabetes [7, 88]. In addition, results indicated that captopril and atenolol were equally effective in reducing cardiovascular outcomes. These results from the UKPDS and several other clinical trials [89, 90], have led to a consensus in many national and internal guidelines to aim for lower BP targets in patients with diabetes.

A key question regarding the cardiovascular protection afforded by antihypertensive agents, including inhibitors of the RAAS is whether these effects are related

primarily to reduction in blood pressure or whether these agents provide additional protective effects. This issue has been addressed in a number of clinical trials involving hypertensive patients with type 2 diabetes.

The Appropriate Blood Pressure Control in Diabetes (ABCD) trial compared the effects of moderate and intensive blood pressure control using a dihydropyridine calcium channel blocker, nisoldipine, and an ACE inhibitor, enalapril, on hypertension in patients with type 2 diabetes [91]. While achieved blood pressure was equivalent with these two interventions for both the moderate and intensive treatment protocols, the incidence of MI was substantially higher in the calcium channel blocker-treated group compared with the ACE inhibitor group [92]. Although cardiovascular outcomes were a secondary endpoint, this study suggests that ACE inhibition may have protective effects against MI that go beyond its blood pressure-lowering effect. Similar results were reported for the Fosinopril Versus Amlodipine Cardiovascular Events (FACET), an open-label study that randomly assigned 380 patients with type 2 diabetes and hypertension to fosinopril or amlodipine. Although the ACE inhibitor and calcium antagonist were similarly effective in blood pressure reduction, the risk of major cardiovascular events was significantly lower in the ACE inhibitor-treated group [5]. The Swedish Trial in Old Patients with Hypertension-2 (STOP-2) compared treatment of hypertension between ACE inhibitors, calcium channel blockers, and beta-blockers with diuretics. In a post hoc subgroup analysis of patients with type 2 diabetes, achieved blood pressure was equivalent among the three groups, but as in the ABCD trial, the risk for myocardial infarction was significantly lower in the ACE inhibitor group compared to the other two treatment arms [93].

The Heart Outcomes Prevention Evaluation (HOPE) study was a large, randomized placebo-controlled trial with wide entry criteria which examined the hypothesis that ACE inhibition using ramipril and vitamin E would reduce cardiovascular events in patients with multiple risk factors. The MICRO-HOPE (Microalbuminuria, Cardiovascular, and Renal Outcomes-Heart Outcomes Prevention Evaluation) trial was a substudy of the HOPE trial that recruited 3577 patients with diabetes (the majority with type 2) age 55 years and older with evidence of vascular disease or other cardiovascular risk factors (hypercholesterolemia, hypertension, microalbuminuria, smoking) randomized to receive placebo or ramipril for 5 years. The ACE inhibitor component of the trial was discontinued after 4.5 years because of a significant reduction in the composite primary endpoint of myocardial infarction, stroke, and death from cardiovascular disease in the ramipril group. The beneficial effects of ACE inhibition occurred in both type 1 and type 2 diabetic patients and was independent of its effects on blood pressure [4]. Ramipril also reduced the development of overt nephropathy in subjects with microalbuminuria. While it is likely that multiple mechanisms contributed to the reduction of cardiovascular endpoints following RAS inhibition, another substudy of the HOPE trial has shown that the ACE inhibitor-treated group had a reduced rate of progression in carotid intimal-medial thickness [94], which is consistent with a reduction in atherosclerosis. The Losartan Intervention for Endpoint reduction in hypertension (LIFE) study was a

double-blind, prospective, parallel group study designed to compare the effects of losartan with those of atenolol on the reduction of cardiovascular morbidity and mortality in approximately 8300 patients with hypertension and left ventricular hypertrophy. A subgroup of this study compared the effects of losartan and atenolol in diabetic patients [95]. Patients were followed for a mean of 4.7 years. This study reported the primary composite cardiovascular endpoint, including cardiovascular death, stroke, and myocardial infarction, was lower in the patients assigned to the losartan treatment group (relative risk 0.76, $P = 0.031$). Since similar reductions in blood pressure were observed with losartan and atenolol, this study suggests that AT_1 receptor antagonism can provide beneficial cardiovascular effects beyond blood pressure control.

The potential clinical benefits of dual blockade of RAAS with an ACE inhibitor and ARB in patients with vascular disease or high-risk diabetes without heart failure was investigated in the ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) [96]. Patients were randomly assigned to ramipril, telmisartan, or their combination ($n = 25620$). At a median follow-up of 56 months, the primary outcome had occurred in 1412 patients in the ramipril group (16.5%), as compared with 1423 patients in the telmisartan group (16.7%; relative risk, 1.01; 95% confidence interval (CI), 0.94–1.09). In the combination therapy group, the primary outcome occurred in 1386 patients (16.3%; relative risk, 0.99; 95% CI, 0.92–1.07) as compared with the ramipril group, and there was an increased risk of hypotensive symptoms (4.8 vs. 1.7%, $P < 0.001$), syncope (0.3 vs. 0.2%, $P = 0.03$), and kidney dysfunction (13.5 vs. 10.2%, $P < 0.001$). These findings suggest that the combination of the two drugs was associated with more adverse events without an increase in benefit [96].

In recent years, there has been growing interest in the heptapeptide Ang-(1–7), given its ability to counteract many of the effects of Ang II. The inhibitory effects of Ang-(1–7) on Ang II-induced vasoconstriction, and its growth inhibitory, antiarrhythmogenic, and antithrombogenic effects imply that Ang-(1–7) may be a potential therapeutic target for development of new drugs [22]. Ang-(1–7) binds to MasR (Ang-(1–7) G-protein coupled receptor Mas) to trigger eNOS and Akt phosphorylation [97], and stimulates the release of NO and prostaglandins. Overexpression of catalase or administration of Ang-(1–7) normalizes oxidative stress and systolic hypertension in Akita diabetic mice (a mouse model of type 1 diabetes) and the effect of Ang-(1–7) can be reversed after treatment with MasR antagonist A-779, indicating that the antihypertensive effect of Ang-(1–7) is mediated at least partially through suppression of oxidative stress in diabetes [98, 99]. Administration of a non-peptide Ang-(1–7) receptor agonist, AVE0991, rescued cardiac function under diabetic conditions as indicated by a normalization of blood pressure and contractility parameters in rats [100, 101]. A recent study has demonstrated a role for endogenous Ang-(1–7) as an exogenous treatment with the peptide, reducing ischemia-induced cardiac dysfunction in diabetic hypertensive rats [102]. These findings are consistent with the hypothesis that the RAS is capable of self-regulating its activity through the formation of Ang-(1–7).

Effects of ACE Inhibition Following Acute Myocardial Infarction on Cardiovascular Outcomes in Diabetes

The role of ACE inhibition in patients with acute myocardial infarction (MI) and diabetes has been evaluated by post hoc analyses of some large clinical trials. The GISSI-3 study evaluated the effects of ACE inhibition on short-term clinical outcomes following acute myocardial infarction (MI) in a study population of 18,131 patients, of whom 2790 had a history of diabetes. Patients with suspected acute myocardial infarction were randomized to treatment with lisinopril with or without nitroglycerin within 24 h and continued for 6 weeks. A retrospective analysis showed that treatment with the ACE inhibitor was associated with a decrease in 6-week mortality in diabetic patients that was more pronounced when compared to patients without diabetes [2]. The overall risk reduction by ACE inhibitor treatment for the diabetic group was 32%, compared with a risk reduction of 5% for non-diabetic patients. Within the diabetic group, ACE inhibitor treatment reduced mortality rates for both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) patients by 49% and 27%, respectively. The survival benefit in diabetic patients was mostly maintained at 6 months despite withdrawal from treatment at 6 weeks. While this report indicates that the benefit of ACE inhibitor treatment in the diabetic group was greater than that for the non-diabetic group, the basis for this difference is unclear. Although the baseline characteristics for the treated and untreated groups were closely matched, overall, the diabetic group appeared to have had worse baseline characteristics than the non-diabetic group. The subgroup analyses performed in this report did not reveal an association between ACE inhibitor effects and baseline characteristics or physiological responses. Characterization of the diabetic population did not include measures of glycemic control, duration of diabetes, kidney function, or for IDDM, classification of type 1 vs. type 2 diabetes. Thus, while this provocative study suggests the ACE inhibition provided selective protective effects for the diabetic subgroup, the absence of information regarding glycemic control and kidney function among treated and placebo groups limits the interpretation of these results.

The Survival of Myocardial Infarction Long-Term Evaluation (SMILE) trial was a randomized, double-blind, placebo-controlled study that evaluated the effect of an ACE inhibitor zofenopril in reducing the morbidity and mortality of patients with anterior MI not undergoing thrombolysis and treated within 24 h of onset of symptoms. A post hoc analysis of this study compared the efficacy of the ACE inhibitor in patients with and without diabetes [103]. Among the overall study population of 1512 patients, 303 (20%) had diabetes. After 6 weeks of treatment, zofenopril resulted in a more significant reduction in the composite outcome of death and severe congestive heart failure in diabetic patients compared to patients without diabetes. Interestingly, 1-year mortality was significantly reduced among non-diabetic patients, whereas in the diabetic population, the decrease did not achieve

statistical significance. The lesser impact on 1-year mortality indicates that long-term treatment is probably required to maintain the benefits of early ACE inhibition in patients with diabetes.

A retrospective analysis of data from the Trandolapril Cardiac Evaluation (TRACE) study compared the effects of ACE inhibitor therapy in diabetic and non-diabetic patients with left ventricular dysfunction following acute MI. In this study, ACE inhibitor was given 3–7 days after acute MI with a mean follow-up time of 26 months. Treatment with trandolapril resulted in a 36% reduction in risk of all-cause mortality in the diabetic group as compared to an 18% risk reduction among non-diabetic subjects. In the diabetic group, trandolapril resulted in an even more impressive reduction in the risk of progression to severe heart failure by just over 60% compared with a non-significant effect in the non-diabetic group [1]. ACE inhibitor treatment was associated with a trend for a greater relative risk reduction for cardiovascular and sudden death in the diabetic group compared with the non-diabetic group. Thus, data from this study indicated that patients with diabetes mellitus who have suffered an acute MI complicated by left ventricular dysfunction derive a substantial benefit from long-term ACE inhibition.

As with the GISSI-3 and SMILE study, the reason for the larger effects of ACE inhibitors in diabetic patients is unclear. Perhaps, it may be related to worse baseline CVD in the diabetic group. Alternatively, differential responses for diabetic and non-diabetic groups may suggest that ACE inhibition normalizes or compensates for specific cardiovascular abnormalities caused by diabetes.

Effects of RAAS Inhibition in Heart Failure

Several observational studies have consistently shown a two- to fourfold increased risk of heart failure in individuals with DM compared with those without DM. The risk may be higher in younger individuals and in women compared with men [104]. It is clear from multiple clinical trials that included substantial numbers of patients with diabetes that RAS inhibitors have similar efficacy in patients with and without diabetes. An early study, the Consensus clinical trial, evaluated the influence of ACE inhibition on the prognosis of severe congestive heart failure. The addition of enalapril to conventional heart failure therapy reduced mortality by as much as 31% at 1 year and improved heart failure symptoms [105]. A meta-analysis of six clinical trials of ACE inhibitors in heart failure with reduced ejection fraction (HFrEF) that stratified data by diagnosis of diabetes included 2398 patients with diabetes and 10,188 patients without diabetes. The analyses showed that the survival benefit of ACE inhibitor therapy was virtually the same for patients with and without diabetes (RR 0.84 and 0.85, respectively) [106]. It is important to note that the absolute reduction in mortality with ACE inhibitors in individuals with DM is substantial because of their higher baseline mortality risk. Similar results were noted in major heart failure trials involving angiotensin receptor blockers. For

instance, in the Candesartan in Heart failure-Assessment of Reduction in Mortality and morbidity (CHARM) study that involved patients with symptomatic heart failure and a broad range of EF, the effect of candesartan in reducing cardiovascular mortality and morbidity was not modified by diabetes status [107]. Further, a recent subgroup analysis of PARADIGM-HF (Prospective Comparison of ARNI with an ACE inhibitor to Determine Impact on Global Mortality and Morbidity in Heart Failure) demonstrated similar benefit on outcomes with the angiotensin receptor blocker neprilysin inhibitor (ARNI) sacubitril–valsartan in patients with and without DM [108].

Role of Mineralocorticoid Receptor Antagonists in Heart Failure

The mineralocorticoid receptor antagonists (MRAs), spironolactone and eplerenone, are recommended for patients with symptomatic heart failure who have an ejection fraction of 35% or less [109]. They are also recommended to reduce morbidity and mortality following an acute MI in patients who have an ejection fraction of 40% or less who are symptomatic or who have a history of diabetes mellitus [109]. Although the role of MRAs in patients with ejection fraction greater than 35% is unclear [110, 111], they may benefit patients with comorbidities, such as diabetes, hypertension, or kidney disease [112].

Despite guideline recommendations and evidence from clinical trials, MRA use in patients with heart failure with reduced ejection fraction is underutilized in clinical practice [113]. This is largely related to the risks of hyperkalemia and worsening kidney function in patients with heart failure who have diabetes and/or chronic kidney disease [114–116], although these medications are potentially beneficial in these higher-risk populations.

The major clinical trials of MRA therapy in heart failure have produced conflicting results with respect to the benefits of this therapy in high-risk subgroups. The RALES (Randomized Aldactone Evaluation Study) study of patients with reduced ejection fraction and severe symptomatic heart failure showed that spironolactone, in addition to standard therapy, substantially reduced the risk of both morbidity and death among these patients with median serum creatinine of 1.2 mg/dl or greater [117]. Subgroup analyses from EMPHASIS-HF (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure) that included patients with reduced ejection fraction and mild symptoms also showed that eplerenone had a benefit for the primary endpoint of cardiovascular death or heart failure hospitalization in patients with a history of diabetes mellitus and in patients with an estimated glomerular filtration rate less than 60 ml/min/1.73 m^2 [118]. However, among patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure, the EPHESUS study (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study) did not find a significant benefit for eplerenone for the outcomes of all-cause mortality and death

or hospitalizations from cardiovascular disease in patients with diabetes mellitus or those with serum creatinine of 1.1 mg/dl or greater. This trial, however, was not designed with sufficient power to draw statistical conclusions about individual subgroups [119].

The TOPCAT trial (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) evaluated the effects of spironolactone in patients with heart failure and a preserved left ventricular ejection fraction. Treatment with spironolactone did not significant reduce the primary composite outcome of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for heart failure including in the subgroup of patients with a history of diabetes mellitus or with an estimated glomerular filtration rate less than 60 ml/min/1.73 m^2 [110]. However, in a post hoc analysis, an unusually large difference was identified in the placebo group primary event rate among the patients randomized from Russia and Georgia compared with those enrolled from the United States, Argentina, Brazil, and Canada (the Americas) [110]. Further, in patients randomized in the Americas, the rates of cardiovascular death and hospitalization for heart failure were significantly reduced by spironolactone. This regional variation was likely related to marked dissimilarities in baseline variables between the study populations from the two regions [120]. Further analysis of patients with heart failure and preserved ejection fraction enrolled in the TOPCAT trial showed that natriuretic peptide levels were independently associated with an increased risk for all-cause mortality, death from cardiovascular disease, or hospitalizations for heart failure. Interestingly, there was a significant interaction between the effect of spironolactone treatment and natriuretic peptide levels, with most of the beneficial effects of spironolactone seen in patients with low levels of natriuretic peptides and no effect noted in the patients with high levels. However, a degree of caution is warranted in accepting these results given the post hoc subgroup nature of this analysis.

A more recent study used registry data linked to Medicare claims and analyzed patients hospitalized with heart failure between 2005 and 2013 with a history of diabetes mellitus or chronic kidney disease and stratified patients by MRA use at discharge. Among patients with heart failure and diabetes mellitus or chronic kidney disease, MRA use was associated with lower risk of all-cause readmissions to hospital despite a higher risk of hyperkalemia and acute renal insufficiency [112].

Mechanisms of RAAS-Induced Cardiovascular Diseases

Pressure and Hemodynamic Effects

The blood pressure effects of Ang II are mediated via a combination of mechanisms, including vasoconstriction, stimulation of renal tubular sodium resorption, as well as its effects on the central and sympathetic nervous tissues [121]. Since

hypertension exacerbates diabetic vascular complications [122], it is likely that the blood pressure-lowering effects of ACE inhibitors are a major contributor to the reduction of vascular complications in diabetic patients with hypertension [88]. However, there is growing evidence that ACE inhibitors may also provide beneficial vascular effects in diabetes in the absence of systemic hypertension. Several large studies have demonstrated that ACE inhibition can reduce renal, retinal, and cardiovascular complications in normotensive diabetic patients. While a small reduction in systemic blood pressure within the normotensive range may contribute to the vasoprotective effects of ACE inhibition, the magnitude of these effects is greater than that which would be predicted based on the magnitude of these blood pressure-lowering effects alone. Local upregulation or sensitization of the RAAS can result in tissue-specific increases in Ang II action, which may not significantly affect systemic blood pressure. These local changes in the RAAS can affect hemodynamics and pressure within certain vascular structures, such as the renal glomerulus. RAS inhibition has been shown to alleviate glomerular capillary hypertension caused by efferent arteriolar vasoconstriction induced by diabetes ("Effects of Ramipril on Cardiovascular and Microvascular Outcomes in People with Diabetes Mellitus: Results of the HOPE Study and MICRO-HOPE Substudy. Heart Outcomes Prevention Evaluation Study Investigators" 2000) [123]. Thus, in addition to systemic blood pressure control, ACE inhibition can also affect local hemodynamics and pressure. Multiple mechanisms may mediate the detrimental vascular effects associated with mechanical stress caused by hypertension. Mechanical stress stimulates cardiomyocytes to release Ang II, which induces an autocrine hypertrophic response [124]. A recent report has shown that mechanical stretch also induces Ang II-independent activation of the AT_1 receptor [35]. Interestingly, this mechanical stretch response blocked the AT_1 antagonist candesartan but not the Ang II competitive inhibitor (Sar^1, Ile^8)-Ang. In addition, increased shear stress and mechanical stretch can activate vascular calcium transport, TGF-beta, and purinoceptors [125–127].

Intravascular Actions of the RAS

In addition to its potent effects on vasoconstriction and blood pressure control, Ang II also exerts a variety of effects on vascular biology, which are independent of vascular tone and pressure. AT_1 receptors are expressed in most vascular cell types, including endothelial and VSMC, cardiomyocytes, and cardiac fibroblasts [13]. Activation of these receptors affects a diverse array of vascular cell functions, including growth, migration, oxidant production, and gene expression [121]. Overproduction of Ang II and/or increased Ang II sensitivity within the vasculature tissues may stimulate these cellular processes and thereby contribute to vascular remodeling, hypertrophy, fibrosis, thrombosis, and atherosclerosis. Consistent with

this hypothesis, ACE inhibition and AT_1 blockade have been shown to reduce perivascular fibrosis and PAI-1 and matrix metalloprotease expression in normotensive insulin-resistant diabetic rodents [128, 129]. In addition, AT_1 antagonism has been shown to reduce neointimal thickening of balloon catheter-injured vessels in diabetic Wistar fatty rats [130]. Local activation of the RAS may have particular importance at sites of vascular injury or atherosclerosis, which have locally elevated ACE- and chymase-mediated Ang II production as well as upregulation of AT_1 receptors [130]. Activation of AT_1 receptors expressed on monocytes and macrophages may contribute to atherogenesis by increasing arterial thrombosis and inflammatory responses [131–133]. Given that components of Ang II generation and Ang II receptors (AT_1 and AT_2) are co-expressed in RAS target tissues, and the half-life of circulating Ang II is only 14–16 s [134, 135], it is likely that autocrine/paracrine actions of the RAS system play a major role in its blood pressure-dependent and -independent effects in vascular tissues.

Endothelium-Dependent Vasodilatation

Endothelial dysfunction associated with impaired production and/or stability of nitric oxide occurs in both type 1 and type 2 diabetic patients [71, 72], as well as in obese insulin-resistant subjects [136]. Multiple mechanisms contribute to impairment in endothelium-dependent vasorelaxation in diabetes, including the oxidative inactivation of nitric oxide, reduced endothelial nitric oxide synthase (eNOS) expression, reduced eNOS activity, vascular insulin resistance, elevation of circulating levels of asymmetric dimethylarginine (an endogenous NOS inhibitor), and a deficiency in tetrahydrobiopterin, a cofactor for eNOS [137–140].

Both ACE inhibition and AT_1 receptor antagonism improves acetylcholine-induced vasorelaxation and in NIDDM subjects [141, 142]. Treatment of normotensive type 1 diabetic patients with an ACE inhibitor has also been shown to increase acetylcholine-induced vasorelaxation in [143]. In these studies, no difference in vasodilatation induced by nitric oxide donors (sodium nitroprusside) was observed in diabetic vs. control subjects, suggesting that the endothelium dysfunction was related to impairment in the generation of nitric oxide rather than an impaired response potential. ACE inhibition may improve endothelium-dependent relaxation by suppressing Ang II effects on vascular NADH/NADPH oxidase production of superoxide anion and/or vascular insulin signaling [144, 145]. While ACE inhibition improved endothelium-dependent vasorelaxation induced by acute acetylcholine infusion [141], it did not improve endothelial function in response to flow-mediated dilation [146, 147]. Therefore, ACE inhibition appears to selectively affect endothelium response to acetylcholine infusion in diabetes. Additional studies are needed to determine whether ACE inhibition affects endothelial functions in diabetes apart from its hemodynamic effects.

Effects on Cardiovascular Progenitor Cells

Recent studies have suggested that endothelial progenitor cells (EPCs) contribute to re-endothelialization of injured vessels as well as neovascularization of ischemic lesions [148–151], and that a decrease in the number of EPCs is an independent predictor of morbidity and mortality of cardiovascular diseases [152]. Interestingly, a number of studies have shown the potential beneficial effects of AT_1 receptor antagonists on EPCs. A 12-week treatment with olmesartan or irbesartan selectively increased the EPC subpopulation, but not the CD34+ hematopoietic stem cells (HSCs) [153] in patients with type 2 diabetes. A 2-week treatment with candesartan restored the decreased EPC number and function seen in salt-loaded stroke-prone hypertensive rats [154]. In addition, it was shown that 2 weeks of Ang II infusion in Wistar rats resulted in a lowering of EPCs that could be reversed by valsartan treatment [155]. Similarly, in patients with hypertension, Ang II accelerates the onset of EPC senescence by gp91 phox-mediated increases in oxidative stress [156]. In contrast, Ang II stimulates the angiogenic function of adult endothelial cells and increases VEGF-induced proliferation of human EPCs through AT_1 receptor-mediated upregulation of the VEGF receptor. Improvement in VEGF/eNOS function and decreased ROS production through inhibition of the NAD(P)H oxidase system may be important mechanisms that mediate the effects on EPCs [157].

In addition to AT_1 receptor blockade, treatment with ramipril was associated with an approximately 1.5-fold increase in the number of circulating EPCs by 1 week after initiation of treatment which was followed by sustained increased levels to approximately 2.5-fold throughout the 4-week study period in patients with coronary artery disease [158]. Moreover, ramipril treatment leads to increases in the functional activity of EPCs, as assessed by their proliferation, migration, adhesion, and in vitro vasculogenesis capacity. In C57/BL6 mice, ACE inhibition prevents pressure-induced maladaptive cardiac hypertrophy and increases angiogenesis associated with the upregulation of EPC and amelioration of EPC migration [159]. These findings suggest that the RAS contributes to the regulation of EPC bioactivity in patients with CVD.

Role of the RAAS on Glycemic Control, Insulin Sensitivity, and Diabetes Onset

Effect of RAAS Inhibition on Glycemic Control and Insulin Sensitivity

There is growing evidence that inhibition of the RAAS system by either ACE inhibition or AT_1 receptor antagonism can increase insulin sensitivity and glucose utilization. Studies using euglycemic hyperinsulinemic clamps have shown that ACE inhibitor treatment improves insulin sensitivity in most [160–162], but not all [163,

164] individuals with hypertension, obesity, and/or type 2 diabetes. Similarly, while AT_1 antagonism has been reported to improve muscle sympathetic nerve activity and insulin sensitivity in obese hypertensive subjects [165] and increase basal and insulin-stimulated glucose oxidation in normotensive individuals with type 1 diabetes [166], other clinical studies have not observed improvements on insulin sensitivity and glucose homeostasis following treatment with AT_1 receptor antagonists [162].

In experimental rodent models, Ang II induces insulin resistance in rat [167] and cultured skeletal muscle cell line [168]. The increased oxidative stress, possibly through impaired insulin signaling located downstream from PI 3-kinase activation, is involved in Ang II-induced insulin resistance [169]. Further evidence has been obtained from the TG(mREN2)27 rat, which manifests increased tissue RAAS activity, elevated serum aldosterone, and hypertension. The TG(mREN2)27 rat displays whole body and skeletal muscle insulin resistance that is associated with local oxidative stress [170] and specific defects in the insulin signaling pathway in skeletal muscle [171]. Direct renin inhibition attenuates abnormalities and improves systemic insulin resistance and skeletal muscle glucose transport [172]. ACE inhibition has also been shown to enhance skeletal muscle and adipose glucose transport in insulin-resistant obese Zucker rats and spontaneously hypertensive rats [173, 174]. In addition, angiotensin AT_1 receptor antagonism has been shown to improve insulin sensitivity and glucose uptake in skeletal muscle of normotensive diabetic KK-Ay mice [175], partially reduce insulin resistance in Wistar fatty rats [130], and increase 2DG uptake and GLUT-4 expression in skeletal muscle obese Zucker rats [176]. Since insulin resistance and the metabolic syndrome accelerate CVD [177], inhibition of the RAS may improve cardiovascular outcomes, in part, by increasing insulin sensitivity and improving metabolic control.

RAAS Inhibition and New-Onset Diabetes

Multiple large prospective trials involving ACE inhibitors or ARBs have reported an unexpected reduction in the development of new type 2 diabetes mellitus in patients treated with these agents. The MICRO-HOPE study reported a 34% reduction in the risk of new-onset diabetes in the ramipril-treated group compared to the placebo group [178]. The Captopril Prevention Project (CAPPP) trial reported that the relative risk of developing diabetes in the ACE inhibitor-treated group was 0.79 (0.67–0.94) compared with the conventional (diuretics, beta-blockers) treatment group [179]. The Losartan Intervention For Endpoint reduction (LIFE) trial reported that AT_1 receptor antagonism using losartan was associated with a 25% lower incidence of new-onset diabetes compared with patients treated with atenolol, who were similarly matched for initial clinical characteristics and blood pressure control [95]. The Valsartan Antihypertensive Long-Term Use Evaluation (VALUE) trial showed that the angiotensin receptor blocker, valsartan, is associated with a 23% reduction in the risk of new-onset type 2 diabetes compared to the calcium channel blocker, amlodipine in the treatment of hypertensive patients at high cardiovascular

risk [180]. It must be emphasized, however, that the development of new-onset diabetes mellitus was not a pre-specified primary endpoint of these studies. In the MICRO-HOPE study, the diagnosis of diabetes was self-reported by the trial participants and was not verified by glucose measurements. The CAPPP trial and the LIFE study, however, included new-onset diabetes as a pre-specified secondary endpoint. Given the consistency of the results of these large trials, the hypothesis that specific inhibition of the RAAS would reduce the development of new-onset diabetes was formally tested in two large randomized controlled trials. The DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) trial studied the effects of an ACE inhibitor (ramipril) and/or a thiazolidinedione (rosiglitazone) on the development of diabetes or death (primary outcome) and on the regression to normoglycemia (secondary outcome) in adults with impaired fasting glucose and/or impaired glucose tolerance, and no previous cardiovascular disease. The use of ramipril did not reduce the incidence of diabetes or death but did significantly increase the regression to normoglycemia [181]. The NAVIGATOR (Nateglinide and Valsartan Impaired Glucose Tolerance Outcomes Research) trial studied the effects of valsartan or placebo (and nateglinide or placebo) in addition to lifestyle modification on the development of diabetes in a high-risk patient population with impaired glucose tolerance and established cardiovascular disease or cardiovascular risk factors. The use of valsartan led to a relative reduction of 14% in the incidence of diabetes but did not reduce the rate of cardiovascular events [182] (Table 31.1)

Several meta-analyses also support the positive effects of RAAS blockade on diabetes mellitus prevention. Abuissa et al., in a meta-analysis of 12 randomized controlled trials, showed that ACE inhibitors and ARBs were associated with reductions in the incidence of newly diagnosed diabetes by 27% and 23%, respectively, and by 25% in the pooled analysis [183]. In 2007, Elliott and Meyer reported that using network meta-analysis (from 22 trials and >143,000 patients), ARBs, ACE inhibitors, calcium channel blockers, and beta-blockers all reduced the risk for diabetes mellitus relative to diuretics. The odds ratios of incident diabetes mellitus were lowest with ARBs (0.57) and ACE inhibitors (0.67) [184]. Consistent with the clinical finding on the effects of RAAS inhibition on the onset of diabetes, experimental studies have also indicated that ACE inhibition delays the onset of non-insulin-dependent diabetes in Otsuka Long-Evans Tokushima Fatty rats [185]. Both ACE inhibition and AT_1 receptor antagonism improved first-phase insulin secretion and histopathological changes in pancreatic islets from diabetic Zucker rats [186]. These provocative findings suggest that inhibition of the RAAS, by either ACE inhibition or AT_1 antagonism, could provide protective effects against the onset of type 2 diabetes.

Table 31.1 Summary of clinical trials on renin–angiotensin–aldosterone system in individuals with diabetes mellitus

Trial	Patient population	Comparators	Duration (years)	Setting	Key outcomes
PERSUADE [6]	1502 diabetic patients with CVD but no HF	Perindopril vs. placebo	4.3	CVD	Reduction of primary composite of CV mortality, non-fatal MI, and successfully resuscitated cardiac arrest: 19% (95%CI: −6.5–38.2%, $P = 0.131$)
RENAAL [9]	1431 patients with type 2 diabetes and nephropathy	Losartan vs. placebo	5	CVD	Hospitalization for new HF: 0.74 (95%CI: 0.55–0.98)*
LIFE [17]	1147 patients with hypertension, LV hypertrophy, and DM	Losartan vs. atenolol	5	CVD	Hospitalization for new HF: 0.57 (95%CI: 0.36–0.91)*
UKPDS [7, 88]	1148 patients with hypertension and type 2 DM	Captopril vs. atenolol	8.4	Hypertension	Relative risk of major macrovascular or microvascular event reduced by 34% and 37%, respectively, with tight BP control
ABCD [91]	470 patients with hypertension and type 2 DM	Enalapril vs. nisoldipine	5	Hypertension	Adjusted risk ratio for nisoldipine-treated patients vs. enalapril was 7.0 for combined endpoint of fatal and non-fatal MI ($P = 0.001$)

(continued)

Table 31.1 (continued)

Trial	Patient population	Comparators	Duration (years)	Setting	Key outcomes
MICRO-HOPE [4]	3577 patients with DM and vascular disease	Placebo vs. ramipril	4.5	CVD	Ramipril reduced the composite primary endpoint of MI, stroke, and death from CVD by 25% ($P = 0.004$) and overt nephropathy by 24% ($P = 0.027$)
ONTARGET [96]	25,620 patients with vascular disease or high-risk DM	Ramipril vs. telmisartan vs. combination of ramipril+telmisartan	5	CVD	No difference in primary outcome (death from CVD, MI, stroke, or hospitalization for CHF) between the three groups. Combination of ramipril and telmisartan was associated with more adverse events
GISSI-3 [2]	18,131 patients (2790 with DM) following acute MI	Lisinopril and glyceryl trinitrate singly and in combination	6 weeks	CVD	Treatment with lisinopril resulted in a risk reduction of 32% in diabetic patients vs. 5% risk reduction in non-DM patients

Table 31.1 (continued)

Trial	Patient population	Comparators	Duration (years)	Setting	Key outcomes
NAVIGATOR [182]	9306 patients with impaired glucose tolerance and CVD	Valsartan vs. placebo	5	Type 2 DM and CVD	Valsartan reduced the incidence of DM (HR 0.86, 95% CI, 0.80–0.92, $P = 0.001$)
DREAM [181]	5269 patients with impaired glucose tolerance	Ramipril vs. placebo	3	Type 2 DM	Although ramipril did not significantly reduce incidence of DM, it was more likely to result in regression to normoglycemia than placebo (HR 1.16, 95% CI, 1.07–1.27, $P = 0.001$)

*$P < 0.05$

Abbreviations: *CVD* cardiovascular disease, *DM* diabetes mellitus, *LV* left ventricle, *HF* heart failure, *MI* myocardial infarction, *CI* confidence interval, *HR* Hazards ratio

RAAS and Coronavirus 2019 (COVID-19) Disease

Membrane-bound ACE2 is the functional receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus 2019 (COVID-19) pandemic. Preclinical studies have demonstrated upregulation of ACE2 expression by RAAS inhibitors, such as ACEIs and ARBs, raising concern about their safety in patients with COVID-19.

Effect of RAAS Blockade on ACE2 Expression

In the heart, Ang II receptor blockers have been shown to increase ACE2 protein and gene expression in different models of experimental hypertension [187, 188]. In the model of myocardial infarction after left coronary artery ligation in transgenic Ren-2 rats, the ACE inhibitors enalapril and lisinopril increased heart ACE2 expression [189]. In the thoracic aorta of male spontaneously hypertensive rats, ACE2 was increased in association with reversal of vascular hypertrophy in response to olmesartan treatment [190]. In the kidney, both lisinopril and losartan increased ACE2

enzymatic activity in the renal cortex of adult Lewis rats [191]. Aldosterone antagonists (both spironolactone and eplerenone) have been shown to increase ACE2 enzymatic activity in macrophages from humans and mice [192].

Effects of RAAS Blockade on COVID-19: The Clinical Context

The interaction between the SARS viruses and ACE2 may be one determinant of their infectivity, and there are concerns that increased ACE2 expression induced by RAAS inhibitors may enhance severity of COVID-19. Conversely, observational data have demonstrated an association between use of ACEIs or ARBs and better outcomes in patients with COVID-19 [193]. A prospective cohort study, using routinely collected data from 1205 general practices in the UK with 8.28 million participants aged 20–99 years, studied whether patients prescribed these drugs had altered risks of contracting severe COVID-19 disease and requiring intensive care unit admissions [194]. ACE inhibitors and ARBs were associated with reduced risks of COVID-19 disease after adjusting for a wide range of variables. Neither ACE inhibitors nor ARBs were associated with increased risks of requiring ICU care for COVID-19 disease. A recent randomized controlled clinical trial involved 659 patients hospitalized in Brazil with mild to moderate COVID-19 who were taking ACEIs or ARBs prior to hospitalization [193]. The objective was to determine whether discontinuation compared with continuation of ACE inhibitors or ARBs changed the number of days alive and out of the hospital through 30 days. There was no significant difference in the mean number of days alive and out of the hospital for those assigned to discontinue vs. continue these medications. These results were generally consistent across major subgroups and do not support routine discontinuation of ACEIs or ARBs among patients hospitalized with mild to moderate COVID-19 if there is an indication for treatment.

RAAS inhibitors could benefit patients with COVID-19 through effects on angiotensin II expression and subsequent increase in Ang-(1–7) and -(1–9), which have vasodilatory and anti-inflammatory effects that might attenuate lung injury [195]. Data from animal studies suggest an inherent protective effect of ARBs against COVID-19 pneumonia by limiting lung injury in mice infected with SARS-CoV, a close viral relative of SARS-CoV-2 [196]. Based on the available evidence, scientific societies have recommended that patients should not discontinue ACE inhibitor or ARB therapy during the COVID-19 pandemic.

Conclusions and Future Directions

Inhibition of the RAAS by either ACE inhibitors or ARBs has been shown to provide protective effects against cardiovascular outcomes in diabetic patients. These beneficial effects appear to similarly apply to both type 1 and type 2 diabetes.

Although most of our current understanding of the RAAS in diabetes is associated with the ACE → Ang II → AT_1 receptor pathway, a growing body of experimental evidence has implicated important contributions of renin, ACE2, AT_2 receptors, Ang-(1–7), as well as aldosterone in mediating the effects of the RAAS on vascular functions and disease. A limited number of studies suggest that the RAAS contains pathways that are antagonistic; however, clinical evidence for this level of regulation within the RAAS is not currently available. Further studies are needed to characterize "crosstalk" within the RAAS and to determine whether targeting additional components in this system might provide new therapeutic opportunities.

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Chapter 32
Metformin, Sulfonylureas, DPP-4 Inhibitors and Cardiovascular Outcomes in Type 2 DM

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Introduction

Type 2 diabetes mellitus (T2DM) is associated with a high risk of cardiovascular disease (CVD), including heart failure (HF) [1]. Two pharmacological classes of glucose-lowering agents have demonstrated a significant reduction in major cardiovascular events (MACEs) in patients with T2DM and established CVD or multiple cardiovascular (CV) risk factors, glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium-glucose cotransporter type 2 inhibitors (SGLT2is) [2–6]. As a consequence, these agents now occupy a privileged place in the management of T2DM with CVD [7, 8]. Because of different results reported in cardiovascular outcome trials (CVOTs) [4, 5], GLP-1Ras are recommended (as SGLT2is) in patients with atherosclerotic disease, whereas SGLT2is are preferred in patients with or at risk of HF or progressing renal disease with albuminuria [8]. Even if these new glucose-lowering agents were used as add-on therapy in CVOTs, a majority of T2DM patients being treated with other glucose-lowering agents (in particular, background metformin and/or sulfonylureas), some recent cardiology guidelines, both in the US [9] and in Europe [10], proposed to use GLP-1RAs or SGLT2is as first-line therapy in patients with T2DM and at high CV risk. The reason is that uncertainties remain regarding the CV safety and/or efficacy of other glucose-lowering agents such as metformin, sulfonylureas (SUs) and dipeptidyl peptidase-4 inhibitors (DPP-4is) in patients with T2DM [11, 12]. One dilemma is that a majority

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of patients with T2DM do not have established CVD but share several CV risk factors and the question how to treat these patients, i.e. which glucose-lowering agent should be selected first and which combination should be preferred afterwards, remains open.

The aim of the present chapter is to review the effects of metformin, SUs and DPP-4is on CV outcomes in patients with T2DM, based upon data reported in randomized controlled trials (RCTs) or observational studies, including meta-analyses.

Metformin

Metformin has been recommended as a first-line antidiabetic drug for all patients with T2DM even in the presence of high CV risk in the latest consensus reports by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) [7, 8]. However, the first place of metformin has been challenged by cardiologists [9, 10]. Thus, the right place for metformin in the management of patients with T2DM, especially those at high CV risk, is currently a matter of debate [13–17].

Underlying Mechanisms

Metformin does not stimulate insulin secretion and thereby does not expose to hypoglycaemia. It rather acts as an insulin-sparing agent via multiple effects. Its main sites of action are the liver, where it inhibits hepatic glucose production, and the intestine, where it increases glucose consumption, enhances GLP-1 production and positively modifies gut microbiota [18] (Table 32.1).

In both animal and human studies, multiple potential mechanisms have been shown that support the concept of CV protection with metformin beyond those provided by reduced blood glucose, including improvements in haemostatic function (reduction in increased levels of plasminogen activator inhibitor-1), reduced low-grade inflammation and oxidative stress, and inhibition of key steps in the process of atherosclerosis [18, 19].

United Kingdom Prospective Study (UKPDS)

The UKPDS was the first RCT that emphasized the potential beneficial CV effect of metformin. In patients whose body weight was more than 120% of their ideal weight and who primarily received metformin, reductions in the risk of myocardial infarction by 39% ($P = 0.01$) and of all-cause death by 36% ($P = 0.01$) were observed

Table 32.1 Characteristics of the three classes of glucose-lowering agents considered in the present chapter

Classes	Molecules	Glucose-lowering effect	Possible pleiotopic effects	Possible adverse effects
Biguanides[a]	Metformin	Insulin-sparing agent (mainly via liver and gut effects)	Reductions in PAI-1, low-grade inflammation and oxidative stress	Lactic acidosis (rare)
Sulfonylureas[b]	Glibenclamide (glyburide), gliclazide, glimepiride, glipizide	Insulin-secretagogue (glucose-independent)	Inhibition of extrapancreatic K_{ATP} channels	Hypoglycemia Weight gain Inhibition of ischemic pre-conditioning
DPP-4is[c]	Alogliptin, linagliptin, saxagliptin, sitagliptin	Incretin enhancers (increased insulin/decreased glucagon)	Varia, yet clinical relevance needs confirmation	Small increased risk of acute pancreatitis Increased heart failure with saxagliptin

[a]Phenformin and buformin were withdrawn because of an increased risk of lactic acidosis
[b]First-generation agents such as tolbutamide and chlorpropamide are not used anymore
[c]Vildagliptin not commercialized in the United States. Several other DPPis only commercialized in Asia not mentioned here
PAI-1 plasminogen activator inhibitor-1, *DPP-4is* dipeptidyl peptidase-4 inhibitors

[20]. Interestingly enough, in the group previously treated with metformin, the CV protection persisted after an observational follow-up of 10 years after the end of the UKPDS, with a 33% reduction in myocardial infarction ($P = 0.005$) and a 27% reduction in death from any cause ($P = 0.002$) [21]. However, these results were obtained in a limited subgroup of obese patients with newly diagnosed T2DM, i.e. in individuals with a rather low CVD risk [20]. This population is completely different from the patients recruited in recent CVOTs, who generally had a long duration of T2DM (>10 years) and a high/very high risk of CVD, most of them having established CVD [2, 3, 6].

Meta-Analysis of Clinical Trials and Observational Studies

In absence of a dedicated CVOT, meta-analyses of published data have been performed in order to evaluate the potential impact on CVD of the first-line drug metformin. When RCTs were considered, no reduction in the incidence of CV events could be detected, but none of the trials was designed to test this hypothesis and all of them recruited a large majority of patients without established CVD [22–24] (Table 32.2). However, a meta-analysis that combined results from some RCTs and mainly retrospective cohort studies in patients with coronary artery disease showed that metformin significantly reduces both all-cause (−37%) and CV (−19%)

Table 32.2 Meta-analyses of clinical trials and observational studies that investigated the cardiovascular effects of metformin versus comparators (either placebo or another active glucose-lowering agent)

References	Type of studies	Mode of comparison	*N* Metformin/ comparators	All-cause mortality	CV mortality	Myocardial infarction	Stroke	Heart failure	CVD incidence
Lamanna et al. 2011 [22]	12 RCTs	M-H OR	5455/8996	1.103 (0.804–1.513)	0.923 (0.361–2.320)	0.90 (0.71–1.14)	0.92 (0.65–1.29)	1.12 (0.25–9.04)	0.937 (0.820–1.070)
Boussageon et al. 2012 [23]	13 RCTs	Risk ratio	9560/3550	0.99 (0.75–1.31)	1.05 (0.67–1.64)	0.90 (0.74–1.09)	0.76 (0.51–1.14)	1.03 (0.67–1.59)	NA
Griffin et al. 2017 [24]	13 RCTs	Risk ratio	2079/2079	0.96 (0.84–1.09)	0.97 (0.80–1.16)	0.89 (0.75–1.06	1.04 (0.73–1.48)	NA	NA
Han et al. 2019 [25][a]	15 RCTs, 22 cohort studies, 3 case–control studies	HR Inverse variance, fixed	1,068,408 (all patients)	0.67 (0.60–0.75)	0.81 (0.79–0.84)	NA	NA	NA	0.83 (0.78–0.89)
Zhang et al. 2020 [26]	16 cohort studies	OR	701,843/458,411	NA	0.44 (0.34–0.57	NA	NA	NA	0.44 (0.34–0.57)

[a]Patients with coronary artery disease

CV cardiovascular, *CVD* cardiovascular disease, *HR* hazard ratio, *M-H* Mantel-Haenszel, *NA* not available, *OR* odds ratio, *RCTs* randomized controlled trials

mortality as well as the incidence of CV events (−17%) [25] (Table 32.2). Similarly, a recent meta-analysis of 16 studies including 25 comparisons indicated that metformin treatment was associated with a reduction by about half in CV outcomes, when considering both the mortality and the incidence [26] (Table 32.2). However, the heterogeneity among studies may potentially affect the final results. Meta-analyses of RCTs have not demonstrated the benefits of metformin on the risk of or the clinical course of HF [27].

In a post hoc analysis of SAVOR TIMI-53, metformin use was associated with no difference in risk for the composite end point MACEs (hazard ratio or HR 0.92, 95% confidence interval or CI 0.76–1.11), but lower risk of all-cause mortality (HR 0.75, 95% CI, 0.59–0.95) [28].

Finally, observational studies, including the REACH (Reduction of Atherothrombosis for Continued Health) registry [29], have shown that metformin exerts protective effects, with lower mortality rate, even in patients considered at higher risk, such as patients with renal impairment, stable coronary heart disease and stable HF [30].

Potential Modulation of CV Outcomes by Metformin

Previous data suggested that metformin could differently modulate the effects of new oral glucose-lowering therapies: on the one hand, SGLT2is with lower protection in metformin-treated patients [31, 32], on the other hand, DPP-4is with better protection in metformin-treated patients [33, 34]. In order to test this hypothesis, we performed two meta-analyses of published CVOTs and did not find arguments to support a different modulation by metformin background therapy of the effects of DPP-4is and SGLT2is in T2DM patients at high risk of CVD [35]. In both metformin and non-metformin users, DPP-4is did not reduce the incidence rate of MACEs, whereas SGLT2is showed a significant reduction, without any significant interaction between the two subgroups with and without metformin. The absence of modulation by background metformin therapy of CV outcomes with SGLT2is was confirmed in three other recent meta-analyses [36–38]. A possible positive effect of metformin on CV outcomes with DPP-4is was suggested in another meta-analysis [38], but this paper did not include the results from the latest trial CAROLINA, in contrast to our meta-analysis [35].

Perspectives

A definitive evidence base for prioritization of new drugs versus metformin is currently missing because there are no head-to-head RCT data [17]. Even if some evidence suggests that metformin may exert a protective effect on CVD beyond its

glucose-lowering effects [15], future prospective cohort-based studies and dedicated RCTs are needed to identify CV at-risk population who may potentially benefit from metformin [15, 39]. A population-based, longitudinal-cohort study using a nationwide US commercial insurance claims database is ongoing (Metformin And Cardiovascular Effectiveness Versus SGLT2: MACES; ClinicalTrials.gov Identifier: NCT03627039). Its objective is to compare in new users with T2DM the effectiveness of SGLT2is relative to metformin for reducing subsequent CV events (estimated study completion date August 2020, results not available yet). A specific CVOT comparing the effect of metformin versus placebo is also underway. Because it is not possible anymore to perform such a trial in patients with T2DM for ethical reasons, this CVOT will recruit patients with impaired glucose tolerance (VA-IMPAcT: Investigation of Metformin in Pre-Diabetes on Atherosclerotic Cardiovascular OuTcomes; ClinicalTrials.gov Identifier: NCT02915198). This trial will test the hypothesis that treatment with metformin, compared with placebo, reduces mortality and CV morbidity in patients with pre-diabetes and established CVD (estimated study completion date: August 2024).

Sulfonylureas

In the 2018–2020 ADA-EASD consensus reports, SUs occupy a limited place, because they can induce weight gain and hypoglycaemia and apparently do not exert protective CV effects. Their main advantage is a very low cost combined with a long clinical experience [7, 8]. Since many decades, the CV safety of SUs is still contentious [40, 41]. However, recent data, especially the findings of CAROLINA [42], may offer a new revival to SUs in the management of hyperglycaemia in patients with T2DM [43–45].

Underlying Mechanisms

SUs are insulin-secreting agents, which stimulate insulin secretion independently of plasma glucose levels, without any direct effect on insulin resistance [46] (Table 32.1). No consistent effects have been demonstrated on CV risk factors such as arterial blood pressure, lipid profile or low-grade inflammation. Thus, besides their well-recognized glucose-lowering effect, no particular positive effects on CVD risk may be expected. On the contrary, because SUs are associated with a higher risk of (sometimes severe) hypoglycaemia, which results in an activation of sympathetic tone, potential deleterious CV effects may be suspected [40, 41].

SUs trigger insulin release by binding to SU receptors (SUR_1) and inhibiting K_{ATP} channels on the pancreatic beta-cells. Extra-pancreatic K_{ATP} channels and SU receptors exist in abundance in cardiac myocytes (SUR_{2A}) and smooth muscle cells

(SUR_{2B}). Off-target K_{ATP} channel inhibition in the heart and vascular smooth muscle may contribute to adverse CV effects, i.e. inhibition of pre-conditioning ischemia [47]. SUs show different tissue-specific binding affinities. For instance, gliclazide and glipizide are more selective for pancreatic SUR_1 compared with glibenclamide (glyburide), which binds non-selectively to both pancreatic SUR_1 and CV SUR_{2A} and SUR_{2B}. These particularities might explain differences between SUs regarding CV safety (see below) [40].

From UGDP to UKPDS

In the seventies, the University Group Diabetes Program (UGDP) gave intriguing, yet controversial, results with a higher rate of mortality associated with the first-generation SU tolbutamide [48]. Furthermore, the percentage of patients identified during the course of follow-up with a specified nonfatal cardiac event was higher in the tolbutamide-treated group than in the placebo-treated group [49]. However, the study was highly criticized from a statistical point of view, because of rather few CV events and possible interference of confounding factors. Published in 1998, the UKPDS followed 2867 newly diagnosed patients with T2DM, randomized to intensive treatment with a SU [glibenclamide (glyburide), glipizide or chlorpropamide] or insulin or conventional treatment with diet alone. After a median follow-up of 11 years, there was no evidence that SUs (glibenclamide (glyburide) and chlorpropamide) were associated with increased mortality or a higher risk of myocardial infarction or stroke (Table 32.3) [50]. Even if the findings with SUs were less favourable than those noticed with metformin (Table 32.2), as already described above [53], the results of the UKPDS regarding the use of SUs and CV outcomes were reassuring when compared to those of the UGDP [40].

More Recent Randomized Clinical Trials

Three large RCTs deserve careful examination (Table 32.3). First, in the international ADVANCE trial, intensifying glucose lowering-therapy with controlled release gliclazide was not associated with any significant change in the incidence of CV events when compared to standard therapy [51]. Second, in the long-term, pragmatic TOSCA-IT trial, the incidence of CV events was similar with SUs (mostly glimepiride and gliclazide) and pioglitazone as add-on treatments to metformin [52]. Third, in the CAROLINA study that recruited adults with relatively early T2DM and elevated CV risk, the use of glimepiride compared to the DPP-4i linagliptin over a median 6.3 years resulted in non-significant difference in the occurrence of CV outcomes [42] (Table 32.3). Thus, overall, these data are reassuring when comparing SUs with different type of comparators.

Table 32.3 Randomized controlled trials that investigated the cardiovascular safety of sulfonylureas

RCTs	Sulfonylurea	Comparator	Follow-up (years)	*N* SU/ comparator	All-cause mortality	CV mortality	Myocardial infarction	Stroke	MACEs
UKPDS [50]	Chlorpropamide	Diet alone	11.1	619/1138	1.02 (0.82–1.27)	NA	0.87 (0.68–1.12)	1.01 (0.65–1.58)	NA
	Glibenclamide	Diet alone	11.1	615/1138	0.91 (0.73–1.15)	NA	0.78 (0.60–1.01)	1.38 (0.52–2.08)	NA
ADVANCE [51]	Extended release gliclazide (intensified arm)	Standard treatment	5.0	5571/5569	0.93 (0.83–1.06)	0.88 (0.74–1.04)	0.98 (0.78–1.23) nonfatal	1.02 (0.85–1.24) nonfatal	0.94 (0.84–1.06)
TOSCA-IT [52]	Glibenclamide Glimepiride Gliclazide	Pioglitazone	4.8	1493/1535	0.91 (0.62–1.33)	NA	1.15 (0.65–2.08) nonfatal	1.27 (0.65–2.44) nonfatal	1.04 (0.79–1.35)
CAROLINA [42]	Glimepiride	Linagliptin	6.3	3010/3023	1.10 (0.94–1.28)	1.00 (0.80–1.23)	0.97 (0.78–1.22)	1.16 (0.89–1.52)	1.02 (0.88–1.19)

Results are expressed by hazard ratio (with 95% confidence intervals) of SUs versus comparators

HR hazard ratio, *CI* confidence interval, *MACEs* major cardiovascular events, *NA* not available, *NS* not significant, *RCTs* randomized controlled trials, *SU* sulfonylurea

Meta-Analysis of Clinical Trials

– **SUs versus metformin**
In a meta-analysis published in 2013, only two RCTs compared CV outcomes and mortality with SUs versus metformin. SUs were associated with a numerical increase in all-cause mortality compared with metformin, without any increase in the incidence of MACEs [54]. These results were confirmed in two network meta-analyses [55, 56] and were extended in another meta-analysis published in 2017, yet this meta-analysis combined both RCTs and observational studies [57]. Compared with biguanides, SUs were associated with a significant increase in all-cause mortality and a trend for higher risk in CV mortality, acute myocardial infarction and stroke (Table 32.4). In the SPREAD-DIM-CAD study carried out in Chinese patients with T2DM and coronary artery disease, treatment with metformin for 3 years substantially reduced MACEs in a median follow-up of 5.0 years compared with glipizide (HR 0.54; 95% CI 0.30–0.90; $P = 0.026$) [61].

– **SUs versus DPP-4is**
More studies reported CV outcomes in patients treated with SUs compared with DPP-4is [62] (Table 32.4). Monami et al. found a significant higher risk of MACE, mainly attributed to more ischemic strokes, in patients treated with SUs than in those treated with DPP-4is [54]. According to another meta-analysis of 12 head-to-head RCTs, SUs were associated with a significantly higher incidence of CV events as compared with DPP-4is [58]. Another meta-analysis of 8 RCTs data showed an increased risk of myocardial infarction, ischemic stroke, CV mortality and all-cause mortality with SUs compared with DPP-4is, all statistically significant. Again, the most marked difference concerned the risk of ischemic stroke [57].

Network meta-analyses gave divergent results. One showed only a trend for higher incidence of all-cause mortality, CV mortality and myocardial infarctions in SU-treated patients compared to those treated with a DPP-4i [55] while two others reported a significantly increased risk of myocardial infarction [60] or of MACEs [56] with SUs compared to DPP-4is (Table 32.4).

– **Insulin secretagogues versus placebo or active comparators**
A recent meta-analysis suggested that insulin secretagogues (all SUs plus repaglinide and nateglinide: see below) compared with either placebo or active comparators are not significantly associated with an increased risk of MACEs in comparison with controls (14 RCTs, Mantel-Haenszel odds ratio [MH-OR] 1.08, 95% CI 0.96–1.22, $P = 0.20$), but were associated with an increased risk of all-cause mortality (48 RCTs, MH-OR 1.11, 95% CI 1.00–1.23, $P = 0.04$) [63].

Table 32.4 Meta-analyses of clinical trials and observational studies that investigated the cardiovascular effects of sulfonylureas versus comparators

References	Type of studies	Mode of comparison	All-cause mortality	CV mortality	Myocardial infarction	Stroke	MACEs
Versus metformin							
Monami et al. 2013 [54]	2–4 RCTs	MH-OR	1.29 (0.80–2.13)	NA	NA	NA	0.95 (0.34–2.70)
Bain et al. 2017 [57]	RCTs + observational studies	HR	1.37 (1.03–1.84)	1.38 (0.90–2.16)	1.21 (0.78–1.99)	1.40 (0.92–2.22)	NA
Lee et al. 2017 [55]	2 RCTs	RR	1.21 (0.79–1.85)	1.62 (0.72–3.68)	0.82 (0.47–1.46)	NA	NA
Wu et al. 2018 [56]	RCTs	OR	NA	NA	NA	NA	1.19 (0.52–2.78)
Versus DPP-4is							
Monami et al. 2013 [54]	7 RCTs	MH-OR	1.40 (0.74–2.65)	1.50 (0.49–4.52)	NA but NS	4.51 (1.60–12.66)	1.85 (1.20–2.87)
Zhang et al. 2014 [58]	12 RCTs	MH-OR	NA	NA	NA	NA	1.89 (1.15–2.63)
Bain et al. 2017 [57]	RCTs + observational studies	HR	2.03 (1.22–3.58)	4.42 (1.92–13.00)	2.54 (1.14–6.57)	9.40 (3.27–41.90)	NA
Wang et al. 2017 [59]	2 RCTs + 6 cohort studies	RR	1.39 (1.15–1.69)	1.72 (1.22–2.44)	NA	NA	1.41 (1.11–1.79) (nonfatal only)
Lee et al. 2017 [55]	14 RCTs	RR	1.45 (0.97–2.17)	2.08 (0.91–4.76)	1.37 (0.91–2.08)	NA	NA
Chou et al. 2017 [60]	10 RCTs	OR	NA	NA	2.08 (1.10–3.70)	NA	NA
Wu et al. 2018 [56]	28 RCTs	OR	NA	NA	NA	NA	1.27 (1.00–1.61)

CV cardiovascular, *HR* hazard ratio, *MACEs* major cardiovascular events, *M-H* Mantel-Haenszel, *NA* not available, *NS* not significant, *OR* odds ratio, *RCTs* randomized controlled trials, *RR* relative risk

Observational Studies

SUs Versus Metformin

Using US National Veterans Health Administration databases, in patients without chronic kidney disease who initiated metformin or SU therapy for diabetes, the

incidence of MACEs was significantly higher in SU users than in metformin users (adjusted hazard ratio or aHR 1.21, 95% CI 1.13–1.30) [64]. These results were confirmed by the same research group among patients with diabetes and reduced kidney function persisting with monotherapy in whom treatment with metformin, compared with a SU, was associated with a 20% lower risk of MACEs (aHR 0.80, 95% CI 0.75–0.86) [65]. In another study, the relative risk of HF in SU users versus metformin users was 1.17 (95% CI, 1.06–1.29) (5 cohort studies) and 1.22 (1.02–1.46) when restricted to new users (two studies) [66]. A meta-regression analysis was used to evaluate heterogeneity of observational studies. SUs were associated with an increased risk of CV events and mortality in the majority of studies with no major design-related biases [67]. When considering 27 relative risk estimates, the mean relative risk of occurrence of a CV adverse event in patients treated with SUs compared with patients treated with metformin averaged 1.43, with an adjusted relative risk ratio of 1.13 (95% CI 1.01–1.27) [67].

SUs Versus DPP-4is

The comparison of CV efficacy and safety between SUs and DPP-4-is has been extensively discussed in a dedicated review [62]. Overall, the CV safety of SUs appears to be poorer than that of DPP-4is in cohort studies, thus confirming the findings of RCTs. However, the results are somewhat disparate, and such heterogeneity may be explained not only by different patient characteristics across studies, but also perhaps by differences between various molecules in each pharmacological class [62] (see discussion below).

During a median follow-up of 19.6 months of nationwide cohort in Korea and after propensity score matching, there was no significant difference in the risk of ischemic heart disease, ischemic stroke or cardio-cerebrovascular death in the DPP-4i group compared to that in the SU group in combination with metformin [68]. However, in a meta-analysis of eight studies (six of them being retrospective cohort studies), the combination therapy of metformin plus DPP-4i versus metformin plus SU was associated with lower rates of nonfatal CV events (relative risk or RR 0.71, 95% CI 0.56–0.90), CV mortality (RR 0.58, 95% CI 0.41–0.82) and all-cause mortality (RR 0.72, 95% CI 0.59–0.87) [59]. A significant higher incidence of all-cause mortality in patients treated with SU compared to patients treated with DPP-4is (HR 2.03, 95% CI 1.22–3.58) was also reported in another meta-analysis of four observational studies [57].

Are All Sulfonylureas Similar Regarding CV Risk?

As already mentioned, differences in the pharmacological properties of SUs, i.e. different tissue-specific binding affinities to $SUR_{1/2}$ K_{ATP} channels and different risks of hypoglycaemia, may result in different risks of mortality and CV events among SUs [40]. As discussed in several papers, it is important to distinguish

between first-generation SUs (tolbutamide, chlorpropamide), which were associated with increase CV risk, and second-generation SUs, which appear to be much safer [40, 69, 70]. Among SUs of second generation, it has been recommended not to use glibenclamide (glyburide) because this sulfonylurea causes more hypoglycaemia, interferes with ischemic preconditioning and may be associated with an increased incidence of CV events compared with other second-generation SUs [71]. However, this conclusion may be challenged by the results of recent studies. First, in a large observational US study, results of increased risk of MACEs with SUs compared with metformin were consistent for both glibenclamide (glyburide) (aHR, 1.26, 95% CI 1.16–1.37) and glipizide (aHR 1.15, 95% CI 1.06–1.26) [64]. Second, a UK cohort study, which included 17,604 SU initiators with a mean follow-up of 1.2 years, showed no difference in CV safety between nonspecific, long-acting SUs glibenclamide and glimepiride and specific, short-acting SUs gliclazide and glipizide [72]. Third, in a retrospective cohort study using U.S. Medicaid claims from five large states, compared with glipizide, propensity score-adjusted HR for sudden cardiac arrest and ventricular arrhythmia were 0.82 (95% CI 0.69–0.98) for glibenclamide (glyburide) and 1.10 (0.89–1.36) for glimepiride, respectively [73]. Fourth, using patients on glimepiride as the reference group in a nationwide real-world analysis from Taiwan, the adjusted HR of CV event risk was 1.22 ($P = 0.005$) for gliclazide, 1.19 ($P = 0.073$) for glipizide, and 1.32 ($P < 0.001$) for glibenclamide (glyburide), with no obvious differences between the last three compounds [74]. Fifth, another population-based cohort study in the Netherlands reported that the risks of a first-ever acute myocardial infarction (aHR 1.02, 95% CI 0.70–1.50) and all-cause mortality (aHR 0.97, 95% CI 0.80–1.17) were not significantly different when comparing gliclazide use with non-gliclazide SU use (among which glibenclamide) [75].

In a network meta-analysis of 18 studies, the relative risk of CV-related mortality (0.60, 95% CI 0.45–0.84) was significantly lower with gliclazide compared with glibenclamide (glyburide), but not significantly different compared with glimepiride [70]. Recent review papers summarized emerging evidence suggesting better CV profile of gliclazide over other SUs [76, 77]. However, in the absence of a dedicated head-to-head trial, this remains an open question.

Glinides, as an Alternative to Sulfonylureas

Meglitinide derivatives (repaglinide, nateglinide) are insulin-secreting agents whose mechanism of action is almost similar to that of SUs. They have a shorter half-life and thereby should be administered three times a day, before each main meal (‘one meal, one pill’). These pharmacokinetic properties may result in a better control of postprandial hyperglycaemia combined with a lower risk of late hypoglycaemia compared to SUs [78]. However, few data are available regarding the CV safety and efficacy of these compounds [78].

Among persons with impaired glucose tolerance and established CVD or CV risk factors, assignment to nateglinide versus placebo for 5 years did not reduce the coprimary composite CV outcome (a composite of death from CV causes, nonfatal myocardial infarction, nonfatal stroke or hospitalization for HF) in the large prospective NAVIGATOR study [79]. Using patients on glimepiride as the reference group, the aHR of all-cause mortality and CV event risk were, respectively, 1.88 ($P < 0.001$) and 1.69 ($P = 0.001$) for repaglinide in a nationwide real-world analysis in Taiwan [74].

DPP-4 Inhibitors

DPP-4is occupy an increasing place in the management of T2DM [80], progressively replacing SUs in numerous countries. The reasons for this trend are that DPP-4is are not associated with hypoglycaemia or weight gain, have a good safety profile, including in a frailty elderly population and are very easy to use (generally one tablet a day, without titration) [81]. They can be prescribed in patients with moderate to severe chronic kidney disease, provided that the daily dose is adjusted to the estimated glomerular filtration rate, except for linagliptin that does not require dose adjustment because of a biliary rather than a renal excretion [82]. Of note, a small increased risk of acute pancreatitis associated with DPP-4is has been reported, yet it remains a very rare adverse event [80, 81].

Underlying Mechanisms

DPP-4is act as incretin enhancers by inhibiting the enzyme that degrades two gut-derived incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [80, 83]. Thereby, they stimulate insulin secretion and reduce glucagon secretion in a glucose-dependent manner, both effects contributing to the glucose-lowering activity without increasing the risk of hypoglycaemia (Table 32.1).

Beyond the glucose-lowering effect, DPP-4is may positively influence surrogate vascular endpoints and other CV risk factors, as extensively discussed in previous reviews [84–87]. GLP-1 is classically viewed as the primary DPP-4 substrate capable in modulating CV function [86, 88]. However, DPP-4, which is widely expressed in most cells and tissues, exhibits enzymatic activity against dozens of peptide hormones and chemokines with roles in vascular pathophysiology, inflammation, stem cell homing and cell survival [85]. Thus, DPP-4is may exert a possible beneficial action on vessels and heart, via both GLP-1-dependent and GLP-1-independent effects [86, 88].

Clinically, DPP-4is improve several CV risk factors beyond the improvement of glucose control (mainly by reducing postprandial hyperglycaemia). They show

modest weight loss (even if weight neutrality is a classic concept), may be associated with mild reduction (or no significant changes) in blood pressure without increase in heart rate, somewhat improve postprandial lipid profile, slightly reduce inflammatory markers, dampen oxidative stress and improve endothelial function in patients with T2DM [84, 86]. Some positive effects were also described on the heart in patients with ischemic heart disease or congestive HF, yet their clinical relevance remains to be further investigated [11, 84].

CV Outcomes in Meta-Analyses of Phase 2–3 Trials

Several meta-analyses of RCTs with individual DPP-4is generally reported a non-significant trend towards a lower incidence of MACEs compared to placebo or other active glucose-lowering compounds: alogliptin [89], saxagliptin [90], sitagliptin [91], linagliptin [92] and vildagliptin [93]. It is noteworthy, however, that none of these trials were designed to test CV safety/efficacy of the DPP-4i; moreover, patients were at rather low risk of CV disease (primary prevention), the trial duration was quite short (generally ≤1 year) and CV events were not always properly adjudicated. Because of the rather low number of MACEs in each individual DPP-4i meta-analysis, the differences failed to reach statistical significance.

Contradictory results were reported when the results of all studies with DPP-4is were pooled. In one meta-analysis of 70 phase 2–3 RCTs comparing DPP-4is with a placebo or an active glucose-lowering agent [94], significant reductions in the incidence of MACEs, myocardial infarction and all-cause mortality and a trend for lower incidence of stroke and CV mortality were reported. Another meta-analysis separated RCTs using placebo from those using an active glucose-lowering agent as comparator. When compared to placebo in 11 RCTs, DPP-4is did not significantly affect the risk of all-cause mortality, CV death, myocardial infarction, stroke or HF. When compared to active controls in 29 RCTs, DPP-4is showed a significant reduction in the risk of stroke (HR 0.58; 95% CI 0.34–0.99), but did not significantly affect other CV endpoints [95].

Results of Dedicated CV Outcome Trials

Five CVOTs that specifically investigated the CV safety and efficacy of DPP-4is have been published : EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care) with alogliptin [96], SAVOR TIMI-53 (Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction 53) with saxagliptin [97], TECOS ('Trial Evaluating Cardiovascular Outcomes with Sitagliptin'), with sitagliptin [98], CARMELINA (Cardiovascular and Renal Microvascular Outcome Study With Linagliptin in Patients With Type 2 Diabetes Mellitus with linagliptin) [99] and

Table 32.5 Cardiovascular outcome trials comparing a DPP-4 inhibitor with a placebo (or glimepiride in CAROLINA)

Clinical trial	DPP-4 inhibitor Daily dose[a]	DPP-4i versus comparator (placebo, except CAROLINA) *n*	History of CV disease % patients	Median Follow-up years	Primary CV composite outcome[b]	Myocardial infarction (fatal or nonfatal)	Stroke (fatal or nonfatal)	CV mortality	All-cause mortality	Hospitalization for heart failure
SAVOR-TIMI 53 [97]	Saxagliptin 5 mg	8280 versus 8212	78	2.1	1.00 (0.89–1.12)	0.95 (0.80–1.12)	1.11 (0.88–1.39)	1.03 (0.87–1.22)	1.11 (0.96–1.27)	1.27 (1.07–1.51)
EXAMINE [96]	Alogliptin 25 mg	2701 versus 2679	100	1.5	0.96 (≤1.16)[c]	1.08 (0.88–1.33)	0.95 (≤1.14)[c]	0.85 (0.66–1.10)	0.88 (0.71–1.09)	1.07 (0.79–1.46)
TECOS [98]	Sitagliptin 100 mg	7257 versus 7266	100	3.0	0.98 (0.89-1.08)	0.95 (0.81–1.11)	0.97 (0.79–1.19)	1.03 (0.89–1.19)	1.01 (0.90–1.14)	1.00 (0.83–1.20)
CARMELINA [99]	Linagliptin 5 mg	3494 versus 3485	57	2.2	1.02 (0.89–1.17)	1.12 (0.90–1.40)	0.91 (0.67–1.23)	0.96 (0.81–1.14)	0.98 (0.84–1.13)	0.90 (0.74–1.08)
CAROLINA [42]	Linagliptin 5 mg	3023 versus 3010 (glimepiride)	35	6.3	0.98 (0.84–1.14)	1.03 (0.82–1.29)	0.86 (0.66–1.12)	1.00 (0.81–1.24)	0.91 (0.78–1.06)	1.21 (0.92–1.59)

Results are expressed by hazard ratio (with 95% confidence intervals) of DPP-4i versus comparator

[a]Reduction of daily dose if necessary according to estimated glomerular filtration rate

[b]Cardiovascular death, nonfatal myocardial infarction or nonfatal stroke

[c]Upper boundary of the one-sided repeated confidence interval

CV cardiovascular, *DPP-4is* dipeptidyl peptidase-4 inhibitors, *NA* not available

CAROLINA (Cardiovascular Outcome Study of Linagliptin Versus Glimepiride in Patients With Type 2 Diabetes) with linagliptin [42] (Table 32.5). The last two trials with linagliptin are original ones. Indeed, CARMELINA recruited a much higher proportion of patients with impaired renal function and albuminuria compared with other CVOTs [99], while CAROLINA was the only CVOT that used an active comparator (glimepiride, a SU used as reference) instead of the placebo used in all other trials [42]. The main results are summarized in Table 32.4. Overall no significant differences were observed between patients treated with a DPP-4i or a placebo (glimepiride in CAROLINA) regarding the incidence of MACEs, myocardial infarction, ischemic stroke, CV mortality and all-cause mortality. The only exception was a higher risk of hospitalization for HF in the SAVOR-TIMI 53 with saxagliptin compared with placebo [97] (see discussion below).

Similar results were reported with omarigliptin, a DPP-4i commercialized in Japan, in a CVOT trial that enrolled 4202 patients with T2DM and established CVD, but was early terminated (following a business decision not to submit a marketing application for omarigliptin in the United States) after a median follow-up of 96 weeks. No significant differences were observed between omarigliptin arm and placebo arm regarding the incidence of MACEs and hospitalization for HF [100].

Several meta-analyses of the first three prospective CVOTs (EXAMINE, SAVOR-TIMI 53, TECOS) failed to demonstrate any positive effect of DPP-4is compared to placebo on CV outcomes and mortality in diabetic patients with coexisting CVD [95, 101, 102]. Furthermore, because of the characteristics of the studied population, there was a lack of definitive evidence supporting the CV benefits of DPP-4is among diabetic patients free of CVD history [95].

Observational Studies

DPP-4is Versus Metformin

Using a new-user retrospective cohort derived from a US nationwide commercial claims database, DPP-4is exhibited similar risk for MACE compared to metformin, in patients with T2DM, without CVD or renal disease (adjusted HR = 1.07; 95% CI 0.97–1.18) [103].

DPP-4is Versus SUs

A systematic research of published data showed that the combination therapy of metformin plus DPP-4i was associated with a significant reduction in the relative risk of nonfatal CV events, CV mortality and all-cause mortality, compared with the combination therapy of metformin plus SUs [59]. In a retrospective US cohort, DPP-4is exhibited 13% lower risk for MACEs compared to SUs in T2DM patients

without CVD or renal disease (adjusted HR = 0.87; 95% CI 0.78–0.98), with significantly lower rates of acute myocardial infarction, stroke and HF [103].

DPP-4is Versus SGLT2is

In CVD-REAL2, a large, international, observational study, initiation of SGLT2is versus DPP-4is was associated with lower risks of HF, all-cause death, myocardial infarction and stroke [104]. These results confirmed findings reported in the CVD-REAL Nordic, which showed a higher rate of CV events in T2DM patients treated with DPP-4is compared to those treated with the SGLT2i dapagliflozin [105].

DPP-4is Versus Different Comparators

A meta-analysis that combined RCTs and observational cohorts/registries in a total number of 157,478 participants with T2DM showed that treatment with DPP-4is did not significantly increase CV outcomes [106]. In a recent study from Taiwan based on a large nationwide diabetic cohort of 113,051 patients with T2DM, DPP4is as a second- or third-line add-on treatment provided CV benefits (mainly reduction in all-cause mortality and stroke) compared with other glucose-lowering agents, including SUs, acarbose and meglitinide [107].

Concern About a Higher Risk of Heart Failure

The significant increase in the incidence of hospitalization for HF in patients treated with saxagliptin compared to patients having received placebo in SAVOR TIMI-53 (HR 1.27; 95% CI 1.07–1.51; $P = 0.007$) was a surprising finding [97], which rose further dedicated attention to HF [108, 109]. A non-significant trend for such an increase was also observed in EXAMINE [96] and in CAROLINA [42], but not in TECOS [98] and not in CARMELINA [99] (Table 32.4). Whether this risk may be considered as a class effect remains a matter of controversy [110].

The relative effect of DPP-4is [111, 112], in general, and of saxagliptin [113], in particular, on the risk of HF in patients with T2DM remains uncertain. A meta-analysis of RCTs concluded that the use of DPP-4is was associated with a modest and nonsignificant increase of the HF risk (+5%) [114], while another meta-analysis of observational studies (all with sitagliptin) suggested that DPP-4is may increase the risk of hospital admission for HF (aOR 1.41, 95% CI 0.95–2.09), compared with no use, in those patients with existing CVD or multiple risk factors for vascular diseases, but with very low-quality evidence [111]. However, a differential effect of each DPP-4i on the risk of HF has been pointed out in another meta-analysis: the use of saxagliptin significantly increased the risk of HF by 21%, especially among patients with high CV risk, while no signals were detected with other DPP-4is

[115]. Nevertheless, despite pooled data from 79,867 patients, including the data from the first three CVOTs (EXAMINE, SAVOR-TIMI 53, TECOS), whether DPP-4is increase HF overall or exhibit within-class differences remains unresolved [112]. Of note, in a nationwide T2DM cohort, DPP-4i use was not associated with a higher risk of hospitalization for HF even in patients with pre-existing HF [116].

The reason for the increase in hospitalization for HF in patients treated with saxagliptin in SAVOR TIMI 53 is unclear, the statistical analysis has been criticized and a chance finding could not be excluded [113]. Currently, the safety of saxagliptin regarding the risk of HF remains a matter of controversy, which, however, justifies a warning in the label of the compound.

Finally, in the VIVIDD ('Vildagliptin in Ventricular Dysfunction Diabetes') trial in patients with HF (New York Heart Association functional class I to III and left ventricular ejection fraction <0.40), vildagliptin, compared with placebo, had no major effect on left ventricular ejection fraction but did lead to an increase in left ventricular end-diastolic and end-systolic volumes, the cause and clinical significance of which are unknown [117]. In a network meta-analysis that included RCTs as well as a small number of cohort studies, DPP-4is were more strongly associated with a negative impact on left ventricular end-diastolic volume than were placebos [118]. Thus, more evidence is needed regarding the safety of DPP-4is in patients with HF and left ventricular systolic dysfunction.

Clinical Implications

Place of Classical Glucose-Lowering Agents

Although SGLT2is [119] and GLP-1RAs [120] gained much interest in recent years because of the demonstration of CV and renal protection in CVOTs [2–5], metformin, SUs and DPP-4is remain largely prescribed in the population with T2DM, both with and without established CVD. While metformin as first-line medication has been challenged in recent guidelines by cardiologists for T2DM patients with high risk of CVD [9, 10], it remains as initial background therapy (if well tolerated and not contraindicated) in all T2DM patients in the latest ADA-EASD consensus report [8], confirming its privileged place in the previous edition in 2018 [7]. Pros and contras of the use of metformin in patients with CVD remain highly debatable in the absence of a well-dedicated CVOT in high-risk T2DM patients [13–17]. Nevertheless, overall available data from observational studies support a positive CV impact of metformin. Furthermore, metformin, because of its pleiotropic effects, may be associated with a positive impact beyond any CV effect [14].

SUs are best positioned as glucose-lowering agents without any positive impact on CV outcomes. As they are associated with a risk of hypoglycaemia, the use of these agents should be restricted to patients with low risk of hypoglycaemia and should not be considered as an ideal medication in T2DM patients with

CVD. Nevertheless, despite a potentially increased CV risk associated with use of SUs, pre-existing CVD did not decrease clinicians' relative prescriptions of SUs according to the data of a registry in Denmark [121]. Finally, the risk may vary between different SUs (gliclazide and glimepiride being apparently associated with the more favourable profile) and recent findings of CAROLINA [42], which showed a similar safety CV profile of glimepiride compared to linagliptin, may lead to some revival of SUs [41].

The CV safety of DPP-4is has been extensively demonstrated in prospective CVOTs and in numerous observational studies [11]. However, this pharmacological class does not provide CV protection in contrast to GLP-1RAs [87] and SGLT2-is [11]. Thus, they should not be prescribed with the aim to reduce the risk of CVD. A higher risk of hospitalization for HF, reported with saxagliptin [97] and sometimes considered as a class effect [110], remains a matter of controversy [113]; nevertheless, a warning is inserted in the label of saxagliptin. One advantage of DPP-4is, besides their CV safety, is an excellent overall tolerance and safety profile so that these glucose-lowering agents are well suited for elderly and frailty patients with T2DM [80, 81].

Special Focus on Antidiabetic Agents in the COVID-19 Era

An increased risk of CVD has been noticed during the pandemic COVID-19 (coronavirus disease of 2019) [122] and T2DM is associated with a higher risk of more severe SARS-CoV-2 infection [123]. The prognostic factors in patients with T2DM exposed to COVID-19 are many [124], but the potential impact of antidiabetic agents on the course of the disease is unclear [125]. Several observational studies suggested that metformin may exert a positive influence by reducing the need for admission to intensive care units and the risk of mortality [126]. The potential effect of SUs is unknown in the absence of reported data [125]. Several retrospective observational studies compared the clinical outcomes between DPP-4i users versus non-users among diabetic patients with COVID-19. Overall, results regarding the risk of progression towards a severe form of the disease and mortality were heterogeneous, precluding from any definite conclusion [127]. Nevertheless, new expectations arose following recent reports of a significant reduction in admission in intensive care units and mortality in patients treated with sitagliptin [128]. However, because of limitations inherent to observational studies, available results should be considered at most as hypothesis generating hints pointing to potentially substantial benefits of DPP-4is in diabetic patients with COVID-19 [129]. While safe use of metformin and DPP-4is in COVID-19 patients seems an acceptable hypothesis, positive findings should be confirmed in RCTs before any recommendation for clinical practice. As recently emphasized, most of these conclusions are preliminary, and further investigation of the optimal management in patients with diabetes mellitus is warranted [130].

Conclusion

Metformin, SUs and DPP-4is are widely used in patients with T2DM, yet none of these three pharmacological classes has shown a clear-cut reduction in MACEs in patients with established CVD or several CV risk factors. This lack of evidence contrasts with the CV protection consistently demonstrated with SGLT2is and GLP-1RAs in dedicated CVOTs and observational studies. DPP-4is have proven CV safety in several large prospective CVOTs, but no superiority compared with placebo. Thus, their place in the management of T2DM is challenged by the alternative use of medications with proven CV protection. Nevertheless, their excellent safety profile is attractive, especially in elderly and/or frailty patients with T2DM. SUs were generally associated with a higher risk of MACEs and mortality compared with DPP-4is in meta-analyses of RCTs and this difference was confirmed in meta-analyses of observational studies. However, recent RCTs gave more reassuring results when gliclazide or glimepiride were compared with other glucose-lowering agents, including a DPP-4i in CAROLINA. Nevertheless, caution is required in patients with established CVD because of a higher risk of SU-associated hypoglycaemia. The position of metformin is more difficult to be defined. Indeed, while several indirect findings suggested a positive impact on CVD risk, thus confirming the initial observation of the UKPDS, no dedicated prospective CVOT has been performed with metformin in patients with T2DM and high CVD risk. Thus, the question arose whether metformin should be still considered as a first-line glucose-lowering therapy in such patients. Given metformin's importance in the management of T2DM and its widespread use in patients with CVD or HF, the current confidence in its benefits in high-risk patients needs to be re-evaluated in dedicated RCTs or real-life observational studies using extensive high-quality databases. It is of major importance to determine the correct priority of initial drug therapy, define hierarchical best combinations of glucose-lowering medications and/or identify specific patient populations most likely to benefit from the cheap drug metformin or new more expensive medications.

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Chapter 33
SGLT2 Inhibitors and GLP1 Antagonists on Diabetes and Cardiovascular Disease

David Fitchett

Introduction

Cardiovascular disease (CVD) is a common complication of diabetes [1] and results in an important reduction of life expectancy [2] and morbidity due to an increased prevalence of vascular events (MI/Stroke/Amputation) [3], heart failure [4] and diabetic kidney disease [5]. Heart failure in the patient with diabetes is common [6], often unrecognised [7], has a high mortality [8] and can be exacerbated by certain glucose-lowering agents [9]. Diabetic kidney disease is also common, progressive and the most frequent cause of end-stage renal disease [10].

Recently, there has been a paradigm shift in the management strategies for the patient with diabetes. For years, diabetes management had a glucocentric approach, with little focus on CV risk reduction. Subsequent research showed the enhanced benefit of CV risk factor reduction with control of risk factors such as hypertension, LDL cholesterol and lifestyle issues such as weight and smoking. In the past 5 years, glucose-lowering agents were identified, which reduce CV events. Until recently, we had no definitive evidence that any glucose-lowering agent impacted on cardiovascular outcomes. Studies with metformin [11] and pioglitazone [12] had suggested that they might reduce CVD; however, the trials were either too small or only secondary CVD outcomes were reduced. After concern that rosiglitazone might increase myocardial infarction and mortality [13], the FDA mandated that all new glucose-lowering agent be subjected to clinical safety trials in patients with high CV risk (U.S. Food and Drug Administration Center for Drug Evaluation and Research Guidance for Industry: Diabetes Mellitus—Evaluating Cardiovascular Risk in New Antidiabetic Therapies to Treat Type 2 Diabetes. Silver Spring, MD,

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U.S. Department of Health and Human Services, 2008, pp. 1–5). The trials had to include patients at high CV risk and be of sufficient duration to observe adequate numbers of CV events and show an upper confidence interval of the point estimate of hazard ration of the primary outcome to be less than 1.3. All were multicentred, placebo-controlled randomised controlled trials. The statistical design of the trial was to show non-inferiority, and then in the majority of the studies, if non-inferiority was shown, an assessment of superiority. Glucose control was encouraged in both the treatment and placebo arms, with the A1C level aimed at the local guideline target.

In 2015, the results of the EMPA-Reg Outcome trial with the sodium–glucose cotransporter 2 inhibitor (SGLT2i) empagliflozin changed clinical practice. It was the first randomised placebo-controlled clinical trial to show definitively that a glucose-lowering agent could reduce CV events. Subsequently, further studies with SGLT2i (with canagliflozin [14, 15] and dapaglifozin [16]) and three with a glucagon-like peptide 1 agonist (GLP-1 A) (liraglutide [17], dulaglutide [18] and semaglutide [19]) have demonstrated a reduction of CV events. However, there have been studies with agents from both classes of agents that have showed no CV benefit. This chapter will discuss the clinical trial evidence for each class of drugs, their potential mechanisms of action and clinical application.

Sodium–Glucose Cotransporter 2 Inhibitors (SGLT2i)

Glucose filtered by the glomerulus is effectively removed from the filtrate by active uptake in the proximal renal tubule, such that virtually no glucose is present in the urine of normal individuals. Tubular glucose uptake is coupled to sodium transport with SGLT2 driving sodium across the Na+ gradient which is maintained by a sodium–potassium ATPase [20] (Fig. 33.1). SGLT2 is located in segment 1 of the proximal renal tubule and takes up equimolar amounts of glucose and sodium. Any remaining glucose is removed by the SGLT1 cotransporter in segment 3 of the proximal tubule. In patients with diabetes, the expression of SGLT2 is enhanced resulting in a higher threshold of blood glucose to cause glycosuria.

Since the nineteenth century, it has been known that an extract of apple bark (shown to contain the agent phlorizin) caused glucosuria [21]. Phlorizin was later shown to inhibit both the SGLT1 and 2 cotransporters yet had a limited value in diabetes management due to a high incidence of gastrointestinal side effects, likely due to inhibition of the SGLT1 cotransporter. While SGLT2 is almost entirely confined to the renal tubule, SGLT1 is more widely expressed in the heart, GI tract as well as the kidney. Current SGLT2 inhibitors have a much greater selectivity for SGLT2 than SGLT1 than phlorizin (Table 33.1), eliminating the adverse effects attributed to SGLT1 inhibition. Yet the cardiovascular and renal consequences of SGLT1 inhibition in man are largely unknown.

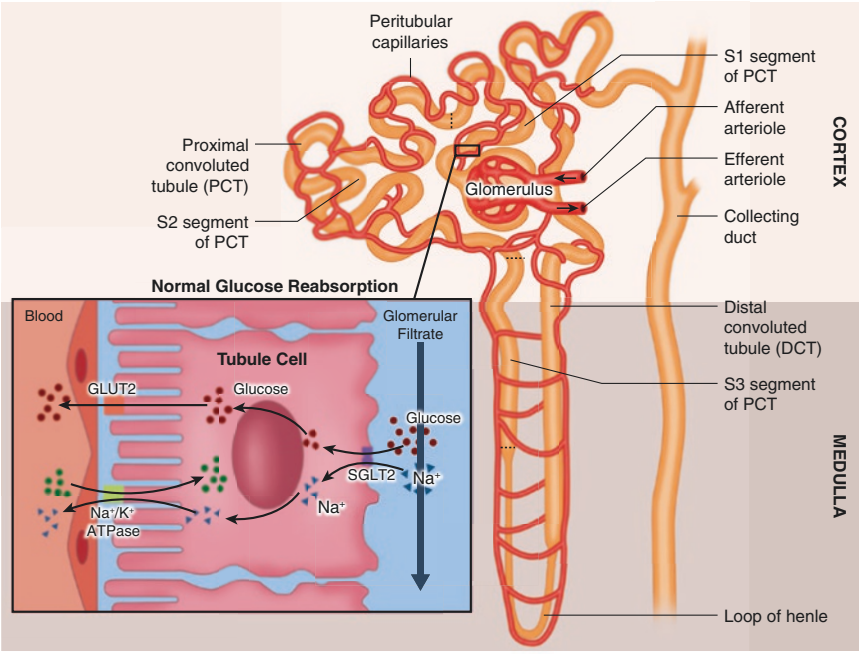

Fig. 33.1 The location and action of the sodium–glucose cotransporter 2. The SGLT2 cotransporter is located in the proximal tubule and transports approximately 80% of the filtered glucose. Any remaining glucose in the tubule is transported by SGLT1 cotransporter located more distally in the proximal tubule. Reprinted with permission from Zelniker and Braunwald 2018 [20]

Table 33.1 Selectivity of SGLT2 inhibitors for SGLT2 and SGLT1

Molecule	SGLT2 (IC50 nM)	SGLT1 (IC50 nM)	SGLT2 selectivity over SGLT1
Empagliflozin	3.1	8300	~2500-fold
Ertugliflozin	0.87	1960	~2000-fold
Dapagliflozin	1.2	1400	~1200-fold
Canagliflozin	2.7	710	~250-fold
Sotagliflozin	1.8	36	~20-fold
Phlorizin	2800	4200	~1.5-fold

Reprinted from https://www.researchgate.net/figure/SGLT2-SGLT1-selectivity-of-main-SGLT-inhibitors-33-37_tbl1_320204564

Pharmacological Effects of SGLT2 Inhibitors

SGLT2 inhibition results in the excretion of approximately 80 g of glucose per day in individuals with normal renal function with a consequent reduction of blood sugar, reducing blood sugar by a similar degree as other oral glucose-lowering agents. As the glycaemic effect of SGLT2 inhibitors is independent of insulin secretion or sensitivity, SGLT2 inhibitors can be effectively combined with any other glucose-lowering agent including insulin for additional glucose lowering. Hypoglycaemia is rarely observed unless the SGLT2i is combined with an agent that may cause hypoglycaemia (i.e. insulin and sulphonylureas). Body weight is reduced by 2–3 kg during the first weeks of treatment but does not usually fall further. Cardiovascular effects include lowering BP (approximately 5–7/2–3 mmHg), without any change of heart rate. Uric acid is reduced and there is a very modest increase of LDL cholesterol (~0.1 mmol/l).

Cardiovascular Safety Trials of SGLT2 Inhibitors

The clinical characteristics of the patients in the individual SGLT2 trials are shown in Table 33.2. The outcomes of the placebo groups of the trials (shown in Table 33.3) vary widely indicating the range of CV risk in the patient groups of each trial.

Table 33.2 Baseline characteristics of patients included in the SGLT2 CVD trials

	Entry criteria	Age	CVD (%)	Heart failure (%)	eGFR < 60 ml/min/1.73 m (%)	Mean eGFR ml/min/1.73 m^2
EMPA REG Empagliflozin	CVD A1C > 7.0% eGFR > 30	63.1	100	10.2	25.9	74.2
CANVAS Canagliflozin	CVD or MRF A1C > 7.0% eGFR > 30	63.3	66.6	14.4	n/a	76.5
CREDENCE Canagliflozin	A1 > 6.5% eGFR 30–90 Proteinuria 300–5000 mg/day	63	50.4	14.8	59.2	56.2
DECLARE Dapagliflozin	CVD or MRF A1C > 6.5% eGFR > 60	64.0	40	10.1	7	85.3
VERTIS Ertugliflozin	CVD A1C > 7.0% eGFR > 60	64.4	100	24	22.4	76.0

MRF Multiple risk factors, *CVD* Cardiovascular disease
CVD: % of patients with atherosclerotic cardiovascular disease, heart failure % of patients with investigator reported history of heart failure, eGFR < 60 ml/min/1.73 m^2: % of patients with eGFR < 60 ml/min/1.73 m^2, eGFR average eGFR

Table 33.3 Baseline ASCVD and CKC with outcomes in placebo groups in SGLT2 inhibitor trials

Agent TRIAL	ASCVD (%)	CKD eGFR < 60 Mean eGFR	MACE	CV death	All cause death
			Events/1000/year		
Empagliflozin EMPA-REG	100	30% 74.2	43.9	20.2	28.6
Canagliflozin CREDENCE	50.4	59% 56.2	48.7	35.0	24.4
Canagliflozin CANVAS	65.6	34.4% 76.5	31.5	12.8	19.5
Dapagliflozin DECLARE	40.6	0% 85.4	24.2	7.1	16.4
Ertugliflozin VERTIS	100	22.4% 76.0	40	19	NA

ASCVD Atherosclerotic Cardiovascular disease, *CKD* Chronic kidney disease, *MACE* Major Adverse Cardiac Events (Cardiovascular mortality, non-fatal myocardial infarction, non-fatal stroke)

EMPA REG Outcome [22]

The EMPA REG Outcome trial is the CV safety study for empagliflozin mandated by the FDA. It included 7020 individuals with established CVD who were randomised to placebo, empagliflozin 10 mg and empagliflozin 25 mg in equal numbers. After a median observation time of 3.1 years the primary combined endpoint of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke was reduced by 14% (HR 0.86 95% CI 0.74–0.99). The significant reduction of the primary endpoint was driven by a 38% reduction of CV mortality (Fig. 33.2). The reduction of mortality in the empagliflozin group was seen early and the benefit persisted throughout the treatment period. Non-fatal myocardial infarction and stroke were not significantly reduced.

The CV mortality reduction was observed in a wide range of subgroups that included gender, ethnic origin, degree of risk factor control, A1C at baseline and during treatment, medications (including insulin and metformin), baseline cardiovascular disease (coronary heart disease, myocardial infarction, stroke, peripheral vascular disease, history of coronary bypass surgery, atrial fibrillation and heart failure), renal function, criteria for metabolic syndrome and the presence of microvascular disease. Benefit was observed in patients with an eGFR down to 30 ml/min/1.73 m^2, despite minimal glucose lowering with this degree of renal impairment.

All-cause mortality was reduced by 32% (HR 0.68 95% CI 0.57–0.82) consequent to the reduction of CV mortality, as non-CV mortality was not changed. Empagliflozin treatment resulted in a projected 2–5 year increase in life expectancy. The number of patients to treat (NNT) for 3 years to prevent one death was 39 which compares favourably with other CV treatments (Simvastatin for 5.4 years in 4S NNT 30; Ramipril for 5 years in HOPE NNT 56; and Liraglutide for 3 years in LEADER NNT 98).

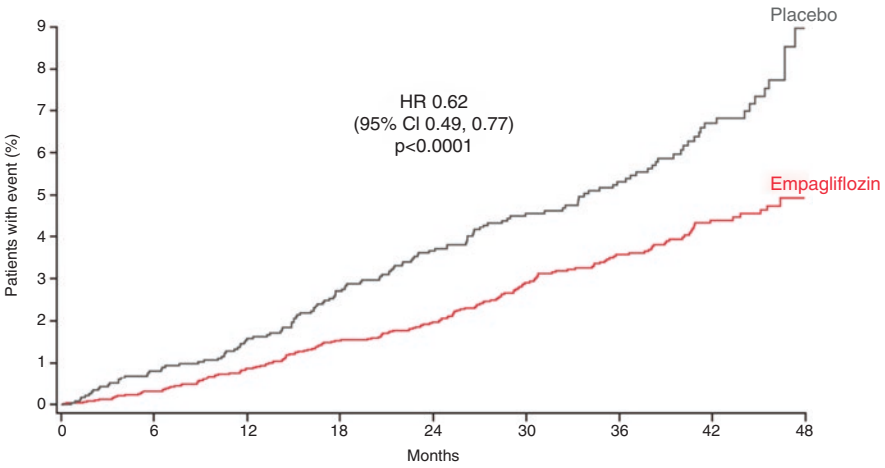

Fig. 33.2 Cumulative cardiovascular mortality in patients receiving empagliflozin and placebo (EMPA REG Outcome). Reproduced with permission from Zinman et al. N Engl J Med [22]

Non-fatal MI was not significantly reduced (HR 0.87 95% CI 0.7–1.09). Non-fatal stroke was nonsignificantly increased (HR 1.24 95% CI 0.92–1.67). Yet most of the small excess of strokes in the treatment group occurred long after empagliflozin was discontinued [23]. Empagliflozin treatment resulted in a slower progression of kidney disease with a 39% (HR 0.61 95% CI 0.53–0.70) reduction of the primary renal endpoint (progression to macroalbuminuria, doubling of serum creatinine, initiation of dialysis or death from renal disease) [24].

Admission to hospital with heart failure was reduced by 35% (HR 0.65 95% CI 0.5–0.85). Empagliflozin reduced recurrent vascular events including myocardial infarction (rate ratio 0.79, 95% CI 0.62–0.99). First plus recurrent admissions for heart failure were reduced by 42% (rate ratio 0.58 95% CI 0.42–0.81) and all-cause hospitalisations by 17% (rate ratio 0.83 95% CI 0.76–0.91).

CANVAS [14]

In the CANVAS trial, patients were randomised to receive either canagliflozin (100 mg or 300 mg daily) ($n = 5795$) or placebo ($n = 4347$). All patients had type 2 diabetes and high CV risk. Those 30–50 years old had to have a history of atherosclerotic vascular disease (ASCVD), whereas patients >50 years old could either have ASCVD or no ASCVD but multiple CVD risk factors (two or more of: diabetes duration >10 years, SBP > 140 on treatment, current smoking, albuminuria or HDL cholesterol < 1 mM/l (<38.7 mg/dl)).

The median duration of follow-up was 3.6 years. Details of baseline characteristics are shown in Tables 33.1 and 33.2. Approximately, two-thirds of patients had a history of CVD and one-third risk factors only. There was a history of heart failure in 14.4% of subjects.

The primary outcome of triple MACE (CV death, non-fatal MI and non-fatal stroke) was reduced by canagliflozin (26.9 vs. 31.5 events per 1000 patient-years; HR 0.86; 95% CI 0.75 to 0.97; $p < 0.001$ for non-inferiority; $p = 0.02$ for superiority). The reduction of the primary outcome resulted from statistically nonsignificant reductions of each of its components. Heart failure admission was reduced by 33% (HR 0.67 95% CI 0.52–0.87). Subjects with chronic kidney disease (eGFR 30–60 ml/min/1.73 m^2) had a similar reduction of the triple MACE primary endpoint to those with normal renal function. The combined renal endpoint (40% increase of creatinine, need for chronic dialysis or renal death) was reduced to 40% (HR 0.60 95% CI 0.47–0.67) by canagliflozin.

DECLARE [16]

The DECLARE study subjects had type 2 diabetes (A1C 6.5–12.0) with either established cardiovascular disease or multiple cardiovascular risk factors (age greater than 55 (men) and 60 years (women), current smoking, hypertension or dyslipidaemia). A total of 17,160 patients received either dapagliflozin 10 mg daily or placebo. There were two co-primary outcomes (1) Triple MACE (CV death, non-fatal myocardial infarction or stroke) and (2) CV death + heart failure hospitalisation. The statistical analysis permitted efficacy analysis of the two co-primary endpoints providing the Triple MACE endpoint was non-inferior.

The trial population had a history of CVD in 40% and only CV risk factors in 60%. Most patients with established CVD had coronary heart disease, and half of this group had had a prior myocardial infarction. A history of heart failure was reported in 10%. A eGFR < 60 ml/min/1.73 m^2 was present in 9.1% and Albumin creatinine ratio >300 mg/g in 6.8%.

After a median follow-up of 4.2 years, 1559 primary MACE events were recorded. The primary MACE endpoint showed non-inferiority ($p < 0.001$), but no superiority (HR 0.93, 95% CI 0.84–1.03). The reduction of non-fatal MI was numerically greater than the other components of the MACE triple primary endpoint (HR 0.89 95% CI 0.77–1.01). However, the co-primary combined endpoint of CV death and heart failure hospitalisation (CVD/HFH) was significantly reduced by 17% (HR 0.83, 95% CI 0.75–0.95, $p = 0.005$). This was entirely driven by the 27% reduction of heart failure hospitalisation (HR 0.73, 95% CI 0.61–0.88), as there was no reduction of CV death (HR 0.98, 95% CI 0.82–1.17).

Similar reductions in the CVD/HFH co-primary endpoint were observed in the group with prior CV as in the group with multiple risk factors. Patients with and without a history of prior MI had similar reduction of the HHF/CVD endpoint. In patients with a prior MI, the triple MACE endpoint was significantly reduced by 16% (HR 0.84, 95% CI 0.72–0.99), whereas in patients with no prior MI, there was no reduction. However, the *p*-value for interaction was 0.107. A similar HHF/CVD benefit was seen in patients with and without a baseline history of heart failure, and with a range of eGFR from <60 to >90 ml/min/1.73 m^2.

VERTIS [25]

The VERTIS study is the most recent CV outcome trial of SGLT2 inhibitors to be reported. It evaluated the CV safety and efficacy of the highly specific SGLT2 inhibitor ertugliflozin. The trial enrolled patients with T2 DM, A1C 7–10.5%, eGFR > 30 ml/min/1.73 m^2 and with established CVD. A total of 8246 subjects were randomised to receive ertugliflozin 5 mg, ertugliflozin 15 mg daily or placebo. The mean duration of follow-up was 3.5 years.

CAD was present in 76%, a history of prior MI in 48% and heart failure in 24.5%.

Ertugliflozin reduced A1C by 0.5%, weight by 2.4–2.6 kg and systolic BP 2.6–3.2 mmHg.

The primary triple MACE endpoint showed ertugliflozin was not inferior to placebo ($p < 0.001$), but not superior, with 11.9% of events in both the ertugliflozin and placebo groups (HR 0.97 95% CI 0.85–1.11). CV death was not significantly reduced. However, an early 30% reduction of heart failure hospitalisation was observed.

The combined renal outcome (renal death, dialysis or transplant and doubling of creatinine) was not significantly reduced by ertugliflozin (HR 0.81 95% CI 0.63–1.04).

CVD Safety Trials Conclusions

The reduction of the MACE primary endpoint across the SGLT2 inhibitor class was modest. The reduction of MACE in the EMPA REG trial was largely due to the reduction of CV mortality with a trend to a reduction of non-fatal MI and a small nonsignificant increase in stroke. In the CANVAS study, MACE was reduced due to individually nonsignificant reductions of CV death, MI and stroke. However, in DECLARE and VERTIS, MACE was not reduced.

CV death was only reduced in the EMPA REG Outcome trial. Yet in the CREDENCE trial, all-cause mortality was significantly reduced. There is a consistent reduction of HHF across the SGLT2 inhibitor class. The HF benefits are independent of baseline CVD, prior HF, across a spectrum of eGFR and independent of glucose lowering.

Differences in outcomes in the individual trials could be due to numerous factors. Differences between agents especially the specificity for the SGLT2 and SGLT1 cotransporters could play a role (Table 33.1). Differences in patient baseline characteristics, especially the presence of CV disease and renal dysfunction, impact on CV event rates. As shown in Table 33.4, the primary MACE and CV mortality rates relate to the proportion of patients with ASCVD and eGFR < 60 ml/min/1.73 m^2. Differences in study design, including sample size, inclusion/exclusion criteria, endpoint definitions and analysis of outcomes may play a role. Despite

Table 33.4 CV outcomes related to baseline presence of CVD and renal function in the SGLT2 CVD outcome trials

Agent TRIAL	ASCVD (%)	CKD eGFR < 60 Mean eGFR	MACE (or other Primary Endpoint)	CV death	All cause death
			Events/1000/year		
Empagliflozin EMPA-REG Outcome	100	30% 74.2	43.9	20.2	28.6
Canagliflozin CANVAS	65.6	34.4% 76.5	31.5	12.8	19.5
Canagliflozin CREDENCE	50.4	59% 56.2	48.7	35.0	24.4
Dapagliflozin DAPA-CKD	37	89% 43.0	145	37	68
Dapagliflozin DECLARE	40.6	0% 85.4	24.2	7.1	16.4
Ertugliflozin VERTIS	100	22.1% 76	40	19	

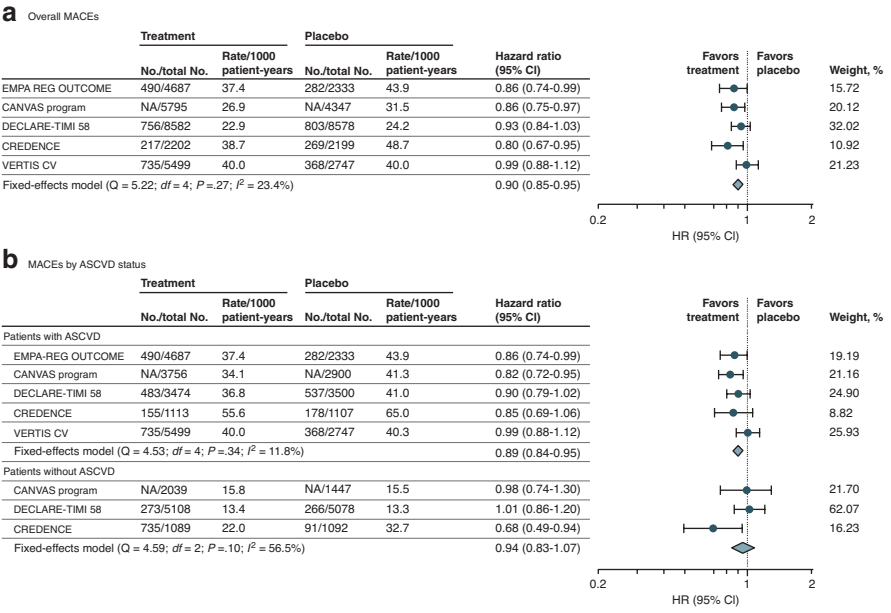

Fig. 33.3 Meta-analysis of SGLT2 CV outcome trials. (**a**) Primary outcome, (**b**) Primary outcome in subjects with and without ASCVD. Reprinted from McGuire D et al. [26]

differences between the trials, meta-analyses show significant reductions of the primary endpoint, CV mortality and heart failure hospitalisation (Figs. 33.3, 33.4, and 33.5)

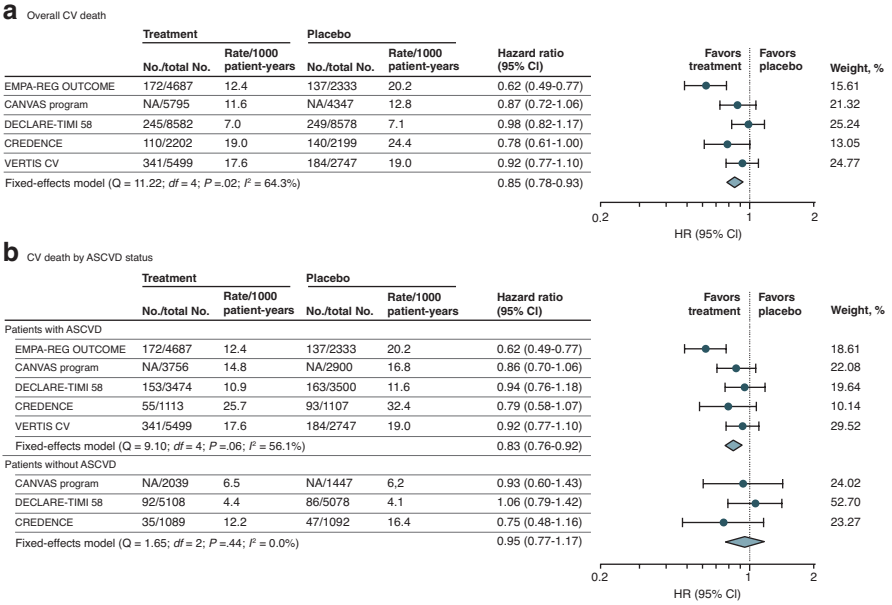

Fig. 33.4 Meta-analysis of SGLT2 CV outcome trials. (**a**) CV mortality (**b**) CV mortality in subjects with and without ASCVD. Reprinted from McGuire et al. [26]

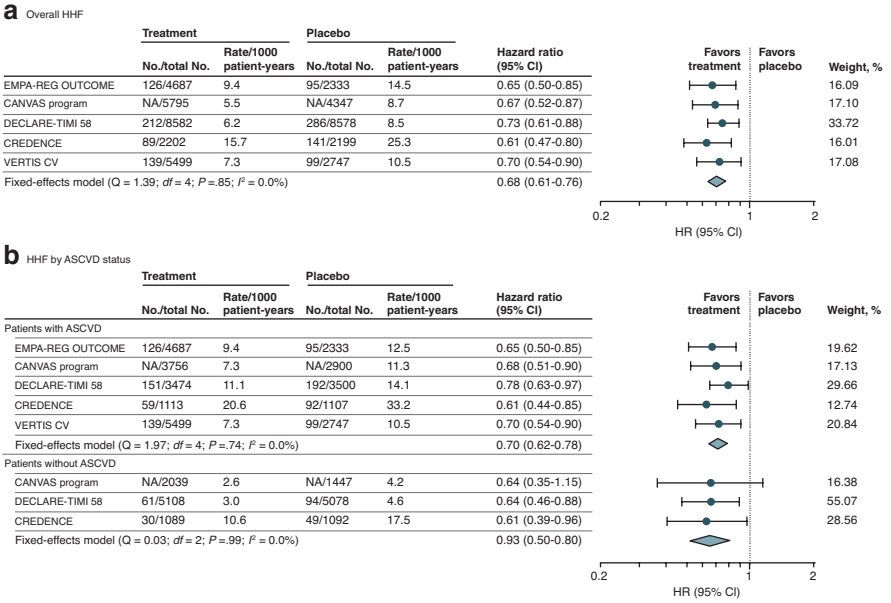

Fig. 33.5 Meta-analysis of SGLT2 CV outcome trials. (**a**) Hospitalisation for heart failure (**b**) Hospitalisation for heart failure in subjects with and without ASCVD. Reprinted from McGuire et al. [26]

Outcomes in Patients with and Without Established ASCVD (Figs. 33.3, 33.4, and 33.5)

In the EMPA-REG Outcome and VERTIS trials, all patients had a history of atherosclerotic cardiovascular disease. In the CANVAS study, 30% had no ASCVD history and in DECLARE 60% had a history of only CVD risk factors and no established CVD. A meta-regression analysis of the trials [26] (Fig. 33.5b) shows that in patients with established ASCVD treatment with a SGLT2 inhibitor significantly reduced the primary CV event endpoint and heart failure hospitalisation. However, in patients with CVD risk factors alone, and no history of established disease, only heart failure hospitalisation and decline of renal function decline were reduced.

SGLT2 Inhibitor Trials in Patients with Chronic Kidney Disease

Chronic kidney disease is a common condition that is associated with a diminished quality of life, reduced life expectancy and increased risk of cardiovascular disease. Angiotensin-converting enzyme (ACE) [27] and Angiotensin receptor blockers are commonly used to slow the progression of CKD, yet there is no strong evidence to show benefits beyond those attributable to blood pressure lowering [28]. CKD is more frequent in patients with diabetes and is present in over half of patients developing end-stage kidney disease and requiring dialysis.

The secondary renal outcome of the SGLT2 CV safety trials indicated that SGLT2 inhibitors slowed the decline of renal function (eGFR), and the combined renal endpoint included doubling of serum creatine, the development of macroalbinuria, the need for dialysis and renal death. The CREDENCE [15] and DAPA CKD [29] trials selected patients with CKD with and without CVD. The DAPA CKD trial included patients with and without diabetes. A meta-analysis indicates that patients with and without ASCVD have beneficial effects from SGLT2 inhibition (Fig. 33.6).

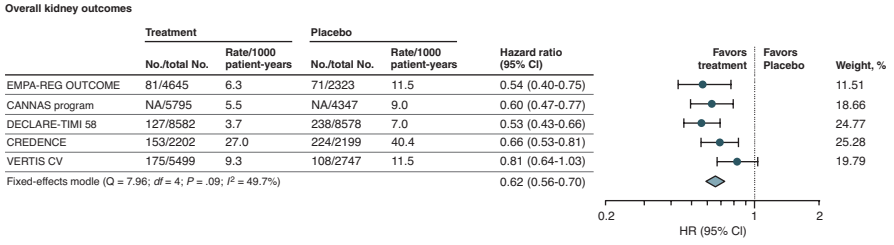

Fig. 33.6 Meta-analysis of SGLT2 inhibitor cardiovascular safety trials: Kidney-related outcomes. Reprinted from McGuire et al. [26]

CREDENCE [15, 29]

The goal of the CREDENCE study was to assess the effects of the SGLT2 inhibitor, canagliflozin on renal outcomes in patients with T2DM and established CKD. A secondary aim was to determine the impact of canagliflozin on cardiovascular outcomes in this very high-risk population. A total of 4401 patients with A1C 6.5–12.0% and eGFR 30–90 ml/min/1.73 m^2 and/or UACR 300–5000 mg/g who were receiving a maximally tolerated dose of an ACE inhibitor or ARB were randomised to receive canagliflozin 100 mg daily or placebo. CV disease was present in 50% and a history of heart failure in 14%. A total of 97% of subjects had a history of hypertension. The primary outcome of end-stage kidney disease, doubling of creatinine or renal or CV death was reduced by 30% (HR 0.70, 95% CI 0.59–0.82). CV outcomes were also reduced by canagliflozin: CV death/MI/CVA 20% (HR 0.80, 95% CI 0.67–0.95), CV death 22% (HR 0.78 95% CI 0.61–1.00) $p = 0.0502$) and hospitalisation for heart failure 39% (HR 0.61, 95% CI 0.47–0.80).

DAPA CKD [29]

DAPA CKD included patients with eGFR 25–75 ml/min/1.73 m^2 (mean 43 ml/min/1.73 m^2 and 89.8% with eGFR < 60 ml/min). A total of 15% had a eGFR, 30 ml/min/1.73 m^2 and urinary albumin creatinine ratio of 200–5000 mg/G. A total of 67% had type 2 diabetes. A total of 37% had a history of cardiovascular disease. Patients received dapagliflozin 10 mg daily or matching placebo.

The primary composite outcome was an eGFR decline of at least 50%, the onset of end-stage kidney disease requiring chronic dialysis or renal transplantation, or death from renal or cardiovascular causes. After a median follow-up of 2.4 years, dapagliflozin reduced the primary composite outcome by 39% (HR 0.61 95% CI 0.51–0.72) with an absolute benefit of 5.3% and NNT to prevent one primary outcome of 19. Similar benefits were observed in patients with and without diabetes. Each of the components of the primary outcome was reduced, with number of patients developing a 50% fall in eGFR nearly halved (HR 0.53 95% CI 0.42–0.67) and the need for dialysis was reduced to 34% (HR 0.66 95% CI 0.48–0.90). Cardiovascular mortality was lower in the dapagliflozin group but the difference did not achieve statistical significance despite the high death rate (17/1000/year). Yet all-cause mortality was reduced by 31% (HR0.69 95% CI 0.53–0.88). The combined outcome of death from CV cause or hospitalisation for heart failure was reduced by 29% (HR 0.71 95% CI 0.55–0.92).

The SCORED trial [30] included 10,584 patients with diabetes and chronic kidney disease (eGFR 25–60 ml/min/1.3 m^2 irrespective of the degree of albuminuria) who were randomised to receive the SGLT1/2 inhibitor sotagliflozin or placebo. The study was prematurely terminated after a median follow-up of 16 months due to a loss of funding. Despite the short duration of the trial, the primary combined

endpoint of CV death, hospitalisation due to heart failure or acute decompensated heart failure was reduced by 26% (HR 0.76, 95% CI 0.63–0.88). The combined endpoint of CV death, non-fatal myocardial infarction or stroke was significantly reduced (HR 0.84 95% CI 0.72–0.99). Most adverse effects occurred with a similar frequency observed in the SGLT2 inhibitor trials. However, diarrhoea occurred more frequently with sotagliflozin, likely due to the inhibition of intestinal SGLT1, and diabetic ketoacidosis was five times as frequent as with placebo.

Following the results of the CREDENCE trial, the ADA-EASD Consensus report recommended the use of SGLT2 inhibitors for patients with type 2 diabetes and CKD irrespective of a history of CV disease. With the DAPA CKD results, it is likely that this recommendation will be extended to include those with CKD and no diabetes. We await the results of EMPA Kidney in early 2023 to provide additional information in patients with CKD with and without diabetes.

SGLT2 Inhibitors in Patients with Heart Failure

Heart failure is a common and often unrecognised complication of diabetes. The cardiovascular safety trials have reminded us the impact of heart failure, with hospitalisation for heart failure predicting a worse survival than hospitalisation for a myocardial infarction. HF occurs both related but often unrelated to the presence of coronary artery disease. ACE inhibitors, ARBs, mineralocorticoid inhibitors and neprilysin inhibitors improve prognosis and reduce symptoms in patients with HFrEF. HFpEF occurs more often than HFrEF in patients with diabetes. Until recently, there has been no therapy to improve outcomes for patients with HFpEF.

The SGLT2 inhibitor CV safety trials and studies in patients with chronic kidney disease have consistently shown that SGLT2 inhibitors reduced the frequency of heart failure hospitalisation (HFH). HFH was reduced in patients with or without a history of prior heart failure and chronic kidney disease. In the safety trials, 10% of patients had a HF diagnosis determined by the investigator without any requirement for a measure of left ventricular function or BNP measurement. The DAPA HF and EMPEROR trials have investigated the impact of SGLT2 inhibitors in patients with heart failure with and without diabetes.

Heart Failure with Reduced Ejection Fraction (HFrEF)

DAPA HF [31]

The DAP HF trial randomised 4744 patients with Class II to IV heart failure symptoms and a LV ejection fraction <40% to treatment with dapagliflozin 10 mg daily or placebo. Over 18.2 months follow-up, the primary endpoint (worsening heart

failure (either hospitalisation for heart failure) or an urgent visit when the patient required intravenous therapy for HF) or CV death was reduced by 26% (HR 0.74 95% CI 0.65–0.85). Worsening heart failure was reduced by 30% and CV death by 18% (HR 0.82 95% CI 0.69–0.98). Similar benefits were observed in more than 50% of patients without diabetes and in those with diabetes or with or without an ischemic cause of heart failure. Improved functional status was reported in patients receiving dapagliflozin [32]. There was no increased incidence of volume depletion, renal dysfunction or hypoglycaemia in the dapagliflozin-treated group compared to those receiving placebo.

EMPEROR Reduced [33]

The EMPEROR Reduced trial included 3730 patients with NYHA class II-IV symptoms of heart failure and an ejection fraction of less than 40% who were randomised to receive either empagliflozin or placebo. Patients receiving empagliflozin and followed for a median of 16 months had a 25% reduction of the primary endpoint of heart failure hospitalisation or CV death (HR 0.75 95% CI0.65–0.86). Heart failure hospitalisation was reduced by 30%; however, CV mortality was not significantly reduced (HR 0.92 95% CI 0.75–1.12). Similar reductions of the primary endpoint were seen in patients with and without diabetes. The decline of eGFR was slower in the empagliflozin-treated patients.

SOLOIST WHF [30]

The SOLOIST WHF trial included 1222 patients with diabetes who had a recent episode of decompensated heart failure. Treatment with the SGLT 1–2 inhibitor sotagliflozin or placebo was started during hospitalisation or shortly after hospital discharge. Unfortunately, the trial was prematurely terminated due to a loss of funding, and median follow-up was only 9.2 months. The combined endpoint of cardiovascular mortality, hospitalisation for heart failure and urgent visit for heart failure was significantly reduced (HR 0.67 95% CI 0.52–0.85).

The results of these heart failure trials are consistent with observations in the safety studies which only included patients with investigator identified heart failure. The initial heart failure studies only included patients with HFrEF. A meta-analysis of the DAPA HF and EMPEROR reduced trials [34] showed a consistent reductions of HFH/CV death (HR 0.74 95% CI 0.68–0.82) and first hospitalisation for heart failure (HR 0.69 95% CI 0.62–0.78) as well as a reduction of all-cause and CV death (HR 0.86 95% CI 0.76–0.98).

EMPULSE (Presented at the American Heart Association Virtual Annual Session on 14 November 2021 and Accepted for Publication in Nature Medicine)

The EMPULSE trial randomised patients hospitalised with acute heart failure (irrespective of the LV ejection fraction or the presence of diabetes) to empagliflozin 10 m daily or placebo. The primary endpoint was a clinical benefit analysis which included death, time to first heart failure event, number of heart failure events and change of Kansas City Cardiomyopathy Questionnaire Total symptom score (KCCQ-TS). At 90 days, the primary endpoint was 36% lower in the empagliflozin patients $p < 0.0054$, with all-cause mortality or hospitalisation for heart failure reduced to 35% (95% CI 0.43–0.99), and all-cause death nonsignificantly lower (HR 0.69, 95% CI 0.45–1.08). The EMPULSE Trial shows that it is safe and beneficial to initiate an SGLT2 inhibitor during hospitalisation for a heart failure.

Heart Failure and Preserved Ejection Fraction (HFpEF)

Studies with sotagliflozin also suggested a benefit in patients with HFpEF. A combined analysis of the SOLOIST WHF and SCORED studies of patients with LVEF > 50% showed a 37% reduction (HR 0.63, 95% CI 0.45–0.89) of cardiovascular mortality, hospitalisation for heart failure and urgent visit for heart failure (Bhatt D et al. AHA Scientific Sessions November 2020). Subsequently, clinical trials dedicated to patients with HFpEF have been published.

EMPEROR Preserved Trial [35] ClinicalTrials.gov Identifier NCT01297257

THE EMPEROR Preserved trial randomised 5988 patients with NYHA class 2–4 heart failure and ejection fraction >40% with or without diabetes, to either empagliflozin 10 mg daily or placebo. Over a follow-up period of 26.2 months, the primary outcome of cardiovascular death or hospitalisation for heart failure occurred in 13.8% of patients in the treatment group and 17.1% in the placebo group (HR 0.79 95% CI 0.69–0.90). The 37% reduction of hospitalisation for heart failure was the driver of the reduction of the primary combined outcome with the reduction of CV death (7.3 vs. 8.2%) not achieving statistical significance. Similar benefits were observed in patients with and without diabetes.

DELIVER (Dapagliflozin to Improve the LIVEs of Patients with Preserved Ejection Fraction Heart Failure): ClinicalTrials.gov Identifier—NCT03619213

This event-driven multicentre placebo-controlled study in patients with HFpEF compares the effect of dapagliflozin 10 m daily compared to placebo in reducing the composite events of CV death and heart failure hospitalisation. The study includes approximately 11,000 patients with NYHA class II–IV symptoms and LVEF > 40% and is due to be report in 2022.

The PRESERVED HF trial [36] examined whether treatment with dapagliflozin would improve symptoms and exercise capacity in patients with HFpEF with and without diabetes. The study evaluated the effects of treatment with dapagliflozin 10 mg daily for 12 weeks on the Kansas City Cardiomyopathy Questionnaire Clinical Summary Score (KCCQ-CS)—a measure of heart failure health-related status. Dapagliflozin improved the KCCQ-CS as well as a range of measures of physical limitations, increased the 6-min walk distance and reduced weight, without any difference in adverse events. However, NT proBNP was not reduced. The result is consistent with the findings of the DEFINE-HF study [37], which showed dapagliflozin improved functional status and quality of life after 12-week treatment despite no change in N-terminal BNP.

Heart Failure Guidelines

The FDA had approved the use of dapagliflozin in patients with diabetes to prevent hospitalisation with heart failure. In May 2020, the FDA approved the use of dapagliflozin for the treatment of patients with heart failure with a reduced ejection fraction and with or without diabetes. In August 2021, empagliflozin 10 mg daily was approved by the FDA to reduce the risk of CV death plus heart failure hospitalisation in patients with HFrEF.

The 2020 update of the Canadian Cardiovascular Society and Canadian Heart Failure Society [38] strongly recommends the use of SGLT2 inhibitors in patients with mild-to-moderate heart failure with a reduced ejection fraction to improve symptoms and quality of life and to reduce the risk of hospitalisation and cardiovascular mortality based upon high-quality evidence.

The 2021 ESC heart failure guidelines [39] recommend either dapagliflozin or empagliflozin (with Class 1 recommendation) as a component of the first-line treatment of patients with heart failure and reduced ejection fraction to reduce the risk of HF hospitalisation and death. However, the guidelines as yet do not comment on the use of SGLT2 inhibitors in patients with HFpEF.

The 2021 Update to the 2017 ACC Expert Consensus Decision Pathway for Optimization of Heart Failure Treatment [40] recommends an SGLT2 inhibitor be used in patients with Stage C HFrEF after initiation of an either an ARNI, ACE inhibitor or angiotensin receptor blocker, a beta-blocker and diuretic.

Adverse Effects of SGLT2 Inhibitors and Recommendations for Their Prevention

The incidence of adverse effects is available from the CV safety trials and from meta-analyses of glucose-lowering efficacy trials. Adverse effects can be classified into those directly related to the pharmacological action of the drug and those causing off-target adverse effects. A more detailed discussion of the safety issues of SGLT2 inhibitors is found in a recent publication [41]. A summary of adverse effects in the CV trials is shown in Table 33.9. Recommendations to reduce adverse effects are summarised in Table 33.6.

Glycosuria due to SGLT2 inhibition could result in adverse effects such as hypoglycaemia, mycotic genital infections, urinary tract infections, Fournier's gangrene, volume depletion and acute kidney injury. Fortunately, a majority of these potential adverse effects are either not observed or rare.

Mycotic genital infections are the most common adverse effects in patients treated with an SGLT2 inhibitor and observed in up to 10% of women and 5% of men, with a frequency of 3–4× more than in the placebo population [42]. They are more common in individuals with a history of genital candidiasis but almost unseen in circumcised men [43]. Usually, the infection causes mild symptoms, responds rapidly to a single oral dose of fluconazole or topical antifungal agents and does not recur. Good genital hygiene may help to reduce the risk of infection.

Urinary tract infections were not increased in the CV safety trials. In a meta-analysis of adverse effects in 7000 subjects, no increase of urinary tract infections was observed [44]. However, SGLT2 inhibitors should probably not be prescribed in patients at very high risk of infection, such as those with recurrent infections, paraplegia or an indwelling urinary catheter.

Fournier's Gangrene is a severe perineal infection that can be fatal and is more frequently observed in older patients, those with obesity, with diabetes [45] and with alcohol abuse. In May 2018, the FDA reported 12 cases over a 5-year period, to have developed Fournier's Gangrene within months of starting an SGLT2 inhibitor. However, none of the CV safety trials reported an increased risk. In the DECLARE study, six cases were reported: one receiving dapagliflozin and five placebo. Consequently, it is uncertain, but unlikely that an SGLT2 inhibitor is either responsible or contributes to the development of Fournier's gangrene.*Hypoglycaemia* is most unlikely to occur in patients receiving an SGLT2 inhibitor as monotherapy. The amount of glucose filtered by the glomerulus and subsequently excreted in a patient receiving an SGLT2 inhibitor is related to the plasma glucose. Consequently, plasma glucose lowering by an SGLT2 inhibitor is self-limiting. However, if an SGLT2 inhibitor is combined with agents that are associated with hypoglycaemia (insulin or a sulphonylurea), the risk of hypoglycaemia is increased.

In the CV safety trials, symptomatic or severe hypoglycaemia requiring third-party intervention was not increased. A pooled analysis of phase 1–3 trials of empagliflozin showed no increase of hypoglycaemia when the SGLT2 inhibitor was combined with insulin, yet the risk was increased when empagliflozin was combined with a sulphonylurea [46].

The risk of hypoglycaemia can be reduced when starting treatment with an SGLT2 inhibitor by adjusting doses of either a sulphonylurea or insulin in those at risk for hypoglycaemia (e.g. a history of hypoglycaemic episodes, A1C < 7.0–8.0, elderly, and with chronic kidney disease). The insulin dose may be modestly reduced; however, insulin should never be discontinued.

Diabetic Ketoacidosis (DKA)usually occurs in patients with type 1 diabetes, yet one-third of DKA episodes are inpatients with type 2 diabetes who are insulin-dependent and poorly controlled. DKA is increased in patients receiving SGLT2 inhibitors, resulting from reduced insulin requirements, increased fatty acid oxidation, reduced ketone clearance and stimulation of glucagon secretion. It is more likely to occur in association with dehydration, an intercurrent infection or insulin deficiency from an inappropriate reduction of dose. DKA in patients taking SGLT2 inhibitors may occur with only mildly increased blood glucose levels [47], likely resulting from the continued renal clearance of glucose, despite relative insulin deficiency.

The CV safety trials showed DKA was a rare occurrence despite half of the population were receiving insulin. No increase in DKA was seen in EMPA REG and CANVAS. However, an increased incidence of DKA was observed in DECLARE (0.3 vs. 0.1%; HR 2.18; 95% CI: 1.10–4.30), CREDENCE (0.2–2.2% HR 10.8 95% CI 1.39–84) and in VERTIS (0.1–0.35%). Observational studies indicate a twofold increase of DKA incidence, yet a meta-analysis showed a nonsignificant decrease (HR 0.66 95% CI 0.30–1.45) [48, 49].

The usual symptoms of DKA are nausea, vomiting and malaise. Laboratory tests show an anion gap metabolic acidosis with increased serum and urinary ketones. However, in patients taking an SGLT2 inhibitor, the blood sugar may be normal or only slightly elevated. The management of DKA includes the administration of insulin and fluid and electrolyte replacement. Prevention of DKA includes temporary discontinuation of the SGLT2 inhibitor in the event of an acute illness, trauma and before major surgery. However, it is important to maintain insulin treatment with no major changes in dose. SGLT2 inhibitors should probably be avoided in patients with a history of DKA as SGLT2 inhibition is more likely to provoke a further DKA.

Fluid depletion and hypotension can occur due to excessive SGLT2 inhibitor-induced osmotic diuresis and natriuresis. SGLT2 inhibitors cause a small reduction of blood pressure of 4-–6/1–2 mmHg, likely the result of both sodium and glucose excretion, and a reduced plasma volume [50]. Weight loss (approximately 2 kg) and increased arterial compliance may also contribute to the reduced BP. In patients with a low baseline blood pressure or volume depleted, an SGLT2 inhibitor might induce important hypotension and a possible acute kidney injury. However, in †he clinical trials, adverse effects due to volume depletion were rare or not observed. In the EMPA REG Outcome, Declare TIMI 58 and VERTIS trials, the SGLT2 inhibitor did not increase events related to volume depletion. In CANVAS, there

was an increase in symptoms due to volume depletion (26.0 vs. 18.5 events/1000 patient years and related to osmotic diuresis (34.5 vs. 13.3/1000 patient years). A combined analysis of four studies with canagliflozin showed increased symptoms related to the diuresis (e.g. increased urine volume and frequency) yet no symptoms reflecting hypovolemia (e.g. orthostatic or postural hypotension) [51]. A study of trials with empagliflozin showed infrequent (1.8%) symptoms related to volume depletion, which were more likely to occur in older patients [52].

The combination of a diuretic and an SGLT2 inhibitor is generally well tolerated provided the patient is not volume depleted [53]. When adding an SGLT2 inhibitor, it is important to assess both blood pressure and volume status and make adjustments to both diuretics and blood pressure lowering medications such as ACE inhibitors and ARBs to avoid hypotensive events. An algorithm to assist the assessment is shown in Fig. 33.7.

Acute Kidney Injury

There has been concern that SGLT2 inhibitors might result in an AKI due to hypotension and fluid depletion. The FDA posted a warning in 2016 that SGLT2 inhibitors could cause AKI after 101 cases were reported. However, subsequent clinical trials have not shown AKI to be a significant risk when initiating an SGLT2 inhibitor. The CV safety trials EMPA REG Outcome, CANVAS, DECLARE and VERTIS showed that the SGLT2 inhibitors were not associated with an increased risk for AKI. In fact, AKI was significantly reduced in dapagliflozin-treated patients in DECLARE (1.5 vs. 2.0% HR 0.69, 95% CI 0.55–0.87).

In an observational propensity matched study [54], comparing SGLT2 inhibitors with other glucose-lowering agents from two chronic kidney disease registries showed a significantly reduced incidence of AKI in patients receiving SGLT2 inhibitors compared to other glucose-lowering agents(HR 0.4–0.5).

Although AKI may occur less frequently in patients receiving SGLT2 inhibitors, it remains essential to optimise fluid status and BP (as shown in Fig. 33.7 and follow the sick-day strategy to temporarily discontinue SGLT2 inhibitors during an acute illness, major surgery or trauma).

Off-Target Adverse Effects

The off-target adverse effects of concern are an increased risk of amputation, cancer and bone fractures. Fortunately, most studies show that SGLT2 inhibitors are not associated with any risk for these adverse outcomes.

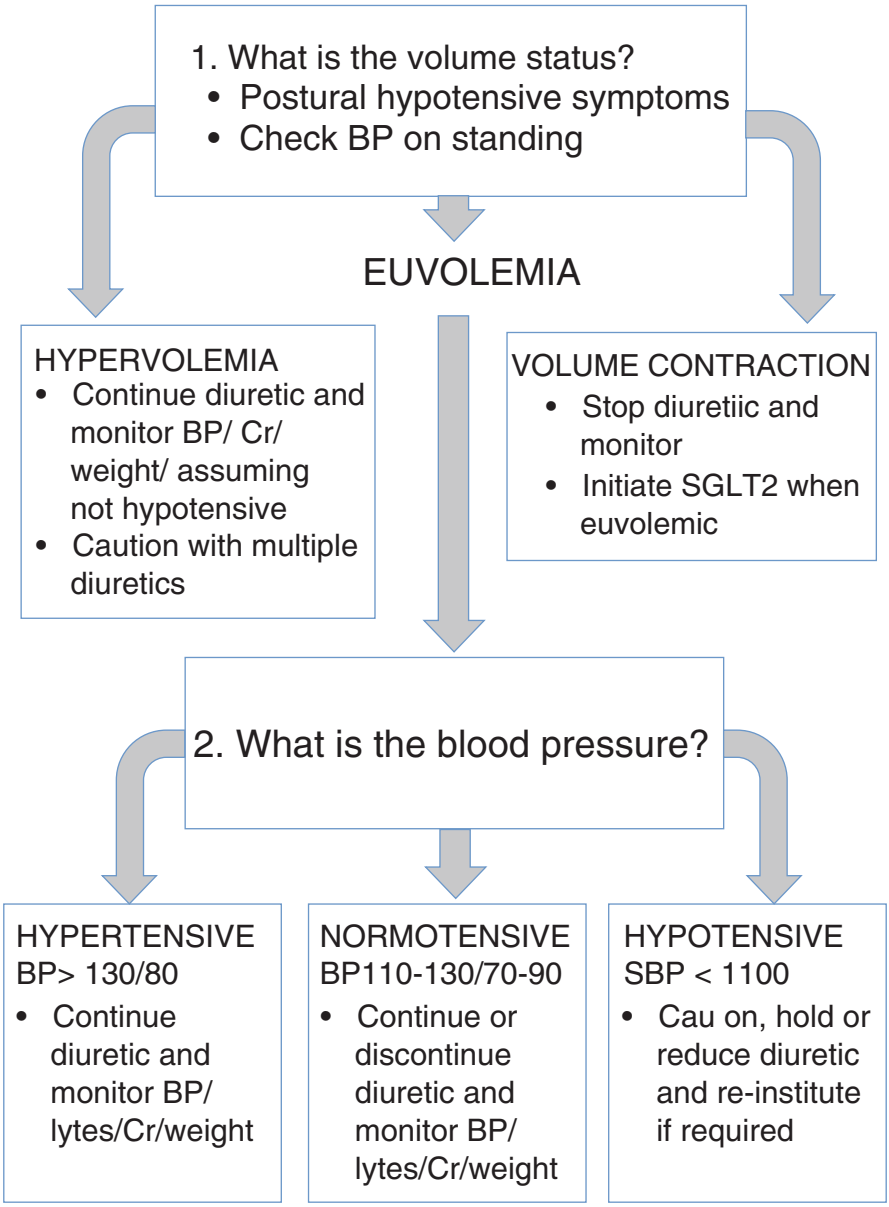

Fig. 33.7 Prevention of excessive volume depletion and hypotension when initiating treatment with an SGLT2 inhibitor. Redrawn from Cherney et al. [53]

Amputation

An increased rate of amputation was an unexpected finding from the CANVAS program [14]. Canagliflozin increased the risk of lower limb (mainly foot and toe) amputation (Canagliflozin 6.3 vs. placebo 3.4 events/1000 patient years). Patients with a prior amputation were at a very high risk of further amputation (96.3 vs. 59.2). Subjects with known peripheral vascular disease (PVD) had a nonsignificant increase (12.09 vs. 8.19 HR 1.39, 95% CI 0.80–2.40). Patients with neither prior amputation nor known PVD remained at increased risk with canagliflozin treatment(no prior amputation HR 1.88; 95% CI: 1.27–2.78, no peripheral vascular disease HR 2.34; 95% CI: 1.58–3.58). Yet in the other CVD safety trials, no increase in amputation rates was observed. Furthermore, canagliflozin did not increase the risk for amputation risk in the CREDENCE trial [15] which included high vascular risk patients.

Observational studies have shown conflicting results. A study in over 900,000 showed a doubling of amputation rates in patients receiving sulphonylureas, metformin or thiazolidinediones, yet a nonsignificant increase in subjects receiving dipeptidyl peptidase inhibitors and GLP1 agonists [55]. Yet in the OBSERVE study [56, 57] with 140,000 patients receiving canagliflozin and 110,000 on other SGLT2 inhibitors, there was no increase in the amputation rate. In a Nordic study, SGLT2 inhibitor treatment was associated with a doubling of amputation rates compared to individuals receiving a GLP1 agonist [58].

The EASEL study [59] was a propensity-matched observational study of patients initiating treatment with a glucose-lowering agent. The study compared outcomes of patients started on an SGLT2 inhibitor (largely canagliflozin) with those receiving other glucose-lowering agents. SGLT2 inhibitor use was associated with a two-fold increase of below knee amputation (0.17 vs. 0.09 events/1000 patient-years: HR 1.99, 95% CI: 1.12–3.51).

No mechanism for an increased amputation risk has been presented. It seems unlikely that fluid depletion or hypotension are responsible.

At the moment, it is uncertain whether the use of SGLT2 inhibitors is associated with a very small increased risk of amputation. Until the situation is better clarified, it is prudent to avoid SGLT2 inhibitor use in patients with prior amputation or with acutely ischemic lower extremities. In addition, canagliflozin should probably not be used in patients with more severe peripheral vascular disease (PVD), especially when there is a higher risk for amputation (e.g. with neuropathy and foot ulcers). However, other patients with PVD have an important CV benefit for SGLT2 inhibitors. In the EMPA REG outcome trial, patients with PVD had a large reduction of CV mortality (7.5 vs. 4.5%, HR 0.51, 95% CI 0.37–0.88) and heart failure hospital admission (6.1 vs. 3.9%, HR 0.56, 95% CI 0.35–0.92) [60]. Consequently, the potential benefit and risks from SGLT2 inhibitor treatment need to be assessed in the individual patient.

Cancer

In 2011, a concern about an increased risk of bladder and breast cancer with dapagliflozin in phase 2 trials leads to the FDA issuing an advisory notice. Yet the phase 3 trial, DECLARE trial [16] with 17,000 patients receiving either dapagliflozin or placebo for 4 years showed no increase of either breast or bladder cancer. Phase 2 and 3 trials with empagliflozin, canagliflozin and ertugliflozin also showed no increased risk for all types of cancer. A systematic review of 46 trials with 34,569 subjects showed no overall increased risk for cancer [61]. However, bladder cancer was increased (OR = 3.87, 95% CI: 1.48–10.08), especially with empagliflozin(OR = 4.49, 95% CI: 1.21–16.73). The controlled clinical trial evidence shows no increased cancer risk. However, the population in trials excludes individuals at a high cancer risk. Observational studies in a large population and post-marketing surveillance are need to determine whether a small cancer risk is real.

Fractures

An increased incidence of fracture in patients receiving canagliflozin in the CANVAS trial was a surprise finding (Canagliflozin 15.4 vs. Placebo 11.9 per 1000 patient-years; HR 1.26; 95% CI:1.04–1.52) [14]. In a pooled analysis of phase II and III clinical trials and in observational studies, as well as the phase III RCT safety trials with dapagliflozin, empagliflozin and ertugliflozin, no increased incidence of fractures was observed [62]. A case-controlled study that included 300,000 subjects compared SGLT2 inhibitors with DPP4 inhibitors and showed no difference of fracture rates with the two treatments [63].

No mechanism for any increased fracture rate has been shown, although SGLT2 inhibition may reduce bone density, increase serum phosphate, parathyroid hormone level, activate a vitamin D axis and possibly increase bone reabsorption, as shown by increased collagen type 1 b-carboxy-telopeptide.

Currently, canagliflozin is the sole SGLT2 inhibitor with a FDA fracture warning (https://www.fda.gov/Drugs/DrugSafety/ucm461449.htm). Yet there may be a small bone fracture risk with other SGLT2 inhibitors which has not been detected in the current trials of relatively short duration. Until, we have post-marketing safety data and large observational studies, it may be wise to restrict the use of SGLT2 inhibitors in individuals at very high spontaneous fracture risk or use of vitamin D supplements in patients at risk and/or with low blood levels. Adverse outcomes observed in the SGLT2 inhibitor trials are summarised in Table 33.5 and recommendations for their prevention in Table 33.6.

Table 33.5 Adverse outcomes observed in SGLT2 CV trials

	EMPA REG outcome	CANVAS	CREDENCE	DECLARE	VERTIS
Hypoglycemia	No increase	No increase	No increase	No increase	No increase
Genital infection (discontinuation)	3–4× (0.6%)	3–4× (NA)	Females 2× (NA)	NA (0.8%)	3–4× (NA)
Volume depletion	No increase	Increased 0.7%	No increase	No increase	No increase
Acute kidney injury	No increase	No increase	No increase	Decreased 1/1000/year	No increase
DKA	No increase	No Increase	Increased 2.0/1000/year	Increased 0.5/1000/ year	Numerically higher 0.1 vs 0.3%
Amputations	No increase	Increased 2 × 3/1000/ year	No increase	No increase	No increase
Fractures	No increase	Increased 25% 4/1000/ year	No increase	No increase	No increase

Updated and redrawn from [41]

Table 33.6 Patients at risk and measures to prevent adverse effects of SGLT2 Inhibitors

	At risk	Measures to prevent adverse event	
Genital mycotic infections	Women, prior candidiasis	Perineal hygiene, changing pads/ tampons frequently, avoid tight synthetic underwear. With recurrent infection: consider treating partner	
Urinary tract infection	Prior UTI	Probably avoid SGLT2i in patients at very high risk	
	Neurogenic bladder, paraparesis, indwelling urinary catheter		
Hypoglycaemia	Currently taking SU and/or insulin with current insulin or SU treatment, higher risk with prior hypoglycemia, in elderly, or with impaired renal function	A1C < 8.0 consider stopping or reducing dose of SU and or reducing insulin dose when initiating an SGLT2i	
		Do not discontinue insulin	

(continued)

Table 31.6 (continued)

	At risk	Measures to prevent adverse event	
Diabetic ketoacidosis	Acute illness, surgery, reduced oral intake, alcohol abuse or inappropriate reduction of insulin dosage	Stop SGLT2i with acute illness/surgery	Advise patient of need for sick day strategy (Fig. 33.2) with acute illness to reduce risk of DKA, acute kidney injury or symptomatic hypotension
		Maintain insulin, if necessary, make only small adjustments to insulin dosage	
		Beware DKA can present with normal or minimally increased blood glucose in patients receding SGLT2i	
		Do not use SGLT2i in patients with type 1 diabetes or with prior history of DKA	
Hypotension	SBP < 100 mm Hg postural hypotension	Assess for volume depletion/hypotension (Fig. 33.1)	
		Consider reducing diuretic	
Acute kidney injury	Hypotension volume depletion	Stop SGLT2i with acute illness/surgery. Maintain euvolemia	
Fractures	Osteoporosis renal impairment	Avoid SGLT2i in patients at very high risk	
		Use of vitamin D is of unproven benefit	
Amputations	Ischemic ulcers, neuropathy Peripheral vascular disease Lower limb ischemia Prior amputation	Avoid SGLT2i in patients with rest ischemia, ischemic ulcers, and prior amputations	

Redrawn and updated from Fitchett et al. [64]
Abbreviations: *DKA* diabetic ketoacidosis, *SBP* systolic blood pressure, *SGLT2* sodium glucose co-transporter 2, *SU* sulphonyurea, *UTI* urinary tract infection

Mode of Action of SGLT2 inhibitors

The mechanisms by which SGLT2 inhibitor reduce both cardiovascular events the development of heart failure and slow the progression of chronic kidney disease are largely unknown.

What is known is that any change of classical CV risk factors such as glycemia, hypertension and lipids is unlikely to play any role. With SGLT2 inhibition, there is a dissociation between glycaemic control and CV risk reduction. The rapid speed of onset of benefit is inconsistent with any CV benefits from glucose control which take many years to be observed [65]. The CV benefits of SGLT2 inhibitors are preserved in patients with renal dysfunction who have attenuated glycosuria and glucose lowering [66]. Finally, in the DAPA HF trial [31], the cardiovascular and renal benefits of dapagliflozin are observed to the same degree in patients with and without diabetes. In patients without diabetes, SGLT2 inhibitors still result in glycosuria and a natriuresis and the associated increase in glucagon and ketones.

An early reduction of heart failure is a common feature of all the SGLT2 inhibitor trials. Consequently, it is likely that SGLT2 inhibitors reduce cardiovascular events mainly through the prevention of heart failure and its consequences such as life-threatening arrhythmias, as opposed to reducing atherothrombotic events. This is supported by the observation that the reduction of heart failure is observed in individuals with and without a history of ASCVD.

Possible mechanisms for the cardiovascular and renal benefits have recently been reviewed [67, 68] and are shown in Fig. 33.8.

The benefits of SGLT2 inhibition can be considered in terms of their impact on (1) increased natriuresis and change in tissue sodium handling and (2) increased glycosuria.

The improved ventricular loading consequent to preload reduction from natriuresis and osmotic diuresis, and reduction of afterload from decreased blood pressure and increased arterial compliance, could be beneficial especially in an individual with diastolic dysfunction. A mediation analysis of the EMPA REG Outcome trial [69] indicated 50% of the reduction of CV mortality, and heart failure hospitalisation was attributable to the increase of haematocrit which is likely a marker of empagliflozin-induced plasma volume depletion. SGLT2 inhibitors may also selectively reduce interstitial fluid and not result in sudden reductions of blood pressure and consequent sympathetic nervous system activation [70].

SGLT2 inhibitors modestly increase plasma ketone body levels consequent to reduced glucose oxidation and increased fatty acid metabolism. The move towards ketone metabolism has been proposed as a benefit for the stressed myocyte, as ketones are more efficiently metabolised than free fatty acids [71, 72]. In addition, increased lipid oxidation might reduce lipotoxicity from fatty acyl CoAs, diacylglycerol and ceramides [73]. However, it is unclear whether ketone body oxidation by the myocardium does have an energetic advantage [74]. Treatment with SGLT2 inhibitor is associated with a reduction of epicardial fat, leptins and pro-inflammatory cytokines such as TNF [75].

Any effect on the myocardium is likely to be indirect as the myocytes have only SGLT1 and very little or no SGLT2 co transporters. In animal models, SGLT2 inhibition reduces inflammatory macrophages and the development of fibrosis [76]. SGLT2 inhibition in one MRI study was associated with a reduction of LV diastolic volume [77], and in another a reduction of LV mass [78] SGLT2 inhibitors inhibit the Na+/H+ exchanger in experimental models and lower myocyte cytoplasmic sodium and calcium [79], and increased mitochondrial Ca++ concentrations [79].

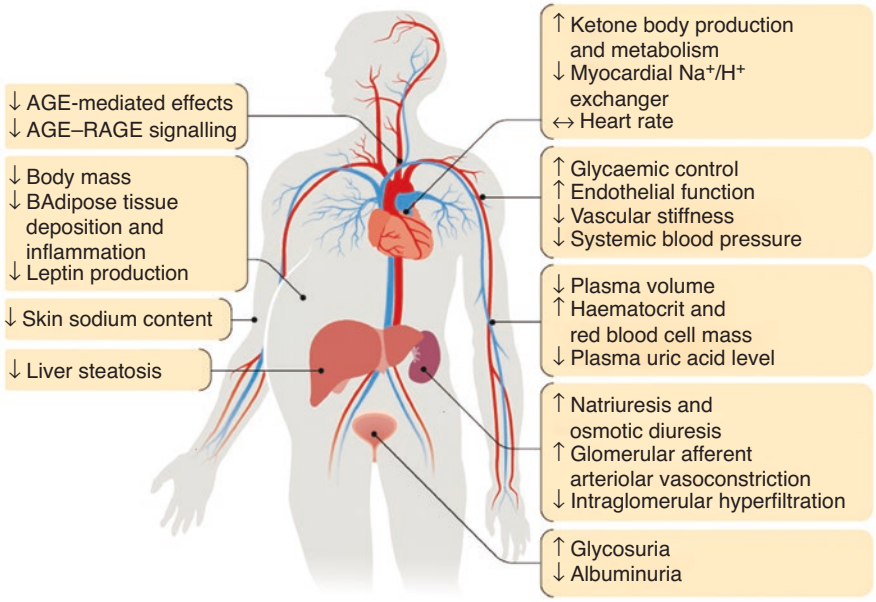

Fig. 33.8 Putative mechanisms of the CV and renal benefits of SGLT2 inhibitors. Reprinted with permission from Cowie et al. [68]. (AGE advanced glycation endproducts, RAGE Receptor for AGE)

These ionic changes could improve excitation–contraction coupling as well as reducing oxidative stress and the development of cardiac arrhythmias [80].

The mechanisms for renal preservation by SGLT2 inhibitors are also not defined. In some patients with chronic kidney disease, dilatation of the afferent renal arteriole results in glomerular hypertension and hyperfiltration, which is a possible cause of progressive renal dysfunction [81]. SGLT2 inhibition reduces the uptake of proximal tubular sodium and intratubular stimulation of the juxtaglomerular apparatus, the release of adenosine and afferent glomerular arteriolar constriction by tubuloglomerular feedback, protecting the glomerulus from the adverse effects of glomerular hypertension. This mechanism is in contrast to the nephroprotective benefits of RAAS inhibitors which reduce glomerular hypertension by dilatation of the efferent glomerular arteriole. The reduction of glomerular pressure with SGLT2 inhibitors results in an early small fall of glomerular filtration with a reduction of eGFR of about 5 ml/min/1.73 m^2 which is reversed within weeks of drug discontinuation.

Future Cardiovascular Trials with Sglt2 Inhibitors

A list of registered SGLT2 inhibitor cardiovascular trials that have not reported outcomes are shown in Table 33.7. The most important trials examine whether SGLT2 inhibitors improve outcomes in patients with heart failure and preserved

Table 33.7 Ongoing cardiorenal trials with SGLT2 inhibitors

Trial name	National identifier	Patient group	Main endpoints
Canagliflozin			
CHIEF HF	04252287	HF	Functional impact, KCCQ
Dapagliflozin			
DELIVER	03619213	HFpEF	CV outcomes
DETERMINE Preserved	03877224	HFpEF	Functional impact, KCCQ and 6-min walk
DETERMINE Reduced	03877237	HFrEF	Functional impact, KCCQ and 6-min walk
Empagliflozin			
EMMY	03087773	MI	HF events
EMPA-Kidney	03594110	Chronic kidney disease	CV and renal outcomes

ejection fraction such as EMPEROR Preserved and EMPERIAL Preserved. Other trials evaluate SGLT2i in patients with recent decompensated heart failure such as EMPA-Response and SOLOIST WHF. Other trials investigate mechanistic questions.

Conclusions

SGLT2 inhibitors have remarkably robust benefits, most of which do not derive from the glucose-lowering properties of the drug class. They have a modest benefit on atherosclerotic adverse cardiovascular events but reduce cardiovascular mortality.

A reduction of heart failure hospitalisation and slowing the progression of chronic kidney disease are consistently observed in patients with and without clinically evident ASCVD. In patients with established heart failure, SGLT2 inhibitors reduce both heart failure admissions and death rates. Progression of renal disease is slowed in patients with CKD, with and without diabetes. The clinical guidelines reflect these benefits by recommending the use of SGLT2 inhibitors as a first-line treatment in a wide range of patients with diabetes and ASCVD, and or chronic kidney disease independent of glucose control, and with heart failure with a reduced ejection fraction irrespective of the presence of diabetes. There is likely no CV benefit from non-selective SGLT inhibitors such as sotagliflozin, beyond the more specific SGLT2 inhibitors, and adverse effects are increased.

Glucagon-Like Peptide-1 Receptor Agonists (GLP-1 RA)

In 1964, it was observed that an oral glucose load resulted in greater insulin secretion than when the same amount of glucose was given intravenously. The observation leads to the discovery of incretin hormones, such as glucose-dependent

insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), released from the distal ileum and stimulating a range of responses that included increasing insulin release. In addition, GLP 1A reduces glucagon release, increases glucose uptake, and glycogen synthesis, delays gastric emptying and increases satiety. GLP-1 could exert cardiovascular benefits by lowering blood pressure, improving endothelial function and limiting atherosclerosis progression and inflammation [82, 83].

Pharmacological GLP-1 agonists were subsequently developed to be resistant to metabolism by the dipeptidyl-peptidase 4 (DPP4) enzyme. The GLP-1 agonists have two different structures: (1) derived from Gila Monster venom (e.g. exenatide and lixisenatide) and (2) analogues of human GLP-1 (e.g. liraglutide, semaglutide and albiglutide). GLP-1 agonists not only are effective glucose-lowering agents but also cause an important weight loss (1–4 kg) due to an increased satiety and decreased food intake. Hypoglycaemia is rarely observed except when GLP-1 agonists are combined with insulin or sulphonyl urea agents. Consequently, they have many features of an ideal glucose-lowering agent. Gastrointestinal adverse effects, especially nausea, vomiting and diarrhoea, occur in up to 5% of patients. With the exception of semaglutide, the GLP-1 agonists are administered by subcutaneous injection. Cardiovascular effects of GLP-1 agonists include a reduction of blood pressure (SBP 2–6 mmHg) and an increased heart rate.

The clinical cardiovascular outcome trials are industry-funded safety trials designed to satisfy the FDA-mandated requirement. Although non-inferiority was the primary statistical goal, the study design of most of the trials allowed evaluation for superiority once non-inferiority had been demonstrated. The entry criteria and baseline characteristics of the cardiovascular safety trials for the GLP1-A agonists are shown in Table 33.8.

Cardiovascular Outcome Trials for GLP 1A Agonists

ELIXA [84, 85]

The lixisenatide in acute coronary syndrome trial (ELIXA)included 6068 patients who had an acute coronary event within 180 days of randomisation. The primary endpoint quadruple MACE (CV death, MI, stroke and hospitalisation for unstable angina) was not reduced by lixisenatide but showed non-inferiority. A total of 25% of subjects discontinued the trial prematurely, largely as a result of gastrointestinal symptoms.

Table 33.8 Cardiovascular safety trials of GLP-1 agonists. Entry criteria, baseline characteristics, follow-up duration and placebo mortality rates

Study	No. of patients	Renal function	% with eGFR < 60 ml/min/1.73 m^2	History of HF %	% with CVD	Baseline A1C (%)	BMI	Median FU (years)	Placebo mortality/100 pt years
ELIXA Lixisenatide	6068	eGFR > 15	23.2%		100	7.7	30.1	2.1	3.3
LEADER Liraglutide	9340	No dosage adjustment	21.7%		81	8.7	32.5	3.8	2.3
SUSTAIN 6 Semaglutide	3297	No dosage adjustment	24.1%		60	8.7	32.8	2.1	1.76
EXSCEL Exenatide	14,752	eGFR > 45	18.6%		73	8.0	31.8	3.2	2.3
HARMONY Albiglutide	9463	eGFR > 15	18.0% nephropathy	20%	100	8.7	32.3	1.6	2.56
REWIND Dulaglutide	9901	No dosage adjustment	22%	8.6%	31.5	7.2	32.3	5.4	2.29
PIONEER 6 Oral Semaglutide	3183	No dosage adjustment	26.5%	12.2%	84.7	8.2	32.3	1.3	2.2

CVD Cardiovascular disease, *BMI* body mass index, *eGFR* estimated glomerular filtration rate, *A1C* glycated haemoglobin, *HF* heart failure, *FU* follow-up

LEADER [17]

The Liraglutide and cardiovascular outcomes in type 2 diabetes (LEADER) trial included patients 50–60 years old with CVD or over 60 years with at least one CV risk factor. A total of 9340 patients were randomised to receive either liraglutide 1.8 mg or placebo daily by daily s/c injection. CVD was present in 81.3% of subjects.

After a median follow-up of 3.8 years, the primary endpoint (three-point MACE (CV death, non-fatal MI or non-fatal stroke) occurred in 13% of patients receiving liraglutide and 14.9% of the placebo group (HR 0.87, 95% CI 0.78–0.97). Consistent benefits were observed in a wide range of subgroups. However, the group of patients with age >50 years and established CVD had a greater benefit (HR 0.83, 95% CI 0.74–0.93) than the group aged >60 and risk factors for CVD (HR 1.2, 95% CI 0.86–1.67). Cardiovascular mortality was significantly reduced by 22% (HR 0.78, 95% CI 0.66–0.93) and all-cause mortality was significantly reduced by 15%. The rates of myocardial infarction were borderline significantly reduced by 14% (HR 0.86, 95% CI 0.73–1.00, $p = 0.046$). Hospitalisation for heart failure was not statistically reduced (HR 0.87, 95% CI 0.73–1.05). Nephropathy (defined as new onset of macroalbuminuria, or a doubling of the serum creatinine and an eGFR of ≤45 ml/min/1.73 m^2, the need for continuous dialysis, or death from renal disease) was reduced by 22% (HR 0.78, 95% CI 0.67–0.92). There was a consistent weight loss of 2.3 kg (95% CI 2.5–2.0). Systolic BP was 1.2 mmHg lower and resting heart rate was 3bpm higher.

Gastrointestinal adverse effects were the most common reasons for discontinuing treatment in 1.6% of the treatment group. Acute gallstone disease occurred in 3.1% of the liraglutide group and 1.9% in patients receiving placebo. There was no significant increase of pancreatic disorders, although pancreatic cancer occurred in 13 of the liraglutide and 5 of the placebo groups.

SUSTAIN-6 [19]

The Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in with Type 2 Diabetes (SUSTAIN-6) included 3297 subjects >50 years old with established CVD or chronic kidney disease and subjects >60 with at least one CV risk factor. They received semaglutide or placebo as a weekly s/c injection. A total of 83% had CVD or CKD stage 3. Semaglutide reduced the incidence of the primary MACE endpoint by 26% (Semaglutide 6.6% Placebo 8.9% HR 0.74 95% 0.58–0.95 *p*-value for non-inferiority <0.001, for superiority 0.002). Individually CV death, non-fatal MI or heart failure hospitalisation were not significantly reduced. There were fewer strokes in the semaglutide group, with 27

compared to 44 in the placebo group (HR 0.61 95% CI 0.38–0.99). New or worsening nephropathy was reduced by 36% (HR 0.64, 95% CI 0.46–0.88).

Gastrointestinal symptoms were the commonest reason for discontinuation of the medication. Gall bladder disease or pancreatitis were not increased. Complications of retinopathy including vitreous haemorrhage, onset of diabetes-related blindness and need for treatment with an intravitreal agent or photocoagulation were increased by semaglutide (HR 1.76 95% CI 1.11–2.78).

It is notable that SUSTAIN 6 had the greatest A1C difference of 0.7–1.0 % between the treatment and placebo groups.

EXSCEL [86]

In the 'Effects of once weekly Exenatide on Cardiovascular Outcomes in type 2 Diabetes' (EXSCSEL) study, more than 80% of the 14,752 subjects had pre-existing CVD. Following 3.2 years treatment with weekly injections of exenatide, there was no difference in MACE event rates between the exenatide and placebo groups. There was no difference in the rates of serious adverse events including severe hypoglycaemia or pancreatic disorders.

HARMONY Outcomes [87]

The 'Albiglutide and CV outcomes in Patients with type 2 diabetes and CVD' (HARMONY Outcomes) included 9463 patients with established coronary artery disease (71%), cerebrovascular disease (25%) or peripheral arterial disease (25%) who received a weekly s/c injection of albiglutide or placebo for 1.6 years. The primary endpoint of triple MACE was reduced by 22% in subjects receiving albiglutide (HR 0.78, 95% CI 0.68–0.90). Fatal or non-fatal myocardial infarction was reduced by 25% (95% CI 0.61–0.91). Cardiovascular or all-cause mortality was not reduced. Serious adverse events such as severe hypoglycaemia or pancreatitis were not increased. Despite the very short duration of the trials, CV benefit of albiglutide was observed in this high-risk population.

REWIND [85]

The Dulaglutide cardiovascular outcomes in Type 2 diabetes (REWIND) trial included patients with both cardiovascular disease and cardiovascular risk factors. A total of 9901 patients received dulaglutide or placebo and were followed for 5.4

years. However, 31.5% had known CVD. The primary MACE endpoint was reduced (HR 0.88, 95% CI 0.79–0.99). All-cause mortality was not reduced but stroke rates were 24% lower. Renal outcomes (new onset albuminuria, a sustained >30% reduction of eGFR or need for dialysis) were reduced by 15%.

The REWIND study was the longest of all the GLP-1 RA safety trials with the lowest risk subjects.

PIONEER-6 [88]

The oral Semaglutide and Cardiovascular Outcomes in patients with type 2 diabetes (PIONEER-6) study included patients over 50 years old with established CVD or renal disease (84.7%) or over 60 years old with risk factors for CVD. Oral semaglutide 14 mg or placebo was given daily to 3183 patients who were followed for 15.9 months. The primary outcome of triple MACE showed non-inferiority but failed to show superiority. Serious adverse events did not differ between the semaglutide and placebo groups. Despite showing no significant benefit, the hazard ratio of the primary endpoint was similar to the SUSTAIN 6 trial using injectable semaglutide (0.79 vs. 0.74).

The seven CV outcome trials for GLP1 agonists show heterogeneity for the CV outcomes. All trials showed non-inferiority, and liraglutide, s/c semaglutide, albiglutide and dulaglutide have shown superiority with significant reductions of the primary outcome. The recent meta-analysis of the seven trials [89] (Fig. 33.9) indicates that the combined primary outcome is reduced by 12%, CV mortality reduced by 12% (HR 0.88, 95% CI 0.81–0.96) and fatal /non-fatal stroke by 16% (HR 0.84, 95% CI 0.76–0.93). Fatal/non-fatal MI is numerically reduced by 9%; however, the reduction is only borderline statistically significant (HR 0.91, 95% CI 0.84–1.00, $p = 0.043$). None of the trials show a significant reduction of heart failure hospitalisation. Combined renal outcomes are reduced in Leader, SUSTAIN-6 and REWIND. However, in ELIXA, there was a nonsignificant reduction of the urinary albumin creatine ratio.

Although patients with no cardiovascular disease appeared to have little or no cardiovascular benefit (Fig. 33.9), statistically there was no interaction between the groups with and without CVD. Hence the analysis indicated similar benefit for patients with and without CVD (Fig. 33.10).

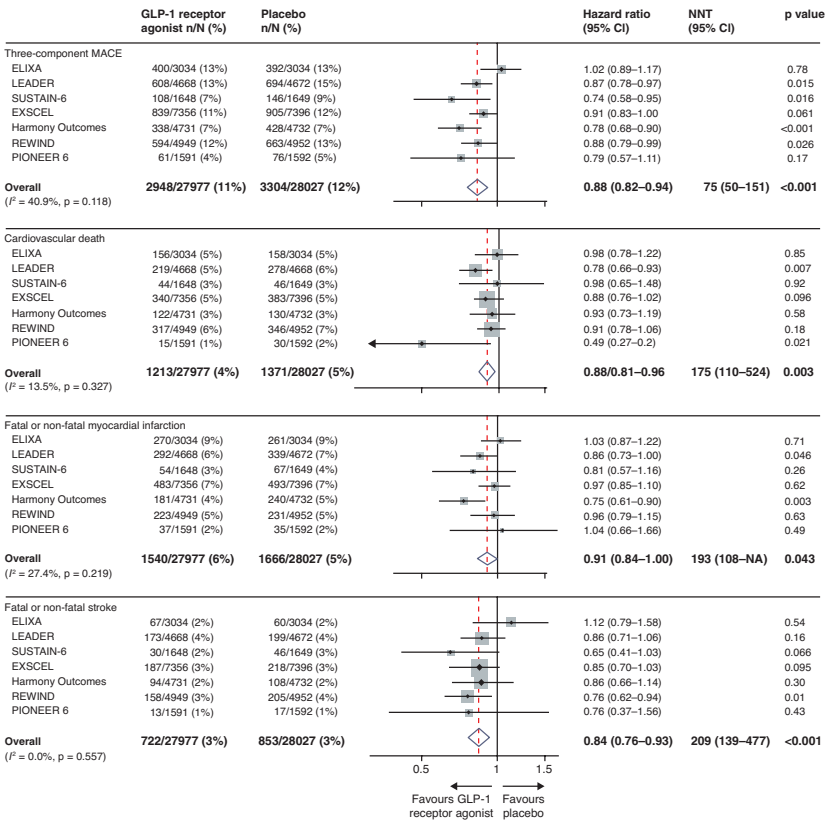

Fig. 33.9 Meta-analysis of GLP-1 agonists cardiovascular trials. Reprinted with permission from Kristensen et al. [89]

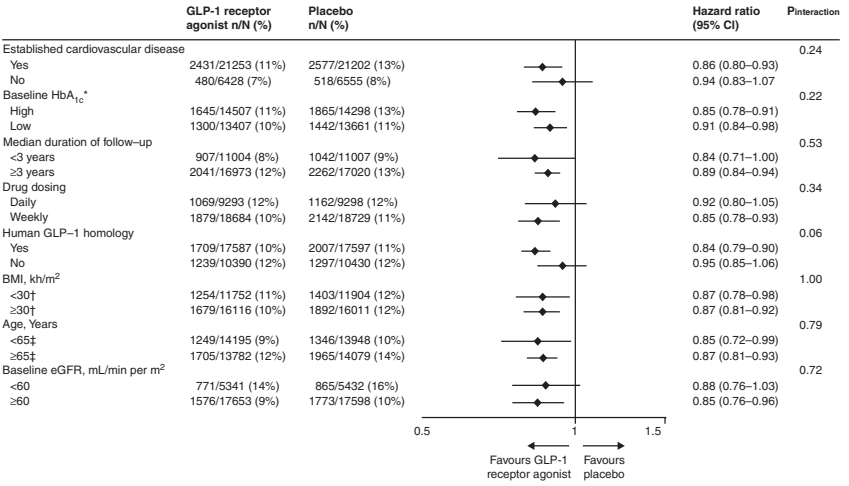

Fig. 33.10 Impact of GLP-1 agonists on subgroups. Reprinted with permission from Kristensen et al. [89]

Impact of GLP-1 RA on Kidney Outcomes

Kidney outcomes definitions varied between the GLP-1 RA trials (Table 33.9). A doubling of creatinine or 40% reduction of eGFR was only significantly reduced in the REWIND trial (HR 0.70 95% CI 0.57–0.85). The composite renal outcome with different definitions was significantly reduced in the LEADER (HR 0.78 95% CI 0.67–0.92), SUSTAIN 6 (HR 0.64 95% CI 0.46–0.88) and REWIND (HR 0.85 95% CI 0.77–0.93). New onset albuminuria was significantly reduced in LEADER, SUSTAIN 6 and REWIND. No study showed a reduction of progression to end-stage kidney disease or death due to kidney disease.

GLP-1 RA and Heart Failure

None of the cardiovascular safety trials of GLP-1 RA have individually shown a significant reduction of heart failure hospitalisation. In SUSTAIN, 623.6% of patients were recorded at baseline as having a history of heart failure. Yet more patients receiving semaglutide were hospitalised for heart failure than those receiving placebo (1.76 vs. 1.61/100,000). In the LEADER trial, 18% of patients had a history of heart failure, and there was a nonsignificant 13% reduction of heart failure hospitalisation. A meta-analysis of the seven GP-1 RA CV trials showed a 9% reduction of heart failure hospitalisation (HR 0.91 95% CI 0.83–0.99) with an NNT to prevent one HF hospitalisation of 311 over the median 3.2 years follow-up.

Table 33.9 Definitions of kidney outcomes by clinical trial

	Worsening kidney function (narrow outcome)	Composite kidney outcome including macroalbuminuria (broad outcome)
ELIXA	Doubling of serum creatinine	New-onset macroalbuminuria
LEADER	Doubling of serum creatinine	New-onset macroalbuminuria, doubling of serum creatinine (eGFR < 45 ml/min/m^2). ESKD, death due to kidney disease
SUSTAIN-6	Doubling of serum creatinine	New-onset macroalbuminuria, doubling of serum creatinine (eGFR < 45 ml/min/m^2), ESKD, death due to kidney disease
EXSCEL	≥40% worsening of eGFR, ESKD, death due to kidney disease	≥40% worsening of eGFR, ESKD, death due to kidney disease, new-onset persistent macroalbuminuria
Harmony Outcomes	No outcomes reported	No outcomes reported
REWIND	≥40% worsening of eGFR	New-onset macroalbuminuria, ≥30% worsening of eGFR, ESKD
PIONEER 6	No outcomes reported	No outcomes reported

eGFR estimated glomerular filtration rate, *ESKD* end-stage kidney disease

Safety of GLP-1 Agonists

GLP-1 receptor agonists (GLP-1 RA) have not been associated with any major safety issues.

Hypoglycaemia is rare unless the GLP-1 RA is used in combination with either insulin or sulphonylureas.

The most frequent off-target side effect of GLP-1 RA is nausea and vomiting. In the CV safety trials, nausea, vomiting or diarrhoea lead to drug discontinuation in approximately 3% of subjects receiving a GLP-1 RA and 0.5% in those receiving a placebo. However, in many patients, the GI symptoms are transient and can be diminished by initiating at a low dose, gradually increasing the dose and advising patients to eat small meals [90].

Treatment with GLP-1 RA may be associated with an increased risk of cholecystitis. GLP-1RA delay gastric emptying and should be used with caution in patients with prior gastric surgery or symptomatic gastroparesis.

Post-marketing reports indicate a possible association between GLP-1 RA use and the development of acute pancreatitis. Yet none of the CV safety trials have shown an increased risk, yet patients at high risk, especially with a prior history of pancreatitis were excluded from the trials. The FDA and EMA have not identified any association between GLP-1RA treatment and pancreatitis or pancreatic cancer [90]. However, treatment with a GLP-1 RA should be discontinued if pancreatitis develops.

Diabetic retinopathy complications, including vitreous haemorrhage, onset of diabetes-related blindness and need for treatment with an intravitreal agent or photocoagulation were increased in the SUSTAIN 6 trial by injectable semaglutide [19]. Although there was a trend to increased retinopathy in the REWIND trial, the complication was not observed in any other study, and the meta-analysis overall risk is not significantly increased. It has been proposed that the retinal complications resulted from the rapid reduction of blood glucose and not any direct effect of the drug. However, patients receiving a GLP-1 RA should have a retinal evaluation prior to and periodically during treatment.

Prior to the GLP-1 RA trials, there was concern that the agents could provoke medullary thyroid cancer. However, no increased risk was observed in the almost 50,000 patients enrolled in these trials.

Mechanisms of CV Benefit of GLP-1 Agonists

GLP-1RA have multiple actions that improve CV risk factors. Weight is reduced by 2–3 kg, blood pressure is reduced and glycaemic control is improved.

GLP-1 receptors are found in both the myocardium and blood vessels (Fig. 33.11). The GLP-1RA modulates systemic inflammation and more localised inflammation in both the myocardium and blood vessesls [82]. GLP-1 RA has anti-inflammatory actions in pre-clinical studies at doses that do not cause weight loss and attenuates

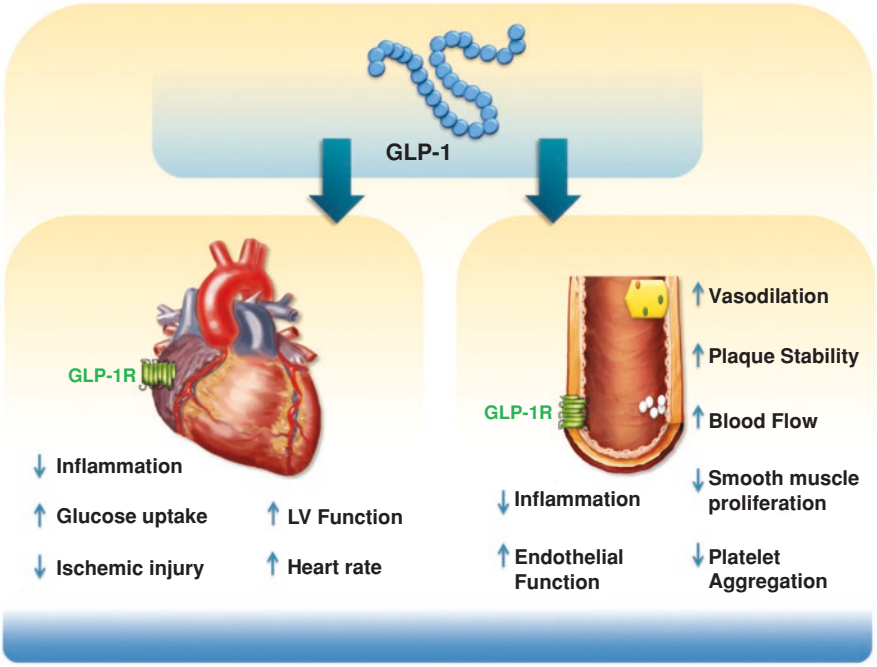

Fig. 33.11 Action of GLP-1 RA on the heart and blood vessels (Reprinted with permission from Drucker et al. [82])

the development of atherosclerosis [83]. Monocyte adhesion to the endothelium and matrix metalloproteinase activity are reduced, thus stabilising the atherosclerotic plaque. In human studies, GLP-1 RA exert both antioxidant and anti-inflammatory effects that appear to be independent from changes in insulin levels. Whether the anti-inflammatory properties of GLP-1 RA are due to a direct effect, or secondary to metabolic changes or weight loss remains controversial [82, 83].

These putative mechanisms translate into possible ways GLP-1 RA might modify CV risk (Fig. 33.12). GLP-1 RA have cardioprotective properties following coronary artery occlusion with smaller infarct size and improved survival [83]. In humans with acute myocardial infarction, GLP-1 RA may reduce myocardial infarct size [91, 92]. Preclinical studies suggest beneficial effects of GLP-1 RA in models of ventricular dysfunction. Human studies have shown mixed results and a larger placebo-controlled randomised study is awaited [82].

GLP-1 RA reduces intestinal chylomicron production. However, the reduction of postprandial triglyceride levels is likely due to an indirect effect of GLP-1 R agonism, from increased insulin and decreased glucagon levels, weight loss and increased insulin sensitivity. Platelet aggregation is inhibited in animal models; however, there is no human data to indicate if GLP-1 RA induces any clinically important change of platelet function or coagulation factors [82]. Endothelial function may be improved [93] and forearm blood flow increased by GLP-1 RA

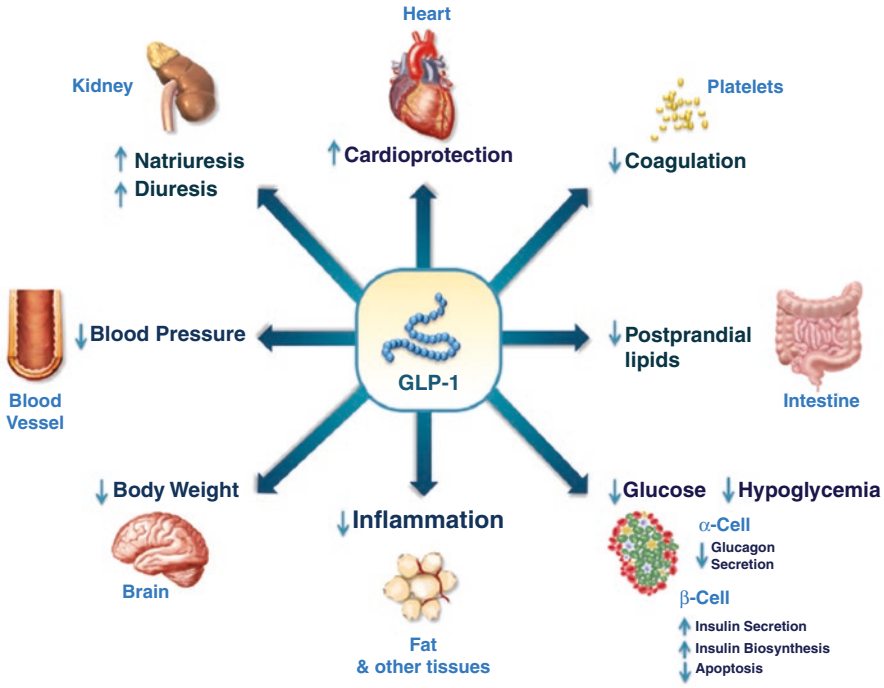

Fig. 33.12 Putative mechanisms for GLP-1 RA modification of CV risk. Reprinted with permission from Drucker et al. [82]

infusion, independent of insulin levels [94]. However, there are several studies showing conflicting results, and it is unclear whether any changes of endothelial function are a direct or indirect effect of GLP-1 RA.

Sustained administration of a GLP-1 RA results in a persistent increase of heart rate of approximately 3bpm, but a fall in systolic BP of approximately 2–3 mmHg. The cause of the increased heart rate is unclear. The mechanism for the fall of blood pressure, which is independent of weight loss, is also not determined, but may be due to natriuresis and vasodilatation. No consistent change in plasma levels of renin, aldosterone or angiotensinogen has been observed.

Summary of Outcomes with GLP-1 RA

Clinical trials with GLP-1 RA showed heterogeneous results, with liraglutide, semaglutide, albiglutide and dulaglutide showing significant reductions of the triple MACE primary endpoint yet trials with lixisenatide, exenatide and oral semaglutide showing no reduction. Cardiovascular mortality was reduced with liraglutide. However, the meta-analysis showed an overall a 12% reduction and an NNT to prevent one CV death of 175.

Liraglutide and albiglutide significantly reduced fatal or non-fatal myocardial infarction. The meta-analysis showed a borderline 9% reduction. In the meta-analysis, stroke was reduced by 16% with only albiglutide showing a significant reduction (HR 0.84, 95% CI 0.76–0.93).

Heart failure hospitalisation was not reduced in any of the individual trials yet the meta-analysis showed a 9% reduction. Renal outcomes were improved with composite renal outcomes reduced in all trials. Yet worsening of renal function was only improved with dulaglutide and the meta-analysis indicated a nonsignificant improvement. Weight loss of 1–2 kg was observed early during treatment and maintained throughout the trials.

The most frequent adverse event was nausea, vomiting and diarrhoea. Yet, strategies can reduce this adverse side effect that leads to approximately 2% of patients discontinuing treatment. Severe hypoglycaemia was infrequent and usually related to the cotreatment with insulin or a sulphonyl-urea.

Application of SGLT2 Inhibitors and GLP-1 Receptor Agonists in Patients with Diabetes

Over the past 5 years, two classes of glucose-lowering drugs have been shown by rigorous randomised placebo-controlled trials to reduce cardiovascular events. The largely consistent results of the trials show that it is likely the cardiovascular benefits are class effects of SGLT2 inhibitors and GLP-1 RA. SGLT2 inhibitors reduce heart failure admission, reduce CV mortality and slow the progression of chronic kidney disease. In contrast, the GLP-1 RA reduce myocardial infarction and stroke, have a small impact on CV mortality, but do not slow the worsening of renal function. Studies of both SGLT2 inhibitors and GLP-1 RA show smaller absolute benefits in patients with no prior atherosclerotic CV disease. However, meta-analyses of SGLT2 inhibitor trials and GLP1-1 RA trials [89] showed no statistically significant interaction between primary and secondary prevention. Yet, guidelines continue to recommend either agent as first-line treatment only in patients with established CVD (Fig. 33.13). It is possible that the combination of an SGLT2 inhibitor and a GLP-1 RA would provide additive cardiovascular benefits.

For patients with diabetes with CVD, chronic kidney disease or multiple CVD risk factors.

An SGLT2 inhibitor or a GLP-1 RA with proven CV benefits should be considered (Fig. 33.13). Yet if heart failure or chronic kidney disease is a concern then a SGLT2 inhibitor is the preferred treatment. If the eGFR is less than 30 ml/min/1.73 m^2, the use of a GLP-1 RA may be preferred. However, the results of the DAPA CKD trial indicate the safety and benefit of an SGLT2 inhibitors even when the eGFR is 25 ml/min/1.73 m^2. For patients with a recent acute coronary event, either a SGLT2 inhibitor or a GLP-1 RA could be prescribed during hospital stay, yet the optimal timing for initiating treatment is not known. Long-term adherence to treatment may be improved by starting treatment prior to discharge and should be considered in patients who are hemodynamically stable.

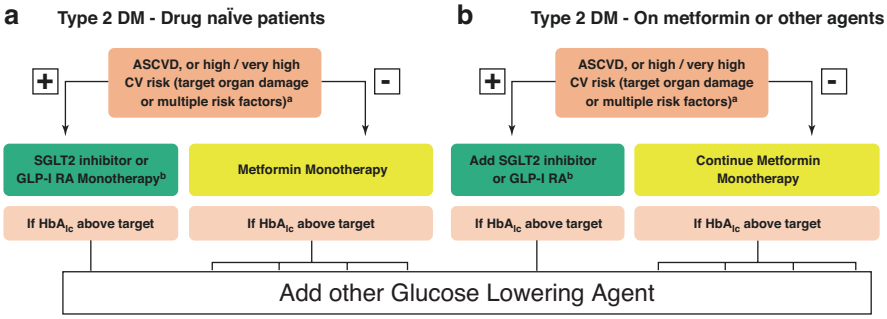

Fig. 33.13 Algorithm for the initial choices of glucose-lowering drugs in patients with type 2 diabetes. (**a**) not taking glucose lowering agents (drug naive) (**b**) receiving metformin or other agents. Reprinted with permission from Cosentino F et al. [95]

In patients at high risk of ASCVD (with multiple risk factors in addition to diabetes), either a SGLT2 inhibitor or a GLP-1 RA should be considered. For individuals at high risk of heart failure or CKD, a SGLT2 inhibitor is selected.

Until recently, glucose-lowering drugs were only prescribed by family physicians and diabetologists/endocrinologists. Today, all physicians play a role in the identification of patients with diabetes, the management of cardiovascular risk factors and initiating drugs with proven cardiovascular benefits such as SGLT2 inhibitors and GLP-1 RA agents. All physicians taking care of patients with diabetes need to be comfortable prescribing SGLT2 inhibitors and GLP-1 RA. Furthermore, now that we have evidence to support the use of SGLT2 inhibitors in patients without diabetes, with heart failure and with chronic kidney disease, SGLT2 inhibitors are no longer just a glucose-lowering drug for the treatment of patients with diabetes. Patients with ASCVD, a history of heart failure or CKD should be screened periodically for diabetes by measuring HbA1c. Patients with ASCVD and chronic kidney disease, and/or heart failure, should be considered for first-line treatment with either a SGLT2 inhibitor or GLP-1 RA with proven CV benefit irrespective of the HbA1c level. In the EMPA-Reg Outcome trial, CV benefit was observed independent of the use of any antidiabetic agents including metformin and insulin. The current European Society of Cardiology/European Association for the Study of Diabetes (Fig. 33.13) now recommends initiating either an SGLT2 inhibitor or GLP-1 RA before metformin in patients with newly diagnosed diabetes and either documented CVD or at very high CV risk [95].

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Chapter 34
Insulin Treatment of Diabetes Mellitus-Tight vs. Conventional Control

Nicholas Emanuele and Peter D. Reaven

Dr. Nicholas Emanuele, my coauthor for this chapter, unfortunately, passed away from COVID-19 while we were working on this project. He is one of the countless, wonderful health care providers and human beings that have perished from this viral scourge. As I thought of him each time I worked on this chapter, I felt it only appropriate to share this information as well as a little about both "Nick" the physician investigator and the man himself

After completing his Endocrine Fellowship at Northwestern University, he joined the military and became Chief of Endocrinology at the Tripler Army Medical Center. Although he never spoke to me of this time, I imagine this was just part of his ever-present desire to give back to society. He subsequently joined the Endocrinology Section, Hines VA Hospital and Department of Medicine, Division of Endocrinology at Loyola University. He eventually became Director, Division of Endocrinology and Metabolism, Loyola University of Chicago Stritch School of Medicine, where he helped establish vibrant clinical and clinical research programs. He was a dedicated educator of fellows and residents and was a strong advocate for his junior researchers/ colleagues. I was always impressed with how hard he worked to support their careers, often taking less prominent positions on papers or presentations to ensure his junior colleagues were highlighted instead

Nicholas Emanuele has died before the publication of this book.

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He managed to conduct science with a strong touch of humanity—never forgetting that we are all humans first, researchers second. He led pioneering research in neuroendocrinology, which along with his other diverse endocrine interests led to publication of several hundred published papers and chapters. One of his many longstanding research interests revolved around understanding the effects of ethanol on the neuroendocrine system, publishing over 50 papers in this area alone. He also became interested in complications of diabetes and identifying the optimal treatment to prevent these from occurring in type 2 diabetes. This stimulated his participation in many large VA Cooperative Study Program studies, including the VA Nephron-D study which tested combination therapy of an angiotensin-converting enzyme inhibitor with an angiotensin receptor blocker among patients with diabetic nephropathy. The main paper from this study demonstrated that combining these agents in type 2 diabetes was, in fact, harmful for renal function. This was a surprising but critical result, since this combination approach was gaining traction among many healthcare providers at the time.

I first came to know Nick as a fellow site investigator on the VA Diabetes Trial, where he and I directed study activities at the Hines and Phoenix VAs, respectively. Over time, we both took on Executive Committee leadership roles within the study and eventually became Co-Chairs of the long-term follow-up study. We shared many a long conference call discussing recruitment, data collection, results, and strategies going forward. These calls occasionally became relatively heated, with many differing opinions forcibly expressed by committee members- not infrequently leading to an uncomfortable gridlock. Nick, however, was unfailingly positive and had a wonderful sense of humor and would make some amusing comment (often about how this conversation reminded him in some way of the sad state of affairs of one of his beloved Chicago sports teams) to lessen the tension, or subtly poke fun at one of us or make a self-deprecating remark, which would invariably get us laughing and lower the temperature of the meeting. He would then tactfully redirect us to the key decisions and actions required to move the study forward in the right direction. I observed this technique repeatedly over years, never quite fully understanding how he knew just when and what to say. Eventually, I just came to accept this as one of his "special talents," which I came to cherish along with his intellect, his deep concern for doing the right thing for his patients and research participants and his persistent commitment to each research project.

This dedication was never more apparent than on the day of our last in-person conversation. We were scheduled to have another one of our many calls that afternoon to discuss progress on this chapter as well as various issues related to the closure of the VA Diabetes Trial follow-up study. Nick called me a little before the meeting time, to let me know that he was not going to make the

call that day. He was heading to the hospital for increasing shortness of breath and informed me of his recent COVID infection. Despite this, he wanted to let me know of recent progress he had made on his sections of the chapter and hoped I could help pick up where he left off—to keep things moving along. Sadly, he died approximately a week later, on December 15, 2020.

Nick—wherever you are, I hope you are proud of this chapter and all the work we have completed together over the years. Working with you on the VA Diabetes Trial was one of my professional highlights and I would not have enjoyed the experience nearly as much without your humor, wit, and genuine warmth. You are greatly missed

Introduction

By the end of the twentieth century, results from three trials reflecting geographically and phenotypically distinct populations reported that good glycemic control prevented or delayed the progression of the microvascular complications of diabetes mellitus, namely retinopathy, nephropathy, and neuropathy. The earliest of these, the Diabetes Control and Complications Trial (DCCT), conducted throughout the US, reported in 1993 that intensive glucose control prevented or slowed the progression of microvascular disease in relatively young people with type 1 diabetes (T1DM) [1]. Subsequently, a small (n = 110) Japanese study, the Kumamoto Study, showed that good control prevented or slowed the progression of microvascular outcomes in thin, middle-aged individuals with T2DM [2]. At the end of the last decade of the twentieth century, data from the United Kingdom Prospective Diabetes Trial (UKPDS) also showed that good glycemic control prevented or slowed the progression of microvascular events in people with newly diagnosed T2DM [3, 4].

While the microvascular disease is important and can greatly affect the quality of life in those with diabetes, the major cause of morbidity and mortality in people with diabetes is the macrovascular disease. Numerous epidemiologic studies have suggested that hyperglycemia also has deleterious effects on cardiovascular disease risk in T2DM [5]. For example, retrospective analyses from the UKPDS showed a 14% decrease in fatal and nonfatal myocardial infarction per 1% decrement in HbA1c and a 12% decrease per 1% decrement in HbA1c [6]. However, no *prospective* trial data had yet shown clear cardiovascular disease (CVD) risk reduction with excellent glycemic control. As a result, what target goals for HbA1c might be optimal for both micro-and macrovascular disease remained unknown. Thus, there was a need for larger, longer studies, especially in people with advanced diabetes. This led to the initiation of three separate studies to examine the effects of improving glycemic control on development of CVD in older T2DM patients with known CVD or at high risk for CVD.

The objective of this chapter is to summarize the data generated by the five major interventional trials (and their observational follow-up phases) of the late twentieth and early twenty-first centuries that helped fill the gap in knowledge on

the question of intensive glycemic control and CVD risk. These studies included the DCCT [1], UKPDS [3, 4], the Action in Diabetes and Vascular Disease: Preterax and Diamicron - MR Controlled Evaluation (ADVANCE) [7], the Veterans Affairs Diabetes Trial (VADT) [8], and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) [9]. Although all trials included analyses examining consequences of intensive glucose lowering on CVD outcomes, the number of events or the time of follow-up was too short in some trials to fully evaluate this outcome. Thus, several of the trials recognized the need for a longer observational follow-up phase. During these observation periods, patients typically returned to care of their own physicians were no longer following rigorous trial protocols, and as a result glycemic separation between treatment arms waned. This permitted investigators to examine if there was evidence that earlier glucose-lowering efforts (during the trial) provided a delayed or legacy effect on CVD events when glycemic separation no longer existed.

To facilitate overview of studies, we have provided a summary that includes relevant participant and trial characteristics and summary results for each study's active interventional and follow-up phase.

Diabetes Control and Complications Trial (DCCT)

Interventional Phase

The DCCT was a study of 1441 male and female individuals with T1DM ages 13 to 39 at entry. Participants included a primary prevention cohort with no retinopathy and a secondary intervention cohort with mild retinopathy. Diabetes duration was 2.6 years in the primary prevention cohort, around 8.8 years in the secondary intervention cohort and entry HbA1c for the whole group was approximately 9% [1]. At baseline, all participants were on insulin (as expected for T1DM) but only 5.8% had prior CVD events. The treatment arms were well balanced with respect to standard CVD risk factors including blood pressure, lipids, body weight, and smoking. They were followed for a mean of 6.5 years to determine the effect of intensive glycemic control on retinopathy and other vascular outcomes. By 6 months into the trial, the average achieved HbA1c in the standard group remained at 9%, while the HbA1c in the intensively treated group fell to 7%; this treatment difference was maintained throughout the study. There was a highly significant benefit of intensive glycemic control on both the incidence and progression of retinopathy, the primary endpoints. The cumulative number of CVD events was relatively low (even combining all major cardiac and peripheral events), and although there was a trend for a decrease in these vascular events in the intensively treated patients compared with those in standard treatment group, this did not reach statistical significance (Table 34.1).

Table 34.1 Overview of major landmark clinical trials of glucose lowering in participants with diabetes mellitus

Landmark clinical trials in diabetes mellitus						
	Early diabetes			Advanced diabetes		
	UKPDS (insulin/ sulfonylurea	UKPDS (metformin)	DCCT	ADVANCE	VADT	ACCORD
DM type	2	2	1	2	2	2
Participant number	4209	342	1441	11,140	1791	10,251
Age (years)	53	53	13–39	66	60	62
Gender (% M/F)	61/39	46/54	50/50	58/42	97/3	62/38
DM duration (years)	0	0	2.6/8,8[a]	8	11.5	10
Baseline HbA1c (%)	7.1	7.3	9	7.5	9.4	8.1
Baseline CV history (%)	7.5	7.5	5.8	32	40	35
Baseline insulin use (%)	0	0	100	1.5	50	35
Achieved HbA1c:STD/INT[b]	7.9/7	8/7.4	9/7	7.3/6.5	8.5/6.9	7.5/6.4
Intervention median duration (years)	10	10.7	6.5[c]	5.0	5.6	3.7[c]
Approximate total follow-up (years)	17	17.7	29	10	10 & 15	9
CV benefit/harm	Benefit[d]	Benefit	Neither	Neither	Neither	Harm
CVD legacy	Yes	Yes	Yes	No	Yes[e]	No

[a]2.6 years in primary prevention group, 8.8 years in secondary intervention group
[b]*STD* standard glycemic control; *INT* intensive glycemic control
[c]Mean duration
[d]CVD benefit was most evident in the follow-up period only, after glycemic separation was no longer apparent
[e]There was CV benefit at the 10-year follow-up, but this was no longer seen at the 15-year follow-up

Observational Follow-Up Phase

Most (93%) of the DCCT cohort was followed passively in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) [10]. After less than 2 years of observation, the separation in glycemic control diminished between the treatment arms, with both groups having HbA1c values stabilizing around 8%.

CVD was defined as nonfatal myocardial infarction, stroke, death from cardiovascular disease, subclinical myocardial infarction, confirmed angina, clinically significant obstruction on coronary angiograph, or the need for coronary-artery revascularization. When data were analyzed after a mean of 17 years of follow-up, those previously in the intensive glycemic control group (in either the primary

prevention or secondary intervention cohorts) showed dramatic CVD benefit. There was a reduction in CVD by 42% (95% CI, 9–63%, $P = 0.02$) and the risk of nonfatal myocardial infarction, stroke, or death (standard 3-point major adverse cardiovascular event (MACE)) by 57% (CI 12–79%, $P = 0.02$) [11].

These data indicated that if the glucose-lowering intervention is substantial (approximately 2% in absolute HbA1c units), started relatively early in T1DM, there is a CVD benefit, but it is delayed. Furthermore, the CVD benefit grew even after glycemic separation between the two groups was lost. This provided strong evidence of a legacy effect of earlier glucose lowering in the intensively treated participants.

The United Kingdom Prospective Diabetes Study (UKPDS)

Interventional Phase

The UKPDS Study enrolled 4209 people with *newly diagnosed* T2DM with an average age of 53 years and an entry HbA1c of 7.1%. A majority of patients were male (61%), only 7% had known CVD at baseline and, not surprisingly, none were using insulin at the time of enrollment. The treatment arms were well balanced in terms of standard CVD risk factors such as blood pressure, lipids, weight, and smoking [3, 4] Study participants were followed for approximately 10 years with the primary aim of determining the impact of glycemic control on "any diabetes related endpoint," a combination of important microvascular and macrovascular events [3]. The primary treatment arm comparison was sulfonylurea and/or insulin compared with conventional therapy of diet or non-intensive pharmacologic therapies if needed. Over the course of the study, the average HbA1c in the conventional arm was 7.9% and in the intensively treated group it was 7%. There was a 12% reduction in any diabetes-related endpoint in the intensive compared to the conventional group, RR 0.88 (CI, 0.79–0.99, $P = 0.029$). However, the main effects of more intensive glycemic control were to reduce photocoagulation from 11 to 8 photocoagulations/1000 patient years and cataract extraction from 7.4 to 5.6 extractions/1000 patient years. Although there was not a comprehensive composite CVD outcome, there was a nonsignificant trend toward a decrease in myocardial infarction in the intensive glycemic control arm (RR 0.84, CI 0.71–1.00, $P = 0.052$; Fig. 34.1). Changes in diabetes-related deaths (RR 0.90, CI 0.73–1.11, $P = 0.34$), all-cause mortality (RR 0.94 (0.8–1.10), $P = 0.44$), or stroke (RR 1.11, CI 0.81–1.51, $P = 0.52$) were not statistically significant.

Thus, in newly diagnosed T2DM patients, moderate glucose lowering (with a HbA1c difference between groups of 0.9%) over a sustained period of time with sulfonylurea/insulin treatment led to microvascular, but not clear macrovascular benefits.

The Metformin Subgroup

Within the UKPDS study, there was a secondary analysis of 342 overweight patients who were randomized to metformin (instead of sulfonylurea/insulin) or

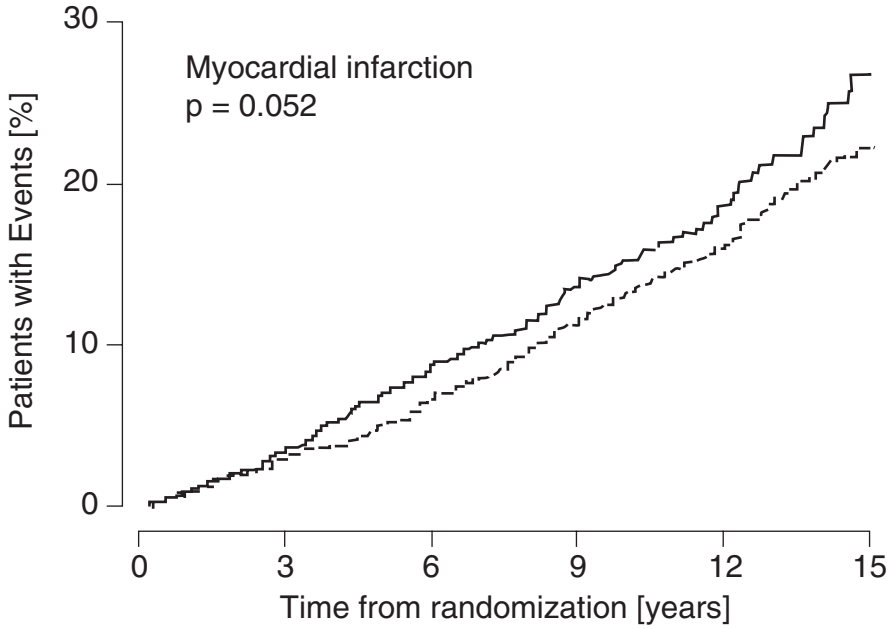

Fig. 34.1 Effects of intensive glucose lowering in type 2 diabetes in the UKPDS study. (Figure is modeled from intensive blood–glucose control with sulphonylurea or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes, *The Lancet*, Vol 352, September 12, 1998 and data are not intended to exactly represent original curves)

conventional therapy [4]. These patients were followed for 10.7 years. The median HbA1c during follow-up was 7.4% in the metformin group and 8·0% in the conventional treatment group. The treatment arms were well balanced in terms of standard CVD risk factors. Patients assigned to more intensive blood–glucose control with metformin had a 32% lower risk (HR 0.68, CI 0.53–0.87), $P = 0.0023$) of developing any diabetes-related endpoint, a combination of important microvascular and macrovascular events, compared with those allocated conventional blood–glucose control. This was also a significantly greater risk reduction than those assigned intensive therapy with sulfonylurea or insulin ($P = 0.0034$). Despite the relatively small sample size for a CVD outcome trial, the metformin group demonstrated a 36% lower risk (HR 0.64, CI 0.45–0.91, $P = 0.011$) in all-cause mortality than the conventional group, which was a greater risk reduction than in the those assigned to intensive therapy with sulfonylurea or insulin ($P = 0.021$). The metformin group also had a 39% lower risk (RR 0.61, CI 0.41–0.89, $P = 0.010$) of myocardial infarction than the conventional treatment group and a 30% lower risk for all macrovascular diseases combined (myocardial infarction, sudden death, angina, stroke, and peripheral disease). Overall, although the cohort was modest in size, metformin appeared to have a surprisingly effective reduction in macrovascular risk.

Follow-Up Phase

After the end of the intervention phase, participants in the UKPDS were followed with yearly clinic visits for 5 years, and then with questionnaires for another 5 years for a median follow-up of 17 years from the beginning of the interventional phase [12]. The HbA1c difference among treatment groups vanished relatively rapidly after completion of the intervention phase, with participants reaching an HbA1c of approximately 8.5%. This equalization of HbA1c allowed study investigators to examine in more detail the legacy effect of prior glucose lowering on subsequent outcome events. In the sulfonylurea–insulin group, trends seen at the end of the intervention trial achieved statistical significant reductions in risk over the approximately 10 years of additional observation for any diabetes-related end point (RR 0.91, CI 0.83–0.99, $P = 0.04$) and microvascular disease (RR 0.76, CI 0.64–0.89, $P = 0.001$). Importantly, risk reductions for myocardial infarction (RR 0.85, CI 0.74–0.97, $P = 0.01$) and death from any cause (RR 0.87, CI 0.79–0.96, $P = 0.007$) also emerged and achieved statistical significance during this follow-up period.

Similarly, in the metformin-treated subgroup, significant risk reductions achieved during the trial persisted during the long-term follow-up for any diabetes-related end point (RR 0.79, CI 0.66–0.95, $P = 0.01$), myocardial infarction (RR 0.67, CI 0.51–0.89, $P = 0.005$), and death from any cause (RR 0.73, CI 0.59–0.89, $P = 0.002$).

These long-term follow-up study results greatly added to the information gleaned from the intervention phase. They demonstrated that if glycemic intervention is started early—at the onset of T2DM—there is a clear CVD benefit. For those randomized to metformin, this occurred during the initial 10 years of treatment, but for those assigned to sulfonylurea/insulin groups, the benefit occurred more slowly, requiring approximately 17 years of median follow-up. Furthermore, the benefit in both these treatment groups becomes apparent or is sustained even after glycemic separation is lost, indicating a legacy effect of prior glucose lowering.

Action in Diabetes and Vascular Disease: Preterax and Diamicron—MR-Controlled Evaluation (ADVANCE)

Interventional Phase

ADVANCE enrolled 11,140 men and women (42%) with T2DM who were on average 66 years old, had a diabetes duration of 8 years, and an entry HbA1c of 7.5%. About 32% had CVD events at entry, but only 1.5% were on insulin [7]. They were followed in ADVANCE for 5.4 years after being randomized to either gliclazide MR (and other additional therapy to achieve glucose targets) or standard glucose-lowering therapy. Although the 8-year diabetes duration in ADAVANCE was not vastly different than 10 years in ACCORD or 11.5 years in VADT (both studies discussed below), the cohort did appear to have somewhat less advanced diabetes. Participants entered the study with a much lower HbA1c and with only a small

fraction receiving insulin. The treatment arms were well balanced in terms of standard CVD risk factors such as blood pressure, lipids, weight, and smoking [7]. Within the first six months of study, there was a modest but rapid fall in HbA1c in both treatment groups to 7.3% in the standard arm and 6.5% in the intensive arm which was largely sustained throughout the study, with an overall separation of 0.8%. These HbA1c levels were close to those achieved in ACCORD (described below). The primary endpoint of ADVANCE, a combination of microvascular and macrovascular events was met, HR 0.90, CI 0.82–0.98, $P = 0.013$), but this was largely driven by improved renal disease, specifically proteinuria. For macrovascular events, there was a modest and not significant CVD benefit, HR 0.94, CI 0.84–1.06, $P = 0.32$. There was no effect of more intense glycemic control on all-cause mortality, HR 0.93, CI 0.83–1.06, $P = 0.28$. These results indicated that improved glucose lowering can improve some microvascular outcomes (primarily proteinuria) but indicates that the benefit on macrovascular disease is less evident.

Follow-Up Phase

During 6 years of observational follow-up, providing a total of greater than 10-year follow-up from the start of the study, no late CVD benefit emerged [13]. There was no benefit for myocardial infarction (HR 1.02, CI 0.89–1.19, $P = 0.75$), stroke (HR 1.01, CI 0.89–1.15, $P = 0.82$), CVD death HR 0.97, CI 0.86–1.10, $P = 0.63$), or all-cause mortality (HR 1.00, CI 0.92–1.08, $P = 0.91$).

In contrast to the more beneficial effects reported in earlier studies (DCCT and UKPDS), these results suggested that glucose-lowering benefits may be less impressive in more advanced T2DM patients, even following extended follow-up. Although stage of diabetes may be one contributor to the reduced benefit of glucose lowering, it is also possible that the relatively good initial glucose control and the more modest decline during the intervention period blunted the improvement in vascular disease. Studies like ADVANCE were also conducted in an era of more comprehensive and aggressive risk factor treatment, perhaps also reducing the ability to show treatment benefits from glucose lowering alone.

Action to Control Cardiovascular Risk in Diabetes (ACCORD)

Interventional Phase

While the achieved level of glycemic achieved in the intensive arm of ADVANCE was near normal, the beginning HbA1c was already relatively good in the whole group and the on trial between group separation in HbA1c was only 0.8%. Thus, it was important to learn if a greater decline in HbA1c and larger separation between

treatment groups would lead to a reduction in CVD. The ACCORD Study enrolled 10,251 people with T2DM with an average age of 62 years, diabetes duration of 10 years, and an entry HbA1c of 8.1% [9]. About 35% of participants had known CVD at entry and 35% were on insulin. There were 62% males and 38% females. So, the ACCORD cohort also reflected a relatively advanced group of T2DM patients, although one that certainly represents a large percent of the patients seen in primary care and specialty clinics currently. They were followed for a mean of 3.7 years with the aim of determining the impact of achieving near normal glycemic control on a cardiovascular composite outcome of nonfatal MI, nonfatal stroke, or CVD death. As in the other studies noted above, the treatment arms were well balanced in terms of standard CVD risk factors such as blood pressure, lipids, weight, and smoking [9]. Within the first 6 months of study, there was a rapid fall in HbA1c in both treatment groups to 7.5% in the standard arm and 6.4% in the intensive arm. This separation of 1.1% was maintained throughout the study. Despite this, there was only a modest and not significant CVD reduction in the primary outcome in the intensively treated group (Fig. 34.2; HR 0.90 (0.78, 1.03) $P = 0.16$). There was a benefit in the secondary outcome of nonfatal myocardial infarction, HR 0.76 (0.62, 0.92), $P = 0.004$. However, alarmingly, all-cause mortality increased with intensive glycemic treatment HR 1.22 (1.04, 1.46) $P = 0.04$. Consistent with this, risk for CVD death was also markedly elevated in the intensively treated arm HR 1.35 (1.04,

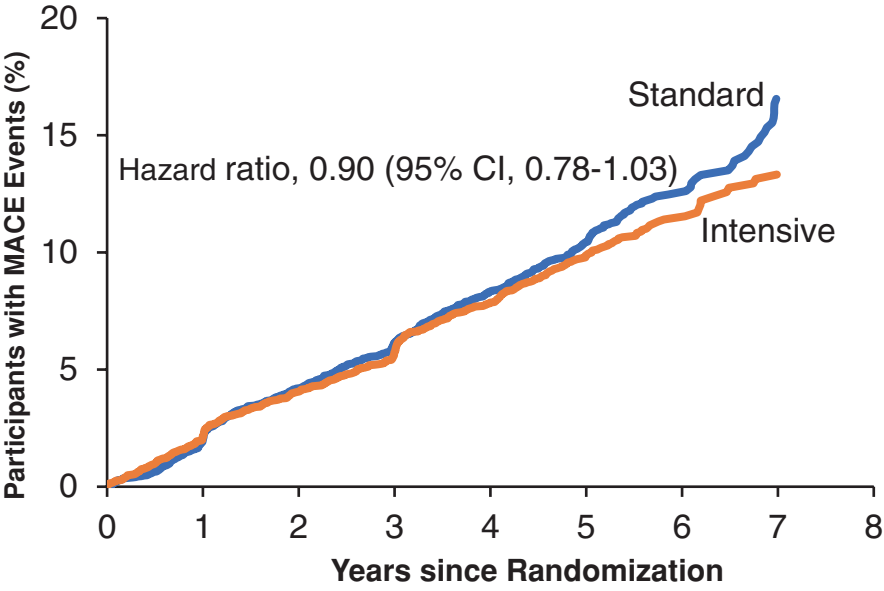

Fig. 34.2 Effects of intensive glucose lowering in type 2 diabetes in the ACCORD study. MACE outcomes included nonfatal MI, stroke and cardiovascular death. (Figure is modeled from Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, et al. *N Engl J Med.* 2008;358(24):2545–2559, and data are not intended to exactly represent original curves)

1.76) $P = 0.02$. As a result, a 10-member data and safety monitoring board concluded that the increased rate of all-cause mortality in the intensive therapy group outweighed any potential benefits and the glycemic part of ACCORD was terminated early. These data clearly indicate that aggressively pursuing near normal glucose control as in ACCORD does not benefit and might cause harm in older people with advanced T2DM.

Despite extensive post-hoc analysis by ACCORD investigators, it is not entirely clear what could explain the surprising results of ACCORD? A reasonable hypothesis is that intensive glycemic therapy increased all-cause and cardiovascular mortality in ACCORD because there was more overall and severe hypoglycemia with intensive therapy. In ACCORD, the individuals in the intensive arm had nearly three times as much hypoglycemia as in the standard treatment arm [9]. Although statistical analyses could not demonstrate that hypoglycemia events accounted for increased mortality, it is recognized that in the absence of continuous glucose monitoring, hypoglycemia events and "time in hypoglycemia" are greatly underappreciated. Hypoglycemia sets up a hemodynamic, thrombotic, and inflammatory situation conducive to cardiac ischemia [14]. Indeed, retrospective analyses of both ACCORD and VADT showed that those individuals who had severe hypoglycemia were more likely to develop a CVD primary endpoint, more likely to have CVD death, and to have higher all-cause mortality [15, 16] . What remains clear is that aggressive glucose lowering to near normal glucose ranges in an older group of T2DM patients with a high prevalence of CVD appears to cause more harm than benefit during the intervention period. By following this same cohort of individuals for an extended period of time after cessation of study, the ACCORD investigators hoped to learn about the long-term implications of this degree of glucose-lowering intervention.

Follow-Up Phase

The final follow-up report from the ACCORD study occurred after a median of 8.8 years, including nearly 5 years of additional observational monitoring [17]. At that point, the HbA1c in both treatment arms was around 8% (7.8 and 8% in intensive and standard groups, respectively) and had slowly approached equal levels over the post-intervention period. The effect of intensive glycemic control over this extended period on death and nonfatal cardiovascular events was now neutral, but cardiovascular-related death remained increased in those who had been in the intensive glycemic therapy arm, HR 1.20, CI 1.03–1.39, $P = 0.02$. These data indicate that even over an extended period of follow-up, the earlier glucose lowering to near normal ranges achieved during the intervention period did not lead to CVD benefit. Thus, consistent with long-term follow-up of the ADVANCE study, there was no evidence of a legacy effect.

As the ACCORD glucose-lowering intervention was stopped early, the duration of glucose lowering was relatively modest as noted above. This raised the

possibility that a longer period of glucose lowering (along with perhaps a greater separation in glucose control) might be needed to yield a CVD benefit.

VA Diabetes Trial (VADT)

Interventional Phase

The VADT enrolled 1791 people with T2DM (93% male) with an average age of 60 years, diabetes duration of 11.5 years, and an entry HbA1c of 9.4% [8]. About 40% had a history of CVD events at enrollment and 50% were on insulin. They were followed for a median of 5.6 years, the longest intervention period of the three trials in advanced T2DM patients. The primary composite CVD outcome included major CVD events, CVD death, myocardial infarction, stroke, congestive heart failure, amputation, interventions for CAD, peripheral vascular disease, and inoperable coronary artery disease. All available glucose-lowering agents were used (GLP-1RA and SGLT2i were not yet available, and DDP4i rarely used at that time) but higher doses of most medications were required to achieve tighter control in the intensively treated group. Lipids, blood pressure, and general health measures were treated identically in both groups during the study. Within the first 6 months of study, there was a rapid fall in HbA1c in both treatment groups to a median of 8.4% in the standard arm and 6.9% in the intensive arm. This separation in HbA1c of 1.5% was maintained throughout the study. Despite the high enrollment HbA1c and relatively large and prolonged separation in HbA1c between groups, there was only a modest and not significant CVD benefit, HR 0.88, CI 0.74–1.05, $P = 0.14$. There was also no clear effect of improved glycemic control on all-cause mortality HR 1.07, CI 0.81–1.42, $P = 0.62$. It was however somewhat reassuring that mortality was not significantly increased as in ACCORD, and it was thought this difference might be due to the less aggressive HbA1c target achieved in the VADT with mean values in the intensive group just over 7%.

Thus, in older people with advanced T2DM, improved glycemic control achieving a reasonably robust difference in HbA1c that was maintained for 5.6 years did not translate into CVD benefit.

VADT Follow-Up Phase

An important caveat for the more recent studies of glucose lowering in T2DM was their shorter duration of intervention. While the median follow-up in the VADT was of moderate duration, 5.6 years, even longer intervention periods had occurred within the UKPDS and DCCT, which had more favorable CVD outcomes.

Fortunately, about two-thirds of the VADT cohort (who had not died and agreed to further observational follow-up) had electronic medical records reviewed and intermittent surveys conducted for prespecified follow-up analyses approximately 10 and 15 years after the start of the intervention [18, 19]. During this observational follow-up phase, all diabetes care was returned to their primary care providers. The 1.5% HbA1c separation that was achieved and maintained during the intervention period waned over several years eventually leading to both former treatment arms having a HbA1c of about 8%. Thus, the intensively treated group potentially benefited from nearly 2–3 years of additional modest but sustained improvements in glucose lowering after completion of the intervention phase. At the 10-year interim analysis [18] those who had been in the intensive treatment group now demonstrated a CVD benefit, HR 0.83, CI 0.7–0.99, $P = 0.04$ (Fig. 34.3). This was driven largely by a reduction in nonfatal myocardial infarctions. In additional post-hoc analyses, it appeared that the reduced CVD outcomes could be largely explained by the cumulative HbA1c separation between treatment groups [18]. This indicated that perhaps prolonged and relatively substantial separation in glycemic control may be needed to affect CVD benefit in those with advanced disease. As HbA1c

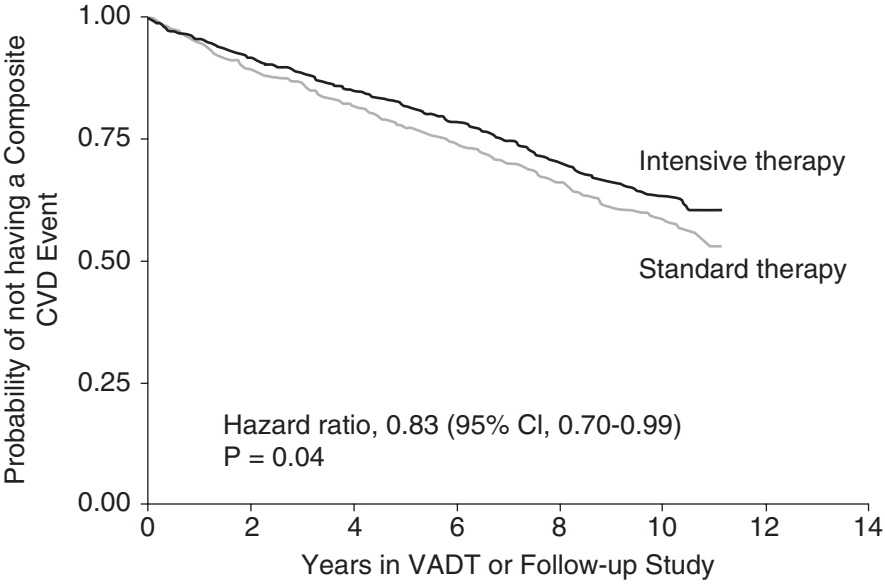

Fig. 34.3 Effects of intensive glucose lowering in type 2 diabetes in the VADT study. Composite outcomes included nonfatal MI, stroke and cardiovascular death, congestive heart failure, amputation. (Figure is modeled from Hayward RA, Reaven PD, Wiitala WL, et al., follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes, *N Engl J Med.* 2015;372(23):2197–2206 and data are not intended to exactly represent original curves)

levels did merge between treatment groups after approximately 8+ years in the study, this also provided an opportunity in subsequent follow-up to examine for evidence of a legacy effect.

Interestingly, at 15 years, CVD benefit in the intensively treated group that had become evident at the 10-year interim analysis was lost [19]. There was no benefit in CVD death, HR 0.88, CI 0.64–1.20, $P = 0.42$ or all-cause mortality, HR 1.05, CI 0.89–1.25, $P = 0.54$.

Interpretation of the VADT and VADT follow-up data would indicate that improved glycemic control led to a modest CVD benefit in people with advanced diabetes; but this conclusion comes with several caveats. First, the decline in HbA1c was substantial, with the intensive group having an absolute drop in HbA1c of nearly 2.5%. Second, the separation in glycemic control between groups during the intervention period needed to be relatively large (a HbA1c difference of 1.5%). Third, the overall duration of improved glucose control was sustained (nearly 8 years). However, even with these robust glucose-lowering results, there was no evidence of a legacy benefit on CVD outcomes or mortality from the prior glucose-lowering intervention.

Placing Glucose-Lowering CVD Trials in Context

The results of these five landmark studies of glucose lowering and complications have revealed several key findings. First, whereas the benefits of glucose lowering on microvascular disease are more consistently observed, and can be relatively robust in some cohorts, the benefits of glucose lowering on CVD events are relatively modest. Reductions in CVD, no matter the nature of the composite outcome utilized, were generally less than 15% and in no case did they achieve statistical significance during the intervention periods. Second, if reductions in rates of CVD do occur, they take a relatively long time to become apparent. In the DCCT, UKPDS, and VADT, favorable effects appeared to take close to 10 years or more. And in each of these trials, benefits only achieved statistical significance after the end of the intensive glucose-lowering periods. Third, there appears a greater potential to modify CVD risk with glucose lowering earlier in the course of diabetes. Glucose-lowering interventions in younger T1DM (DCCT) or new onset T2DM (UKPDS) appeared more successful in reducing both micro- and macrovascular disease than in those individuals with more advanced diabetes. Moreover, more persistent benefits (even after glucose separation had waned between treatment groups) only occurred in these younger cohorts with less baseline prevalence of CVD. A legacy effect of prior glucose lowering therefore only appears possible if treatment is started relatively early in the course of the diabetes. Thus, earlier initiation of improved glucose control appears to have both greater CVD benefits during the intervention as well continued benefits even when glucose control is relaxed.

Additional Explanations for Distinct Differences in Outcomes among Glucose-Lowering Studies

As there were striking differences among studies as pointed out above, it may be useful to consider potential mechanisms that may underly these distinct but related findings and discrepancies among the trials. One key difference in later studies of glucose lowering (ADVANCE, ACCORD, and VADT)—which were all conducted in patients with more advanced T2DM—is that they were conducted in era of more comprehensive risk factor management. Better management of blood pressure and lipids was present, as was therapy to reduce clotting and protect against development or progression of renal disease. This also reflects additional use of numerous medications (e.g., statins, ACE/ARBS) that may also have direct vasculo-protective effects. For example, the percentage of participants using statins or hypertension medications is much higher in ACCORD [9] than in the UKPDS [3, 4].

Older T2DM patients with a longer history of diabetes and hyperglycemia and a greater prevalence of CVD (as present in ACCORD, ADVANCE, AND VADT) may also have a degree and complexity of atherosclerosis that is less influenced by subsequent improved glucose control. While early atherosclerosis plaques may consist largely of reversible lipid-rich lesions, advanced lesions are also enriched in recruited inflammatory cells, cellular debris, fibrous material, cholesterol crystals, and calcium—all much less likely reversible lesion components. Consistent with this, in a substudy of the VADT, glucose lowering was found more effective in reducing CVD in those with lower coronary calcium (i.e., an indication of less advanced atherosclerosis) at enrollment [20].

It has also been suggested that in advanced and longer duration diabetes, the accompanying hyperglycemia and oxidative stress have generated substantial advanced glycation formation (AGEs) in arteries. These products may damage tissues by direct interaction with intra- and extracellular proteins and other molecules and via binding to the receptors for AGE [21, 22]. Through direct binding and cross-linking of structural proteins, such as collagen, vitronectin, or laminin, as well as multiple functional molecules, AGEs interfere with tissue integrity and/or function [23]. These direct binding effects are particularly damaging for long-lived cells, such as nerves and long-lasting proteins such as proteins of lens and cornea [24, 25] and vascular wall collagen [27].

An additional potential limitation of current glucose-lowering efforts has been the singular focus on lowering mean glucose levels, typically tracked by changes in HbA1c. While this approach has in general been quite successful for reducing microvascular disease, especially in earlier stages of diabetes, this may not capture all the risk of hyperglycemia. Importantly, variability in several risk factors, including blood pressure, weight, and lipids, has been linked with multiple vascular outcomes [26–28]. This concept may be particularly relevant for glucose control, as this risk factor has substantial variation over very short-term (minutes to hours), short-term (over days), or long-term (weeks to months) time

frames. Glucose variability, independent of mean or cumulative estimates of glucose control, is strongly linked with CVD and mortality [29, 30]. Of note, in the VADT, the independent relationship of glucose variability with CVD was even greater in those receiving intensive glucose lowering—suggesting controlling glucose variability may even more relevant in this group [30]. This certainly illustrates how simply lowering average measures of glucose may not sufficiently reduce all the risk of hyperglycemia. This also points to the possibility that glucose-lowering interventions need to also consider their effects of on glucose variation.

Another risk of intensive glucose lowering is hypoglycemia. Hypoglycemia has consistently been associated with 2- to three-fold increased CVD risk. Although numerous mechanisms (e.g., increased thrombosis, catecholamine surges) have been proposed to account for acute CVD events, there are also data indicating that hypoglycemia is also linked with increased atherosclerosis and/or cardiac injury [31, 32]. These negative consequences of hypoglycemia could certainly counter the benefits of glucose lowering for this same outcome—CVD. Although severe hypoglycemia was not found to account for the increased mortality associated with intensive glucose lowering in ACCORD, its effect may be underappreciated as it is difficult to accurately capture hypoglycemia events, particularly the less severe but more common episodes of hypoglycemia. Limiting hypoglycemia along with hyperglycemia and glucose variation may be the third leg of the optimal glycemic control stool.

A New Paradigm: Nonglycemic Lowering Drugs with Additional Benefits

The development of several new classes of glucose-lowering medications has dramatically changed the approach to treatment of glucose and diabetes in general. Several of these agents appear to have profound effects on atherosclerosis, heart failure, and/or renal disease that do not appear related to their co-existing effects on glucose. For example, although glucose lowering with GLP-1 receptor agonists (GLP-1RA) can be substantial, the benefits seen in most GLP-1RA cardiovascular outcome studies are far more substantial and occur much earlier than demonstrated in the less drug-specific glucose-lowering studies such as ACCORD and VADT [33]. Similarly, cardiovascular outcome studies with SGLT2i have demonstrated dramatic and relatively early improvements, particularly in the prevention or treatment of heart failure, despite only moderate degrees of glucose lowering [34–37]. Moreover, benefits in several vascular outcomes have been demonstrated with use of this class of medication even in patients without diabetes [38]. How these two medication classes reduce cardiovascular risk remains an active area of investigation, but it is likely multifactorial. However, it is of interest given the above

discussion of intensive glucose-lowering trials in diabetes patients that both these classes of medications lower glucose with reduced glucose variation and risk of hypoglycemia. Thus, it has become clear that selection of the class of glucose-lowering medication is as, or more, important for reduction in CVD than is the degree of glucose lowering achieved for many individuals, and correctly identifying the most appropriate class of medications will be an important component of any personalized diabetes care strategy. Recent guidelines from various diabetes and endocrine organization highlight these points and provide specific algorithms to provide a more patient-centered and risk-based medication selection strategy [39, 40].

Clinical Implications

As a result of these above glucose-lowering trials, we have gained substantial information about how to incorporate more intensive glucose lowering into management of diabetes to reduce CVD. The greatest benefit appears to occur if this is started earlier in the course of diabetes, as reflected in results from the DCCT and UKPDS [1, 3, 4, 12]. Moreover, continued benefits (i.e., legacy effects) may occur in these individuals even if glucose lowering is not sustained [10–12]. In contrast, aggressive glucose lowering in those with more advanced diabetes yields less CVD protection and may be associated with increased mortality [9].

It is also clear that the approach to intensive glucose lowering is critical to successful reduction in CVD outcomes. Picking the right glucose goal for the right patient is a key first step. The greatest benefits in trials occurred in patients who started with relatively elevated initial HbA1c levels and subsequently have these lowered at least by an absolute HbA1c of 1%. Importantly, studies that achieved a HbA1c goal of near 7% were most successful, whereas more aggressive goals failed to achieve significant reductions in outcomes. This may be particularly important in individuals at increased risk of adverse events from aggressive glucose lowering, such as elderly individuals or those with known CVD or significant comorbidities. The most likely—although unproven—explanation for worse results in studies achieving near normal HbA1c targets is increased hypoglycemia. Risk for hypoglycemia is undoubtedly influenced by the extent of within and between day glucose fluctuations. Thus, achieving glucose-lowering targets while minimizing glucose variability and hypoglycemia are the three key components of optimizing glucose management. Importantly, recent diabetes medications, particularly GLP-1RA and SGLT2i, are effective glucose-lowering agents that are associated with decreased glucose variability and rates of hypoglycemia. As these drugs also appear to have CVD protection independent of their glucose-lowering benefits, their early incorporation in glucose-lowering regimens is highly recommended when possible.

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Part VIII
Treatment in Special Populations and Conditions

Chapter 35
Differences of Diabetes Treatment and Care in Various Ethnic Minorities

Yan Emily Yuan and A. Enrique Caballero

Introduction

Most clinicians in the United States (U.S.) and around the world are constantly challenged by having to tailor multiple health-related prevention and treatment strategies to improve the lives of people from diverse races/ethnicities, socio-economic strata, education levels, and cultural backgrounds. Unfortunately, the task of providing optimal care to diverse groups is daunting as health care professionals are not fully aware of practical recommendations they can provide to their patients. In addition, health care systems are often not equipped with culturally oriented programs that fulfill the needs for each of these groups.

Disparities in health care have long been documented in the U.S. and globally. Racial/ethnic minorities have been identified with alarming rates of type 2 diabetes and multiple diabetes-related complications including cardiovascular disease. Some biological factors contribute to these disparities. Abnormalities in the pathophysiology of both diabetes and cardiovascular disease have been identified in Latinos/Hispanics, non-Hispanic blacks, Asians and American Indians, and Pacific Islanders. Specific genetic differences have been postulated to explain some diabetes and cardiovascular disease cases in these populations.

However, genetic and biological abnormalities contribute to explaining disparities in disease rates only to a small percentage. Our current understanding is that social and cultural factors particularly present in racial/ethnic minorities influence the development of both diabetes and cardiovascular disease at a much higher level. Socioeconomic status, neighborhood and physical environment, food environment, health care, and social context are powerful social determinants of health and disease.

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Understanding the interplay of biological differences along with unique cultural and social factors in distinct racial/ethnic groups is crucial to be able to provide optimal health care to all. Health care professionals must embrace the routine evaluation of social determinants of health in order to develop and implement effective prevention and treatment interventions to help these vulnerable populations.

Unfortunately, disparities in health care expand beyond the known differences in racial/ethnic minorities. Gender, age, and sexual orientation are factors that have also been shown to influence diabetes and cardiovascular disease rates in multiple populations across the globe. Groups with lower socio-economic status, education, and health literacy levels are often affected more frequently and severely by diabetes and cardiovascular disease. Understanding how these factors also influence the development of these diseases is extremely important in today's health care environment.

This chapter focuses on detailing the current understanding of how specific biological abnormalities contribute to differential prevalence and incidence rates of diabetes and cardiovascular disease in racial and ethnic minorities in the U.S. as well as in groups divided by gender and sexual orientation. Furthermore, we provide information on the often forgotten social and cultural factors that influence diabetes and cardiovascular disease care in these vulnerable populations. Most importantly, we have aimed at providing some practical recommendations to health care professionals seeking to understand the complexity of biological, psychological, social, and cultural factors that contribute to the disease burden in underserved populations.

Description of Diverse Populations in the U.S.

The main racial/ethnic minorities in the U.S. are Latinos/Hispanics, Blacks or African-Americans, Asians, American Indians and Alaska natives and Native Hawaiian and other Pacific Islanders. These groups have generally grown at a faster pace than the non-Hispanic white population.

At the present time, these groups combined represent approximately 35% of the total population. It is predicted that this figure will increase to 50% by the year 2050 [1]. Current and projected percentage of the U.S. population by race and ethnicity from the year 2014 to the year 2060 is shown in Table 35.1 [2].

The non-Hispanic White alone population is both the largest racial and ethnic group and accounts for greater than a 50% share of the nation's total population. It is currently the "majority" group. However, by 2060, the share of this group is projected to decrease to 44% (see Table 35.1). The point at which the non-Hispanic White alone population will comprise less than 50% of the nation's total population has been described as the point at which we become a "majority–minority" nation. According to these projections, this crossover will occur in 2044.

Table 35.1 Population by race and Hispanic origin: 2014 and 2060 (population in thousands)

Race and Hispanic origin	2014 Number	2014 Percent	2060 Number	2060 Percent	Change 2014–2060 number	Change 2014–2060 percent
Total population	**318,748**	**100**	**416,795**	**100**	**98,047**	**30.8**
One race	310,753	97.5	390,772	93.8	80,020	25.8
White	246,940	77.5	285,314	68.5	38,374	15.5
Non-Hispanic white	198,103	62.2	181,930	43.6	−16,174	−8.2
Black or African American	42,039	13.2	59,693	14.3	17,654	42.0
American Indian and Alaska native	3957	1.2	5607	1.3	1650	41.7
Asian	17,083	5.4	38,965	9.3	21,882	128.1
Native Hawaiian and other Pacific Islander	734	0.2	1194	0.3	460	62.6
Two or more races	7995	2.5	26,022	6.2	18,027	225.5
Race alone or in combination						
White	254,009	79.7	309,567	74.3	55,558	21.9
Black or African American	45,562	14.3	74,530	17.9	28,968	63.6
American Indian and Alaska native	6528	2.0	10,169	2.4	3640	55.8
Asian	19,983	6.3	48,575	11.7	28,592	143.1
Native Hawaiian and other Pacific Islander	1458	0.5	2929	0.7	1470	100.8
Hispanic or Latino origin						
Hispanic	55,410	17.4	119,044	28.6	63,635	114.8
Non-Hispanic	263,338	82.6	297,750	71.4	34,412	13.1

The percentage of foreign-born individuals is expected to gradually increase over the next few decades. In 2014, 13.3% of the population was comprised by individuals born outside the U.S. In the year 2060, this figure is expected to represent 18.8% of the total population [2].

The U.S. population is also expected to include a higher percentage of older individuals over time. The subgroup above the age of 65 is expected to increase by 112.2% from 2014 to 2060. All these changes in the population characteristics will definitely continue to impact our health care system and will increase the demand for tailored programs for diverse populations.

Hispanic origin is considered an ethnicity, not a race. Hispanics may be of any race. In combination means in combination with one or more other races. The sum of the five race groups add to more than the total population, and 100%, because individuals may report more than one race.

Health Care Disparities

Prevalence and Incidence of Diabetes by Race/Ethnicity

The adult U.S. population with diabetes has increased significantly in the past two decades [3]. The Center for Disease Control and Prevention (CDC) estimates the current prevalence of diabetes among adults aged 18 years or older is 34.1 million, or 13.0% of the U.S. adult population [4]. A vast majority of diabetes cases correspond to type 2 diabetes. The prevalence, however, varies among different ethnic and racial groups. From data collected from the National Health and Nutrition Examination Survey (NHANES), American Indians/Alaska Natives have the highest prevalence of diagnosed diabetes (14.7%), followed by Hispanics (12.5%), non-Hispanic Blacks (11.75%), non-Hispanic Asians (9.2%), and non-Hispanic Whites (7.5%) [3, 5]. Of note, within the different racial/ethnic groups, there is a notable heterogeneity across different subgroups. From the CDC data, among U.S. adults of Hispanic origin, Mexican-Americans (14.4%) and Puerto Ricans (12.4%) had higher prevalence than Central/South Americans (8.3%) and Cuban-Americans (6.5%). Similarly, among non-Hispanic Asians, Indian-Americans (12.6%) and Filipino-Americans (10.4%) had higher prevalence than Chinese-Americans (5.6%) and other Asian-American groups [4].

The prevalence of diabetes has been on the rise, and it is in large part driven by aging and increased rates of obesity [3, 4, 6, 7]. Although diabetes has increased in every subgroup, the rate of growth highlights notable disparities across differential racial and ethnic groups. When comparing 2015–2016 to 1999–2000, there is a statistically significant difference in the percentage increase of diabetes prevalence by race [3]. Among non-Hispanic Whites, the prevalence of diabetes increased by 4.8% points—from 6.7 to 11.5%, compared to 7.4% point increase for non-Hispanic Blacks—from 10.9 to 18.3%, compared to 10.1% point increase for Mexican Americans—from 8.3 to 18.4% [3].

The CDC estimates 1.5 million new cases of diabetes in the U.S. adult population in 2018. Despite the increase in prevalence, the overall incidence of diabetes in age-adjusted adults was similar in 2000 (6.2 per 1000 adults) and 2018 (6.7 per 1000 adults) [4]. However, the incidence of diabetes in adults of Hispanic origin (9.7 per 1000 adults) and non-Hispanic Blacks (8.2 per 1000 adults) was higher when compared to non-Hispanic Whites (5.0 per 1000 adults) [4, 8].

The racial disparities in diabetes prevalence affect not only the adult population, but is also present among children and adolescents [4, 9, 10]. In a study using the Pediatric Diabetes Consortium Type 2 Diabetes Clinic Registry, the prevalence of type 2 diabetes was highest in children of Hispanic origin, and about 80% of the population with diabetes were from racial or ethnic minority groups [11]. Projection models estimate that the racial and ethnic disparities in all diabetes prevalence will persist over the next three decades with the highest in non-Hispanic Blacks (1.63 per 1000 children), American Indian/Alaska Native (1.28 per 1000 children), and

Hispanics (0.96 per 1000 children), and the lowest in non-Hispanic Whites (0.28 per 1000 children).

These data shed light on the pervasive illness burden of diabetes on racial and ethnic minority groups. Social, psychological, cultural, biological, and systemic factors all contribute to the disparities in prevalence as well as in the indicators of diabetes care.

Prevalence and Incidence of Complications of Diabetes by Race/Ethnicity

Diabetes is associated with significant comorbidities that affect health outcomes [12–14]. Minority populations have been shown to suffer more diabetes-related complications when compared to their White counterparts [13, 15–17].

Microvascular Complications

Microvascular complications significantly contribute to morbidity and illness burden in patients with diabetes.

Nephropathy

According to the CDC, 37% of U.S. adults with diabetes between 2013 and 2016 had chronic kidney disease (CKD), and more than half had stage 3 or 4 (moderate or severe) CKD [4]. During this period, diabetes nephropathy was the leading cause (38.6%) of end-stage kidney disease (ESRD) [4].

Non-Hispanic Black and Hispanic people are more likely to have ESRD than their White counterparts [18]. In a study of insured population, the adjusted hazard ratio of non-Hispanic Black and Latino individuals for developing ESRD relative to White individuals was 2.03 and 1.46, respective ($p < 0.01$) [18]. Additionally, these two groups are seen to develop CKD earlier, and Blacks in particular have shown a more rapid progression toward ESRD after developing proteinuria when compared to Whites [19]. In a study using multiple national databases to compare the changes in diabetes-related complications between 1990 and 2010, it was found that while the rates of major diabetes complications—acute myocardial infarction, death from hyperglycemic crisis, stroke, amputations and ESRD—all decreased, ESRD had the smallest absolute decline [20]. The authors posited that this observation may be related to the rise of diabetes among non-Hispanic Black individuals, in whom the rates of ESRD is double that of the non-Hispanic White population [20].

The rates of CKD in American Indian and Asian-Americans have been less studied compared to other minority groups. However, in a 10-year population health

study in Hawaii, it was found that individuals of Filipino and Native Hawaiian descent had a higher risk for CKD when compared to non-Hispanic Whites [21].

Retinopathy

Retinopathy is another major contributor to diabetes associated morbidity. Among adults diagnosed with diabetes, 11.7% reported some vision disability, including blindness [4]. In U.S. adults 18–64 years of age, diabetes is the leading cause of new cases of blindness [4]. Diabetic retinopathy disproportionately affects the racial and ethnic minorities. The INSIGHT study screened 1894 persons with diabetes from four urban sites (Birmingham, AL, Miami, FL, Philadelphia, PA, Winston-Salem, NC)—99% of whom were ethnic minorities—and found that one in five screened positive for diabetic retinopathy [22]. In a case-controlled study using data from the Los Angeles Latino Eye Study, Gao and colleagues found that American Indian Ancestry in Latino subjects with type 2 diabetes mellitus was significantly associated with severe diabetic retinopathy ($p = 0.002$) [23].

There has also been consideration that Black Americans may be at an increased risk for microvascular complications of dysglycemia below the diagnostic threshold of diabetes. In a meta-analysis that stratified HbA1c to prevalence of diabetes-related microvascular complications, Butler and colleagues found that in Black-Americans older than 55 years of age, there was notable rise in retinopathy even when HbA1c was <6.5% [24]. This suggests the need to amend screening guidelines for at risk populations to offer appropriate preventive management.

Neuropathy

Population differences in peripheral diabetic neuropathy are more difficult to elucidate due to potential language and cultural differences that can impact clinical assessment and evaluation. Some studies have suggested regional differences in disease prevalence [25]. For example, among American Indians, the prevalence of diabetic neuropathy was 22% in those living in Arizona, 9% in those living in the Dakotas, and 8% in those living in Oklahoma [25].

One study examined the differences in reports of painful diabetic peripheral neuropathy symptoms in Non-Hispanic Black, Hispanic, and White populations [25]. Using a survey to assess symptoms such as numbness, quality and intensity of pain as well as sensitivity, the authors found that Non-Hispanic Black (65%) and Hispanic individuals (49%) were less likely to rate pain as moderate or severe relative to their Non-Hispanic White (87%, $p < 0.05$) counterparts [25]. However, more Non-Hispanic Black and Hispanic individuals also reported difficulty communicating with their healthcare team as compared to the Non-Hispanic White individuals [25], which clouds assessment of true prevalence this complication.

Macrovascular Complications

Cardiovascular disease (CVD) includes heart disease, cerebrovascular disease, and peripheral vascular disease, all common complications in people with diabetes. CVD continues to be the leading cause of death in the U.S. [12].

Heart Disease

According to the CDC in 2017, the prevalence of heart disease was the highest in non-Hispanic white adults (11.5%), followed by non-Hispanic Black adults (9.5%), Hispanic adults (7.4%), and non-Hispanic Asian adults (6.0%) [26]. Although the prevalence of heart disease was higher in non-Hispanic white adults, the trend in overall death rate attributable to CVD has been higher in ethnic minorities than in non-Hispanic white adults [26, 27]. In 2017, the age-adjusted rate of death for heart disease was higher in non-Hispanic Black adults (208 per 100,000 persons) than in non-Hispanic White adults (168.9 per 100,000 persons) [26]. Non-Hispanic Black men, in particular, have the highest rate of death attributable to CVD [27]. As discussed below, these disparate health outcomes are the result of differences in risk factors as well as inequities in health care access and utilization.

Cerebrovascular Disease

Cerebrovascular disease is a cause of significant morbidity and mortality in the U.S., and the risk of stroke is impacted by race and ethnicity. Non-Hispanic Black and Hispanic individuals have a higher age-adjusted incidence for first ever stroke than non-Hispanic White and Asian individuals [27, 28]. While death attributable to stroke has declined between 2019 and 1999 for most racial and ethnic groups, the stroke death rate has increased in Hispanic people in the U.S. [4]. These finding highlight the need to develop tailored screening and management programs to reach the most at-risk populations.

Peripheral Artery Disease and Amputations

Diabetes is a known to be associated with the development of peripheral arterial disease (PAD) [29]. Few studies have examined the prevalence of PAD across a multi-ethnic population. In a cross-sectional study of 2343 adults, Criqui and colleagues examined PAD rates in non-Hispanic Whites, Blacks, Hispanics, and Asians [30]. In weighted logistic models, the authors used non-Hispanic Whites as the reference group and found that non-Hispanic Blacks had significantly higher prevalence of PAD (OR = 2.30, $p < 0.024$), but no statistical differences in Hispanics and Asians. Although the non-Hispanic Black adults also had higher blood pressure and diabetes, including these variables did not significantly change the effect size [30].

Nontraumatic lower extremity amputation is a complication of PAD that is associated with diabetes. Since 1990, the rates of amputation have declined significantly in patients with diabetes [20, 31, 32]. However, despite the declining overall amputation rate, this complication continues to be seen at higher rates in racial and ethnic minorities [31, 32]. In a study of Medicare patients with diabetes between 2002 and 2012, it was found that major lower extremity amputation rate was 1.78 per 1000 per year for Black patients, 1.15 per 1000 per year for Hispanic patients, and 0.56 per 1000 per year for white patients ($p < 0.001$). In a separate analysis of Medicare patients with diabetes between 1999 and 2006, high-risk patients—those with end-stage renal disease or more than three comorbidities—contributed to a growing percentage of all amputations: 33% in 1999 and 50% in 2006 ($p < 0.00$) [31]. However, notably Black patients had the higher rates of amputations in both high-risk and low-risk groups [31].

Lower extremity amputation incidences varied among subgroups of Asian Americans [33]. In a prospective cohort study of patients enrolled in the Kaiser Permanente Northern California Diabetes Registry between 1996 and 2006, Chinese Americans were among the lowest incidence rates for first-time lower extremity amputation. Pacific Islanders, however, were among those with the highest age- and sex-adjusted incidence rates [33]. These results highlight not only the disparities in outcome for this life-altering complication but also highlight the need for careful subgroup analysis to better understand the scope of disease risk factors for different populations.

Mortality

From 1999 to 2017, the death rate for heart disease decreased across the U.S. population for all racial groups [26]. Non-Hispanic Black adults continue to have the highest age-adjusted death rates for heart disease [26]. In 2015, death caused by heart disease represented 23.5% of all deaths for non-Hispanic Black adults, 23.7% for non-Hispanic Whites, 21.4% for Asian-American or Pacific Islander, 20.3% for Hispanics, and 18.3% for American Indian or Alaska Natives [26]. Across the different racial and ethnic groups, the percentage of all deaths caused by heart disease was higher in men than in women [26].

Race/Ethnicity Differences in Cardiovascular Risk Factors

Diabetes is associated with the development of cardiovascular and cerebrovascular diseases as discussed above [34–36]. This risk is magnified by high BMI [35], hypertension, and hypercholesterolemia [36].

Obesity

Obesity has a strong association with CVD. Based on data from the Framingham Heart Study, Fox and colleagues found that the lifetime risk of CVD over a 30-year period was significantly impacted by both diabetes and high BMI [35]. Among normal weight women without diabetes, the lifetime risk of CVD was 34.3% compared to 46.7% among women with obesity and without diabetes. Among women with diabetes, the 30-year risk of CVD was 54.8% in those with normal weight compared to 78.8% in those with obesity. Men as a whole had higher lifetime CVD risk; and a similar relationship with BMI and obesity was observed: 49.2% in normal weight men without diabetes, 66.8% in men with obesity and without diabetes, 78.6% in normal weight men with diabetes, 86.9% in men with obesity and diabetes [35]. See section "Body Mass Index and Fat Distribution" for discussion of racial and ethnic differences in the prevalence of obesity in the U.S.

Hypertension

Hypertension is a risk factor for CVD as well as for the development of diabetic nephropathy and chronic kidney disease. In 2017, the American College of Cardiology/American Heart Association changed the hypertension guidelines to define stage I hypertension as systolic pressure ranging from 130 to 139 mmHg or a diastolic pressure ranging from 80 to 89 mmHg [37]. Based on this updated guideline, there was a significant increase in the prevalence of hypertension from 36% to estimates of 42–63% of the U.S. adult population [38–40]. Given the high prevalence of hypertension, it is important to recognize the significant racial and ethnic differences.

Using NHANES data from 2011 to 2016, Kibria and colleagues applied the new hypertension criteria for defining hypertension to the sample population [40]. Their analyses showed prevalence of hypertension was 46.9% in the overall population. The highest prevalence of hypertension was among non-Hispanic Blacks 59.0% (95% CI: 57.4–60.6%), compared to 46.1% (95% CI: 43.8–48.3%) among Mexican-Americans, 45.7% (95% CI: 44.1–47.3%) among non-Hispanic Whites [40]. This study did not include separate analyses for the prevalence of hypertension in non-Hispanic Asians or American Indian/Alaska Native population. According to the U.S. Department of Health and Human Services Office of Minority Health, in 2018, the age-adjusted percentage of American Indian/Alaska Native adults with hypertension was 27.2% (compared to 24.0% in non-Hispanic Whites) [41]. However, some have argued that collection of data through national surveys likely leads to an underestimation of major diseases including hypertension and cardiovascular disease in the American Indian/Alaska Natives [42].

Non-Hispanic Asian Americans had been reported to have lower prevalence of hypertension compared to other racial and ethnic groups [4]. However, disaggregate

data shows significant heterogeneity among Asian subgroups, with some being among the population with the highest prevalence for hypertension. The New York City Health and Nutrition Examination Survey 2013–2014 found that the age-standardized prevalence of hypertension was 43.5% for non-Hispanic Blacks, 38% for Asians, 33% for Hispanic adults, and 27.5% for Whites [43]. In subgroup analysis, hypertension prevalence was significantly higher in South Asian adults (43%), East/Southeast Asian adults (39.9%) and adults from the Dominican Republic (39.5%) [43].

Redefining hypertension has important implications in cardiovascular risk assessment. In the U.S., the pooled cohort equation is used for estimation of 10-year risk of developing atherosclerotic cardiovascular disease. The current model includes "African American" and "White" in the risk calculation but does not provide specific adjustments for Asian, Hispanics, or American Indian/Alaska Natives. The lack of racial and ethnic-specific risk assessment could lead to undertreatment of hypertension and underestimation of true atherosclerotic cardiovascular disease risk in certain populations [44].

See section "CVD Target Achievement" for discussion of racial and ethnic differences in meeting CVD target achievements including blood pressure control.

Dyslipidemia

High cholesterol is a risk factor for CVD and has been a target for both primary and secondary prevention. The Multi-Ethnic Study of Atherosclerosis (MESA) included 6814 multi-ethnic adults from six U.S. cities: 38% non-Hispanic white, 28% black, 12% Chinese, and 22% Hispanic [45]. They observed that highest total cholesterol concentrations were in non-Hispanic White women (201.3 mg/dL), while lowest in Black men (181.4 mg/dL); LDL cholesterol concentration was highest in Hispanic women (119.6 mg/dL) and again lowest in Black men (113.5 mg/dL) [45]. The prevalence of dyslipidemia was comparable among Black, Hispanic and non-Hispanic White adults. However, among those who had dyslipidemia, men were less likely than women to have controlled dyslipidemia; Black and Hispanic adults were less likely to be treated when compared to non-Hispanic White adults [45]. See section "CVD Target Achievement" for discussion of racial and ethnic differences in meeting CVD target achievements including management of hypercholesterolemia.

Racial and ethnic differences have been identified in analyzing the different components of the lipid profile. Hispanic adults with dyslipidemia have been shown to have high prevalence of both elevated LDL-C and low HDL-C [46]. Non-Hispanic Black adults, on the other hand, have been shown to have low serum triglyceride levels and high HDL-C [47–49]. Elevated serum triglycerides are typically common in dyslipidemia and thought to contribute to metabolic syndrome, insulin resistance as well as other CVD risks factors. However, demographic differences in lipid profile challenge this association and complicates our understanding of the risks for developing cardiovascular disease.

Based on data from the Multi-Ethnic Study of Atherosclerosis (MESA), Lin and colleagues confirmed prior studies that showed that non-Hispanic Black men and women lower prevalence of elevated serum triglyceride levels compared to non-Hispanic white adults. However, among adults who did not have elevated serum triglyceride levels, non-Hispanic Black men and women had higher elevated fasting glucose levels than their non-Hispanic White counterparts [50]. Additionally, while serum triglycerides levels were associated with weight circumference in non-Hispanic White men and women as well as non-Hispanic Black men, no statistically significant association was seen in non-Hispanic Black women [50]. This finding has implications for both assessments of risk factors and effectiveness of intervention efforts to improve cardiovascular health. Because measurements of serum triglycerides and HDL-cholesterol are used as a part of assessment for cardiovascular health and metabolic syndrome, it is important to consider racial differences to more accurately approximate risk for different racial and ethnic groups [51].

Race/Ethnicity Differences in Management of CVD Risk Factors

Glycemic Control

Among adults with diabetes, racial and ethnic differences have been shown to significantly impact glycemic control [52–55]. Hemoglobin A1c (HbA1c) has been accepted for the diagnosis of type 1 and type 2 diabetes in the adult population, and as a measure for diabetes control [56, 57]. Studies have shown that HbA1c significantly varies by race and ethnicity, with Hispanics and non-Hispanic Blacks reporting higher HbA1c than non-Hispanic Whites [7, 52, 53, 55]. While other studies have cited inequities in healthcare access, the differences in glycemic control seem to persist even when controlling for medication adherence, education level, and health care access (Heisler, 2007 #78) [52, 58].

Hivert and colleagues examined how differences in genetic ancestry markers may affect HbA1c between Black and non-Hispanic White participants of the Diabetes Prevention Program [59]. They observed that Black participants had approximately 0.4%-unit higher HbA1c than the non-Hispanic White participants. The authors used principal component analysis (PCA) to determine if admixed populations carry varying proportional contributions from ancestral populations from continents of Africa, Europe, and Americas. Their results found that the first PCA factor of genetic ancestry was associated with a large proportion of the HbA1c difference between Black and non-Hispanic White participants [60]. Therefore, there may be significant genetic variants in individuals of African descent that may influence HbA1c.

In addition to the observations of racial and ethnic disparities in HbA1c measurements, studies have shown that the probability of reaching the HbA1c target over time varied by race [61]. Using the electronic health record at Cleveland Clinic, researchers identified patients with uncontrolled diabetes (HbA1c >9%) and

observed the change in HbA1c over the course of 1 year. In the subgroup of patients who achieved HbA1c <8%, a higher proportion were White (72.2% vs. 65.6%; $P < 0.001$) and non-Hispanic or Latino (92.0% vs. 89.8%; $P < 0.004$). This is consistent with NHANES data from 1999 to 2010 showing that among adults ≥65 years of age who were diagnosed with diabetes, Hispanics were less likely to reach HbA1c <7% as compared to non-Hispanic Whites [52]. The difference in HbA1c goal attainment may be explained in part by inequities in access to diabetes care. Minority patients are less likely to receive the recommended diabetes care including routine HbA1c and annual cholesterol screening [17].

However, healthcare access does not resolve disparities in diabetes outcomes. Using data from the Surveillance Prevention and Management of Diabetes Mellitus cohort, American Indian/Alaska Native individuals in commercial integrated delivery systems were found to have similar rates of annual HbA1c screening when compared to non-Hispanic Whites [62]. However, the American Indian/Alaska Native individuals were significantly more likely to have HbA1c >9% and be less likely to take their diabetes medications as prescribed [62]. These findings led the authors to conclude that population-specific system-level barriers and facilitators need to be identified to address diabetes care [62].

Age is known to be an independent risk factor for increase in HbA1c, independent of body mass index (BMI) [63]. According to the 2017 National Population Projections, all baby boomers will be older than 65 years of age by the year 2030, nearing a population of 77 million (, #82). Understanding racial and ethnic differences among our older population is therefore imperative for addressing glycemic control among those with diabetes. Using NHANES data from 2003 to 2014, a study found that the disparities in glycemic control persist with age: there was a statistically significant difference between non-Hispanic White and non-Hispanic Black adults ≥65 years of age (+0.5%; $p = 0.043$) as well as between non-Hispanic Whites and Mexican American adults ≥65 years of age (+0.4%; $p = 0.006$) [55].

Taken together, ethnic and racial minorities have higher HbA1c than non-Hispanic White counterparts. Additionally, the minority subgroups are less likely to achieve HbA1c goal over time. Unfortunately, recent studies show that these trends will persist as the population ages, which will have significant impact on our health care system as the incidence and prevalence diabetes continues to rise with the growing aging population in the U.S.

CVD Target Achievement

In people with diabetes, blood pressure management and lipid control have been shown to reduce cardiovascular disease rates [64]. Therefore, in addition to recommendations for HbA1c targets, the American Diabetes Association recommends targets for blood pressure <130/80 mmHg, LDL cholesterol <70 mg/dL and initiation of statin therapy for individuals with diabetes of all ages who have atherosclerotic CVD [65]. Racial and ethnic minorities have been shown to be less likely to meet these targets [34, 66]. In a study using NHANES data over three time periods:

2005–2008, 2009–2012, 2013–2016, Kazemian and colleagues found that achievement of CVD targets by race has not significantly improved over the past decade [34]. Hispanic individuals diagnosed with diabetes were less likely to be linked to care when compared to non-Hispanic Whites, and were less likely to achieve combined HbA1c, blood pressure and LDL-cholesterol targets. Non-Hispanic Black individuals with diabetes were not less likely to be linked to diabetes care, but were nonetheless less likely to reach CVD target achievements [34].

Similar trends have been observed in the management of hypertension. In a study of patients with hypertension from 143 primary care clinics, Black women and men with hypertension were 1.18 (95% CI 1.07–1.30) and 1.20 (95% CI 1.05–1.34) times more likely to have uncontrolled hypertension when compared to White women and men [67]. The study also found that Black women and men with hypertension and an indication for statin therapy were 1.23 (95% CI 1.05–1.45) and 1.25 (95% CI 1.03–1.51) times more likely to not have an active statin prescription [67].

Statin therapy has been shown to be an important intervention for patients with atherosclerotic CVD [65, 68, 69]. Lower adherence to statin therapy was associated with increased risk of mortality in patients with atherosclerotic CVD [69]. Unfortunately, minorities have been consistently shown to have a lower adherence to statin therapy [66, 69, 70]. In an analysis of NHANES data among adults diagnosed with diabetes, authors Stark Casagrande and colleagues found the use of statins increased significantly during 1988–2010. However, the prevalence of Hispanic and Non-Hispanic Black individuals on statin therapy was less than their non-Hispanic White counterparts [66]. In 2015, the American Diabetes Association expanded statin therapy recommendation to include all adults with diabetes between 40 and 75 years old. The updated recommendation guidelines have not yet been shown to have a significant impact on the proportion of patients receiving statin therapy [70].

It is important to highlight that a lack of disease awareness is likely to be contributing to the disparities in health outcome. Studies have shown that between 25 and 50% of adults are not aware they had hypercholesterolemia [71, 72], and unawareness was highest in Black individuals [71]. The Hispanic Community Health Study/Study of Latinos found that among U.S. Hispanic/Latino adults with high cholesterol, nearly half (49%) were not aware of the condition [73]. Younger age and men were more likely to be unaware of the diagnosis. Additionally, individuals of Central American and Cuban descent had among the lowest rates of being aware of the condition. In this study, less than one-third of those with high cholesterol were receiving treatment [73].

Data on Diabetes and CVD by Gender

From estimates by the CDC, there is a higher prevalence of diabetes in men (14.0%) as compared to women (12.0%) [4]. A study using NHANES data showed that between 1976 to 1980 and 2007 to 2010, the prevalence of diabetes increased

significantly in both men (4.7–11.2%, $p < 0.001$) and women (5.7–8.7%, $p < 0.001$) [74]. However, after adjusting for age, race/ethnicity and body mass index (BMI), the increase in diabetes prevalence in men was halved (6.2–9.6%, $p < 0.001$) and the increase in prevalence in women was no longer significant (7.6–7.5%; $p = 0.69$) [74]. High BMI has long been associated with the development of metabolic syndrome and as an important risk factor for developing type 2 diabetes [74, 75]. For women, the change in BMI over time seemed to be the most important variable in estimates of diabetes prevalence [74].

CVD is a leading cause of death among women [12]. There is now an emphasis to better characterize sex-specific differences across a woman's lifetime that may confer an increased risk for developing cardiovascular complications. For example, premature menarche is associated with increased CVD, whereas lactation has been associated with a lower risk for developing hypertension and metabolic dysfunction [76]. In the post-menopausal state, lower estrogen levels have been associated with vascular dysfunction, increased inflammation, and upregulation of renin–angiotensin–aldosterone system, all leading to an increased CVD risk [77].

Unfortunately, disparities in diabetes and diabetes-related comorbidities related highlight the intersectionality of race, ethnicity, and gender. In a study of mortality rates attributed to cardiometabolic diseases, Black women with diabetes, for example, have a twofold higher age-adjusted mortality rate between 1999 and 2017 when compared to White women with diabetes [78]. Interestingly, although the age-adjusted mortality rate for hypertension increased in most sex-race groups in the study, Black women had an unchanged hypertension age-adjusted mortality rate [78], suggesting other less well-characterized mechanisms may be influencing the increased rate of mortality. Black men consistently demonstrated the highest age-adjusted mortality rate [78].

Data on Diabetes and CVD by Sexual Orientation Group

The sexual minority—lesbian, gay, bisexual, transgender and queer or questioning (LGBTQ)—population in the U.S. is another marginalized group that experiences health disparities [79–83]. A Gallup poll estimates that in 2021, 5.6% of U.S. adults identify as LGBTQ [84]. Understanding the unique healthcare disparities faced by this group is important to addressing overall health in the U.S.

In 2020, the American Heart Association released a scientific statement addressing cardiovascular health in LGBTQ adults [79]. The authors proposed a conceptual model that posits that the stress related to minority sexual identity is the primary driver of LGBTQ health disparities [79]. The stressors can then impact psychosocial factors (depression, anxiety), behavioral factors (tobacco use, poor diet quality), and physiological factors (increased inflammation), which all contribute to an increase risk for developing cardiovascular risk factors including diabetes [85].

Studies have shown that diabetes and worse glycemic control is more common in sexual minority women than compared to heterosexual women, an effect that seems to be largely driven by high BMI [86]. Sexual minority women have been found to have an increased risk for obesity [82, 87–89]. Additionally, Hispanic lesbian women had increased odds for having obesity and diabetes when compared to non-Hispanic White lesbian women. Sexual orientation differences in men have not been consistently shown to be associated with increased prevalence of diabetes [90]. However, Black sexual minority men have been shown to have higher HbA1c when compared to White heterosexual men [85]. Taken together, the studies suggest that the intersectionality of race and sexual orientation has an impact on cardiovascular risk factors.

The transgender population undergoing gender-affirming hormone therapy has been a point of focus in the understanding the development of cardiovascular risk factors and CVD. Transgender men undergoing female to male transition may take testosterone to induce virilization and progestins for menstrual suppression; transgender women undergoing male to female transition may take estrogen for feminization. In studies on aging in cis men and postmenopausal transition in cis women, sex hormones have been shown to affect body composition, metabolism, and cardiovascular health [77, 91].

Among the transgender population, transgender women have been shown to have increased risk of CVD and a higher prevalence of diabetes [80]. One proposed mechanism is in the effect of feminization therapy on insulin sensitivity. In a study of transgendered adults before and after 1 year of gender-affirming hormone therapy, insulin sensitivity decreased with feminization therapy in transgender women, but increased with masculinization therapy [92]. Transgender women on gender-affirming hormones have also been shown to have higher incidence of major cardiovascular events including venous thromboembolism, ischemic stroke, and myocardial infarction [93]. It is unknown how the effects of behavioral factors and environmental stress impact these risks, but further studies may help to elucidate modifiable risks for this particularly vulnerable population.

Data on Diabetes and CVD in Immigrants in the U.S.

Immigrants have been a part of the history of the U.S. and continue to contribute to population growth. In 2019, international migrants represented 15.4% of the total U.S. population [1]. The health status among immigrants is complex, owing to the large heterogeneity of the immigrant population. Some have suggested that foreign-born individuals have better health profiles when compared to U.S.-born individuals despite fewer resources and higher socioeconomic risks—the "immigrant paradox" [94, 95]. In Hispanic immigrant population, studies have examined migration selection and the "salmon bias"—arguing that Hispanics tend to return to origin country

nearing the end of life and therefore are not included in the mortality statistics in the U.S. [96]—as a way to understand this paradox [94, 95, 97, 98]. However, the studies have not been able to fully explain the health outcome patterns.

More recent studies have examined the variable of underdiagnosis of disease to challenge the validity of the "immigrant paradox." According to the CDC, of the 34.1 million U.S. adults who met laboratory criteria for the diagnosis of diabetes, 7.3 million (21.4%) did not report having the diagnosis or were not aware of it [4]. Hsueh and colleagues used NHANES 2011–2016 data and found that across different racial and ethnic groups, being foreign-born had 48% increased odds of having undiagnosed diabetes ($P < 0.001$) [99]. Additionally, they found that immigrants were less likely to perceive the risk of diabetes and prediabetes [99], which may have significant implications for future health outcomes.

Even in individuals with known diagnoses, pharmacological treatments for diseases vary depending on immigration status. Among those with diabetes, being foreign-born was associated with decreased odds of being treated with insulin [100]. Another study found that the immigrant population who were noncitizens had lower treatment rates of hypercholesterolemia, hypertension, and diabetes when compared to U.S.-born and foreign-born citizens [101].

An important variable in addressing health care equity among immigrants is immigration status as it relates to access to health insurance and health care services. Large-scale data on the health status of undocumented adults in the U.S. is limited as undocumented immigrants may be less likely to be included in data collection or may overreport citizenship [101]. Studies inferring undocumented immigrant status—in which foreign-born participants report neither U.S. citizenship or legal residency—have tended to suggest poorer health outcomes related to lack of access, provider mistrust, underutilization of services and lower levels of self-care [101–103].

However, others have shown that the disparities may be influenced primary by access to health care services. Iten and colleagues analyzed data from Immigration, Culture and Health Care and compared the health experiences of documented Mexican immigrants, undocumented Mexican immigrants and U.S. born Mexican Americans with diabetes who sought care at safety-net clinics in sanctuary areas where immigration status is not ascertained [104]. They found the three groups not only had similar outcomes in glycemic control, lipid control, and systolic blood pressure, but also had no differences in physician communication [104]. This suggests that if access to health can be achieved, foreign-born adults—either with documented or undocumented legal status—can achieve improved health outcomes.

Biological Factors That Impact the Difference in Risk and Disease

According to the Pediatric Diabetes Consortium Clinic Registry, 92% of the children with type 2 diabetes had a positive family history of type 2 diabetes [11]. While the pathogenesis of type 2 diabetes is heterogeneous, the strong predisposition of family history has long raised the question of the contribution of biological and genetic factors.

Body Mass Index and Fat Distribution

A high body mass index (BMI) is highly associated with the development of diabetes and cardiovascular disease. The prevalence of BMI in the obese range (BMI >30.0) varies by race and ethnicity. From the 2017 to 2018 CDC data, the prevalence of obesity was highest among non-Hispanic Black (49.6%), followed by Hispanic (44.8%), non-Hispanic White (42.2%), and lowest in non-Hispanic Asian (17.4%) adults [105]. Although less data is available for American Indians/Alaska Natives, from the 2018 National Health Interview Survey, 48.1% of American Indian/Alaska Natives had BMI in the obese range, compared to 31% in Whites [106]. The overall prevalence of obesity was similar between men and women with the exception of non-Hispanic Black women whose obesity prevalence (56.9%) was notably highly than their non-Hispanic Black men counterparts [105].

Although obesity is a strong predictor of metabolic syndrome [107], obesity alone, however, may not explain the higher prevalence of diabetes in racial and ethnic minority groups. Non-Hispanic Black women, for example, have been found to develop diabetes at a higher BMI when compared to non-Hispanic White women [108]. In contrast, non-Hispanic Asian in the U.S. have been found to develop diabetes at lower BMI when compared to Whites as well as other racial and ethnic minority groups [109, 110]. This effect has been explained by differences in body fat distribution. Non-Hispanic Black women have a relatively higher subcutaneous adipose tissue and lower visceral adipose tissue have been associated in [108, 111, 112], whereas non-Hispanic Asian women had higher visceral adipose tissue when compared to non-Hispanic Blacks and non-Hispanic Whites with similar BMI [113]. In 2015, the America Diabetes Association recommended the BMI cut point for diabetes screening in Asian-Americans to be BMI $\geq$23 kg/m^2 (compared to BMI

≥25 kg/m^2 in the general population) [110]. As the obesity epidemic rises among all groups in the U.S. [105], additional research is needed to better understand the physiological impact on the racial/ethnic minority population in order to provide tailor screening and treatment guidelines.

Young Hispanic adults with parental history of type 2 diabetes usually have insulin resistance and endothelial dysfunction and vascular inflammation, particularly when they also have abdominal obesity [114]. Similarly, Hispanic children/adolescents above their ideal body weight have been found to have insulin resistance and vascular dysfunction even when their blood glucose levels are normal [115]. All these findings call for early identification of individuals and families with overweight and obesity to implement diabetes and cardiovascular prevention programs.

Glucose Metabolism

Studies have demonstrated racial and ethnic differences in glucose metabolism that may contribute to the increased risk for developing type 2 diabetes [8, 116]. B-cell function and insulin sensitivity have been extensively studied in understanding the pathophysiology of type 2 diabetes. In a review by Aguayo-Mazzucato and colleagues, Hispanic populations have been found to be at higher risk for β-cell failure, leading to insulin resistance [8]. In animal models, insulin resistance has been shown to accelerate β-cell senescence and aging, which leads to the progression of diabetes [8].

In a meta-analysis of studies measuring insulin sensitivity, Kodama and colleagues found that populations with African ancestry had significantly lower insulin sensitivity [116]. Even among a multi-ethnic group of healthy individuals without diabetes in the U.S., racial and ethnic minorities were found to have reduced insulin sensitivity when compared to the White individuals. These effects had previously been seen in a hyperglycemic clamp study in prepubertal children without diabetes: Black children had lower insulin sensitivity index when compared to their White counterparts ($p = 0.02$) [117].

In addition to lower insulin sensitivity, studies have also shown ethnic differences in hepatic insulin clearance as early as childhood [118]. In a study of over 200 children, aged 7–13 with mean BMI 19 kg/m^2, it was found that the fractional hepatic insulin extraction was lower in Black children when compared to White children [118]. There were no significant differences in extra-hepatic insulin

clearance (kidney, muscle) between the groups [118]. The causal relationship between hepatic insulin clearance and the development of diabetes is not yet fully elucidated. Insulin clearance by the liver is closely associated with hepatic glucose production and lipid content, which can contribute to glucose dysregulation and decreased insulin sensitivity [119].

Gender has repeatedly been shown as an important intersecting variable in the differences in glucose metabolism between racial and ethnic groups. Using the Atherosclerosis Risk in Communities (ARIC) study data, researchers found that non-obese Black women had higher fasting insulin levels than non-obese White women [120]. The Study of Women's Health Across the Nation (SWAN) study in women without diabetes examined ethnic differences in glucose metabolism using homeostasis model assessments of insulin sensitivity and beta-cell function. They found that insulin sensitivity was lower in non-Hispanic Black women when compared to non-Hispanic White women even after correcting for waist circumference, impaired fasting glucose and other social and behavioral factors [121]. Additionally, the results showed East Asian women (Chinese-and Japanese-Americans) had lower levels of beta-cell function when compared to non-Hispanic White women after adjusting for covariates [121]. The authors argued that the results suggest diabetes prevention strategies should consider ethnic background to target decreased insulin sensitivity and beta-cell function in certain minority groups.

Genetics

In the past two decades, significant research has focused on better understanding the genetic underpinnings of type 2 diabetes and how genetic differences may contribute to the racial and ethnic disparities. With the availability of common variant genome-wide association studies, more than 200 genetic variants associated with type 2 diabetes have been identified [122, 123]. Although the majority of GWAS analyses have focused on individuals of European ancestry, highlighted here are the diabetes-associated SNPs that have been identified in racial and ethnic minority populations (Table 35.2). It is unclear to what degree these genetic differences confer risk for developing diabetes. However, better understanding of the susceptibility genes of diabetes could be an important step toward providing better patient-centered and equitable care.

Table 35.2 Diabetes-associated SNPs identified in non-European populations

At risk group	Trait	GENE	SNP	References
African Ancestry	T2DM	TCF7L2	rs7903146	[124]
		KCNQ1	rs231356 rs2283228	[124]
		HMGA2	rs343092	[124]
		HLA-B	rs2244020	[124]
		INS-IGF2	rs3842770	[124]
	T2DM-ESRD	RBM43 RND3	rs7560163	[125]
		SLC44A3 F3	rs7542900	[125]
		RYR2 MTR	rs4659485	[125]
		GALNTL4 LOC729013	rs2722769	[125]
		TMEM45B BARX2	rs7107217	[125]
	Fasting insulin	SC4MOL	rs17046216	[126]
	Insulin resistance	TCERG1L	rs7077836	[126]
Mexican Ancestry	T2DM	*UBQLNL/OR52H1*	rs979752	[127]
		RORA	rs7164773	[127]
		LINGO2	rs981864	[127]
		CSN3	rs3775745	[127]
		HTR4/ADRB2	rs1833714	[127]
		RALGPS2	rs2773080	[127]
		EGR2	rs1509957	[127]
		RALGPS2/ANGPTL1	rs3922812	[127]
		LCORL/NCAPG	rs10516322	[127]
		UTRN	rs6929370	[127]
	T2DM, low HDL-C	ABCA1	rs9282541	[128]
	MetS, low HDL-C	SIDT2	rs1784042	[129]
Mexican Ancestry Latin American Ancestry	T2DM	SLC16A11	rs13342691	[130]
Latin American Ancestry	T2DM	TCF7L2	rs7903146	[131]
		KCNQ1	rs2283228	[131]
American Indian Ancestry	T2DM	*DNER*	rs1861612	[132]
		TBC1D4	rs7330796	[133]

Table 33.2 (continued)

At risk group	Trait	GENE	SNP	References
East Asian Ancestry	T2DM	GLIS3	rs7041847	[134]
		PEPD	rs3786897	[134]
		FITM2-R3HDML-HNF4A	rs6017317	[134]
		KCNK16	rs1535500	[134]
		MAEA	rs6815464	[134]
		GCC1-PAX4	rs6467136	[134]
		PSMD6	rs831571	[134]
		ZFAND	rs9470794	[134]
		KCNQ1	rs2237892	[135]
		PAX4	rs10229583	[136]
		UBE2E2	rs7612463	[137]
		C2CD4A–C2CD4B	rs1370176 rs1436953	[138]
		NKX6–3	rs33981001	[139]
		ANK1	rs62508166	[139]
South Asian Ancestry	T2DM	VPS26A	rs1802295	[140]
		HMG20A	rs7178572	[140]
		AP3S2	rs2028299	[140]
		TMEM163	rs6723108 rs998451	[141]
		RAB3GAP1	rs6730157	[141]
	T2DM Insulin sensitivity	GRB14	rs3923113	[140]
	T2DM Pancreatic beta-cell function	ST6GAL1	rs16861329	[140]
		HNF4A	rs4812829	[140]

T2DM type 2 diabetes mellitus, *MET-S* metabolic syndrome, *HDL-C* high-density lipoprotein-C

Social, Psychological, and Cultural Factors in Diabetes and CV Care

Social Determinants of Health

The social determinants of health (SDOH) are the conditions in which people are born, grow, live, work, and age. These circumstances are shaped by the distribution of money, power, and resources at global, national, and local levels. The social determinants of health are mostly responsible for health inequities—the unfair and avoidable differences in health status seen within and between countries [142]. SDOH are known to contribute to health disparities in diabetes and cardiovascular care [10, 143–146]. Minority populations disproportionately face challenging SDOH, which contribute to the disparities in the prevalence, management, and outcomes in diabetes and cardiovascular care [147].

Hills-Briggs and colleagues recently reviewed the different social determinants of health as they relate to the management and progression of diabetes [148]. In this paper, they focus on five social determinants of health: socioeconomic status, neighborhood and physical environment, food environment, health care, and social context as all relating to the health outcomes in people with diabetes [148].

Socioeconomic status is a strong predictor of the development and progressions of disease, including diabetes and other risk factors for CVD. Education status, closely related to socioeconomic status has been shown to be related to the prevalence of diabetes. The National Diabetes Statistics Report 2020 from the CDC reports that 13.3% of U.S. adults with less than a high school education had diabetes, compared to 9.7% in those with a high school education level and 7.5% in those with more than a high school education [4]. The effect of education on diabetes appear to span across generations. From Pediatric Diabetes Consortium Clinic Registry, 70% of the children with type 2 diabetes had parents with a high school education or less [11].

Low education level relates to lower income, which has been a strong predictor of diabetes onset, worse glycemic control, and higher association with cardiometabolic risk factors in both adults and children [10, 11, 145, 149, 150]. In a study of 2662 individuals with self-reported diabetes, difficulty paying bills was independently associated with an increase in HbA1c over time both before and after adjusting for demographic variables [149].

Environmental conditions, such as the built environment, have also been cited as a contributor of healthcare disparities [151]. The built environment has been referred to as the characteristics physical space in which people inhabit: “walkable” neighborhoods, open spaces, infrastructure, access to essential resources [146, 151]. Studies on neighborhood walkability and access to green space have not had a clear association with the incidence and prevalence of diabetes in the U.S. However, availability of and access to healthy food options has been shown to decrease the risk of obesity and type 2 diabetes [152].

The food environment (food insecurity, food access, food availability, and food affordability) has important implications in people’s lifestyle. Food insecurity has been closely linked to higher obesity, type 2 diabetes and cardiovascular disease rates and negatively impacts mental health [153]. Intervention to improve nutritional habits among racial/ethnic minorities must consider cost and address cultural and social factors [154].

Health care access is certainly a crucial factor that influences diabetes and cardiovascular disease disparities [148]. Racial and ethnic minorities usually have lower access to health care services. Although it is true that health care access has gradually improved in the U.S., the gap between the mainstream white population and other groups still exists [8]. The cost of medications to people with diabetes and cardiovascular disease represents a huge burden to many of them. Even if patients have health insurance coverage, the out-of-pocket expenses related to the medications that are often prescribed to them may be insurmountable. In fact, people with diabetes may not adhere to their treatment plans in order to save money and redirect it to food or other living expenses [155].

The overall social context where people live is fundamental in determining people's health status. Specific terms have been used to address some particular aspects: social capital—the features of social structures that serve as resources for collective action (e.g., interpersonal trust, reciprocity norms, and mutual aid); social cohesion- the extent of connectedness and solidarity among groups in a community; social support—the experiences in individuals' formal and informal personal relationships as well as their perceptions of those relationships. Taken together, emotional support, tangible support, informational support, and companionship [148] can all influence the development and progression of diabetes and cardiovascular disease.

Psychological Factors

There is a strong association between diabetes and depression [156–159]. In a study of older adults (age 67–90 years old), the prevalence of current depressive symptoms was found to be high in those with diabetes (11%) as compared to those without diabetes (5.4%; prevalence ratio 2.04, 95% CI 1.60, 2.48) [156]. However, depression can affect adherence to diabetes management plans and be associated with worse glycemic control and diabetes-related complications [159, 160]. In minority youths, life stressors have been associated with higher HbA1c [10].

The bidirectional link between diabetes and depression is multifactorial and likely influenced by the social, biological, and cultural factors. However, it is important to note that while having either diabetes or depression have disease-related complications, but those with both diabetes and depression can experience a functionally limiting burden of disease. In a study of Black Americans in the Jackson Heart Study, Kalyani and colleagues found that having diabetes and depression was associated with increased functional disability than those with only diabetes or depression [157]. Depression is a commonly missed diagnosis [161], but studies demonstrate the importance of appropriate screening for intervention and prevention of both worse diabetes outcomes and functional disability. Recent research highlights the role of moderate intensity exercise and decrease in sedentary behavior can decrease the occurrence of depression in adults with diabetes and obesity [162].

Culture Aspects

Culture alludes to beliefs, behavior patterns, and all other products of human thought and work in a certain community [163]. Health care providers and patients with often very distinct cultures interact in clinical encounters without fully understanding each other in many ways. Language concordance does not guarantee understanding. The lack of understanding an be bi-directional. Health care professional

do not fully understand (and sometimes respect) patients' points of view and cultural habits and vice versa.

Respecting our cultural differences is essential for a meaningful and productive interaction with each other. Cultural competence (awareness) alludes to health care providers' knowledge and skills to understand, respect, appreciate, and interact with patients from cultures other than their own [164, 165].

People with diabetes and cardiovascular disease may have particular views on their own body weight, fears to particular medications and a whole host of personal views on treatment interventions that influenced by personal and cultural factors [166, 167].

Establishing a good patient–provider communication is key to help patients improve diabetes and cardiovascular self-care behaviors [168]. Being genuinely curious and interested in patients' points of view about their own health is extremely important. Respecting their ideas and values is necessary in order to fully engage in a meaningful interaction with them.

Conclusions and Recommendations

There have been fascinating scientific advances in the fields of diabetes and cardiovascular disease in the last several decades. We practice medicine in an era where there is enhanced understanding of the pathophysiology of these conditions and their impact on different populations. We also have better prevention and treatment strategies that have helped reduce the burden of these conditions over time.

However, these improvements have not impacted populations in the same way. Unfortunately, racial/ethnic minorities, individuals with lower socio-economic status and education levels in the U.S. and around the world have lagged behind in their diabetes and cardiovascular disease care. Multiple factors on the patient, health care provider, and health care system domains have long contributed to the suboptimal care provided to these groups. Deep understanding of the complex interactions of these three crucial areas is necessary in order to develop and implement effective and sustainable prevention and treatment programs that address the needs of these populations.

There is an overwhelming need to improve the way in which we provide diabetes and cardiovascular disease care to people affected by these two important and closely related conditions. The traditional biomedical model implemented in clinics and hospitals has fallen short in improving the lives of most people with metabolic and cardiovascular diseases [169]. We are at a crucial time when we need to think "outside the box" and fully integrate the evaluation of psychological, social, and cultural factors in routine clinical care and create more comprehensive prevention and treatment programs that address them.

Combining the fascinating latest scientific information in the field of diabetes and cardiovascular disease with practical, realistic and feasible strategies in the

prevention and management of these conditions in vulnerable populations is necessary in order to better achieve treatment targets in these communities [170].

The current COVID-19 pandemic has helped us all realize that not being able to see patients in the clinic or hospital and having to increase the contact time with them through virtual communication may not be as ineffective as we initially thought. In fact, it has become a great opportunity to address many important clinical points when people are at home, in their own community. Returning to the traditional model of only "seeing" patients in the clinic or hospital may not be ideal. Instead, incorporating telemedicine and virtual contact with people affected by diabetes and cardiovascular disease may prove to be a significant improvement in the quality of care we provide to our communities. Certainly, not all individuals have equal access to telemedicine- and technology-dependent communications. This area needs to be improved at a societal level [171].

Improving the lives of all people in our society regardless of race/ethnicity, gender, sexual orientation and many other factors is not only the right thing to do, but rather it is the way to grow as individuals, as health care professionals, and collectively, as a society.

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Chapter 36
Diabetes and COVID

Magdi Zordok and Michael Johnstone

Introduction

In December 2019, the news of a novel form of pneumonia observed in Wuhan, China made headlines. The new form of pneumonia was dubbed Corona Virus Disease 2019 (COVID-19), and the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus was isolated from lower respiratory tract samples. The World Health Organization (WHO) reported the first cases of COVID-19 to be linked to a livestock market in Wuhan [1]. The SARS-CoV-2 virus had some similarities to the bat coronavirus, and bats were thought of as a possible reservoir [2]. The human-to-human transmission was established, as the number of patients continued to grow with no evidence of exposure to the wildlife market in Wuhan [3]. Later on, and in March 11, 2020, COVID-19 was labeled a pandemic.

The USA had its first confirmed case on January 31, 2020. The index patient was a 35-year-old male who just returned to the US after visiting his family in Wuhan, China [4]. With an excess of 47 million cases (around 18% of the world's COVID-19 cases) and more than 760,000 deaths as of November 16, 2021, the US has become the global epicenter for the pandemic [5] (Table 36.1).

Among patients with higher rates of mortality and morbidity, a constellation of comorbidities was reported. This includes diabetes, cardiac disease, hypertension, history of malignancy or renal disease, and chronic obstructive pulmonary disease (COPD). These comorbidities not only increased the risk of patients to develop COVID-19 pneumonia but were also associated with higher risk for death [6]. In this

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Table 36.1 Percentage of patients with obesity and diabetes in the general population and in different degrees of severity of COVID-19

	General population (%)	COVID-19 +ve total positive (%)	COVID-19 +ve with not severe disease (%)	COVID-19 +ve with severe disease (%)		
				Hospitalized	Require ICU admission	Mortality
Obesity						
China	6.2	–	–	22.0	25.5–27.0	88.2
France	21.6–25.8	–	–	–	47.6	–
United States	34.0–42.4	–	14.4	14.0–53.7	19.0–45.7	–
Diabetes						
China	9.2–10.9	2.0–22.0	4.5–11.0	7.4–19.0	13.8–34.6	7.3–31.0
Italy	5.0–9.0	33.9–35.5	–	–	17.0	33.9–35.5
Spain	6.9	–	–	–	–	12.0
United States	9.8–10.8	5.4–10.9	5.3–24.0	15.0–37.8	58.0	–

Adapted from Mechanick JI, Rosenson RS, Pinney SP, Mancini DM, Narula J, Fuster V. Coronavirus and Cardiometabolic Syndrome: JACC Focus Seminar. J Am Coll Cardiol. 2020;76(17):2024–35 [8]

chapter, we study the role of diabetes in orchestrating a cascade of events in conjunction of other risk factors that culminate to worse outcome in patients with COVID-19.

Risk Factors

A team of researchers from the UK conducted a large-scale study of the relationship of different comorbidities with diabetes and the effect on mortality. In this study, diabetic patients of male sex, old age, prior history of kidney disease, stroke, and heart failure, as well as patients of non-white ethnicity, and low socioeconomic status had increased mortality [7].

As for the increased incidence in the nonwhite ethnicities, non-biological factors were more likely incriminated and rather lifestyle and socioeconomic factors. In socioeconomically disadvantaged population groups, overcrowded housing as well as well as higher rates of exposure with employment in jobs that require greater human interactions result in greater transmission. Interestingly though, a study in countries from southeast and southern Asia including India, Bangladesh, and Pakistan showed lower than expected mortality in patients with diabetes who developed COVID-19 which would suggest that there might be other geopolitical and climate factors that affect virus transmission [5].

Metabolic syndrome in concert of COVID-19 results in a coronavirus disease-related cardiometabolic syndrome (CIRCS). Patients with acute CIRCS have worse disease outcome with higher inflammatory cytokine levels, hypercoagulability, severe insulin resistance, as well as other evidence of end-organ disease/failure. The

figure below was adapted from a literature review by Mechanick et al. It compares the percentages of patient with obesity and diabetes in the general populations from the countries within which the individual data was compiled, to patients with COVID-19 within the same countries. It further breaks down the analysis to different degrees of severity of COVID-19 [6].

Within the first week of infection, patients with COVID-19 have a mild picture of the disease with constitutional symptoms; however in susceptible hosts, the disease can progress over the following weeks to a severe picture with multiorgan failure. This pattern was noticed primarily in patients >60 years of age, and the presence of initial signs of organ damage as depicted by lymphopenia, or elevated levels of C reactive protein (CRP), D-dimer, interleukin (IL)-6, troponin-I, and lactate dehydrogenase (LDH) [7].

Potential Interplay of Diabetes in the Pathophysiology of COVID-19

SARS-CoV-2 enters cells through the ACE2 receptor. In the human body, the ACE2 receptors are expressed in type II alveolar, myocardial, ileal epithelial, renal proximal tubules, bladder urothelial, liver, as well as endothelial cells [9, 10]. Binding of a viral glycoprotein to ACE2 receptor is followed by receptor-mediated endocytosis, whereby the virus is internalized into the cell by an endosomal compartment. Subsequently, cathepsin L activates viral (S) glycoprotein, and thereafter viral membrane fusion with releases of ssRNA out of the endosome. Proteolytic cleavage of the viral (S) protein by the Transmembrane Serine Protease 2 (TMPRSS2) protease on the surface of cells can be an alternative route, through which viral ssRNA can be released directly into the host cytoplasm [11]. In patients with diabetes, glycosylation of the viral spike protein enhances viral binding and subsequent entry into the cells [12].

The infected cells undergo either apoptosis or necrosis, which in turn activates an inflammatory response, with further recruitment of inflammatory cells, including CD4+ and Th1. SARS-CoV-2 hence moves to the next phase of infecting circulating CD3, CD4, and CD8 T-cells. The infected cells undergo a process of apoptosis with ensuing lymphocytopenia [13, 14]. Studies suggested that diabetes is among the factors that reduced viral clearance from the body [15] (Fig. 36.1).

In patients with diabetes mellitus (DM), there is an increased expression of ACE2 which predisposes to higher risk of infection or more severe form of COVID-19 [17]. Abu Saleh and others also noted that patients with type 2 diabetes had higher levels of renin and lower levels of angiotensinogen at baseline, and that normalization of glucose level did not seem to improve the renin-angiotensin system (RAS) proteins [18]. In patients with COVID-19, elevated levels of Ang II correlated with worse outcomes with greater degrees of lung injury, due to ventilation/perfusion mismatch caused by pulmonary vasoconstriction, and oxidative damage [19, 20]. The baseline elevated level of Ang II in patients with DM would thus predispose to more severe lung injury in patients with COVID-19.

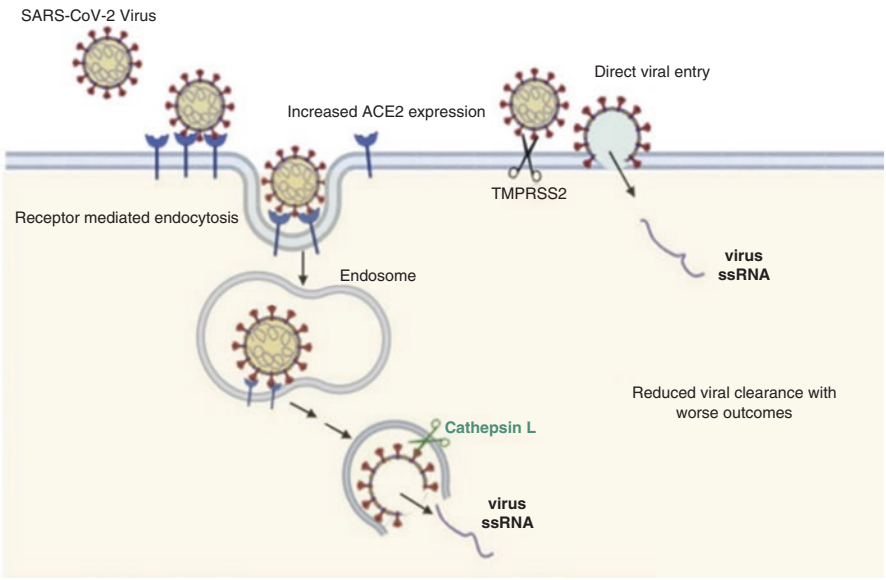

Fig. 36.1 SARS-CoV-2 can enter host cells via endocytosis or directly. In diabetic patients, there is a greater expression of the ACE2 receptor as well as reduced viral clearance that result in poor outcome. (Adapted from Mahmoud IS, Jarrar YB, Alshaer W, Ismail S. SARS-CoV-2 entry in host cells-multiple targets for treatment and prevention. Biochimie. 2020;175:93–8 [16])

Patients with obesity have dysregulation of the immune system with an impaired leptin/adiponectin ratio as well as lipotoxicity [21, 22]. In this subset of patients, there is also an imbalance between the ACE2-Ang-[1-7]-MAS, and the ACE-Ang II-AT1 axis. The ACE2/Ang-[1–7]/Mas axis has anti-inflammatory effects impeding leukocyte migration, cytokine expression, and the activation of fibrogenic pathways. This is contrary to the pro-inflammatory effects seen with the activation of the Ang II pathway, whereby inflammation and fibrosis are promoted with a cascade of events that result in calcium mobilization, free radical production, as well as recruitment of inflammatory cells [23, 24]. The cohort of diabetic patient with obesity was found to have risk for more severe COVID-19 and higher rates of mortality [25, 26]. Patients with a BMI >35 had at least a seven times greater risk for invasive mechanical ventilation [27]. Obesity and diabetes are both conditions that increase thrombosis, and in patient with COVID-19, higher rates of disseminated intravascular coagulation (DIC) and deep venous thrombosis (DVT) were reported [28, 29] (Fig. 36.2).

On the other hand, β-cells damage of pancreas by the virus can potentially lead to insulin deficiency. This theory can be used to explain a finding by Huang and his team who evaluated COVID-19 patients who required inpatient level of care, 6 months after discharge. In this cohort of over 1700 patients, 58 were found to have

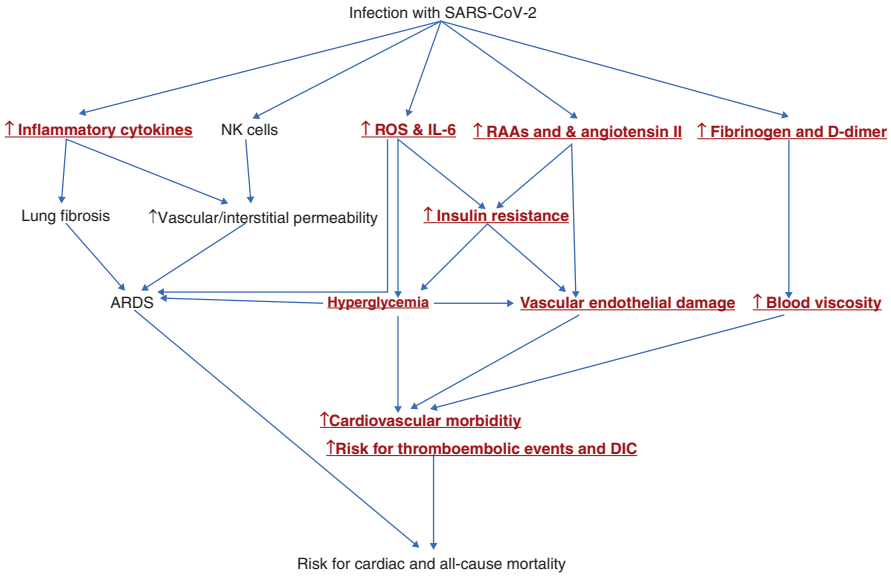

Fig. 36.2 Pathogenesis of infection of COVID-19 in patients with diabetes. Infection with SARS-CoV-2 leads to an increased production of inflammatory cytokines, a change in the natural killer (NK) cells activity (either increased or decreased), increased production of reactive oxygen species (ROS) and interlukin-6 (IL-6), increased activation of the renin–angiotensin–aldosterone system (RAAS) as well as increased fibrinogen and D-dimer production. These events result in a cascade of events that lead to increased insulin resistance and vascular permeability as well lung fibrosis. This in turn lead to an increased risk for cardiovascular morbidity and higher risk of thromboembolic events. The events highlighted are ones that are accentuated in patients with diabetes infected with SARS-CoV-2. (Adapted from Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nat Rev Endocrinol. 2021;17(1):11–30 [30])

newly diagnosed diabetes based on HbA1C measure [31]. B-cell damage in COVID-19 patient can exacerbate pre-existing diabetes, with severe diabetic ketoacidosis (DKA) being present at the time of presentation, or whereby the patient's insulin requirement is significantly increased [32].

Multiorgan Failure in Patients with Diabetes and COVID-19

Diabetes causes an inhibition of neutrophil chemotaxis and phagocytosis. Intracellular oxidation of antigen-presenting cells causes an imbalance of the Th1 cells, which causes a cascade of events leading to a hyperinflammatory response [33].

Overt hyperglycemia in patient with poorly controlled diabetes causes glycosylation and subsequent dysfunctional immunoglobulins production [34]. This

dysfunction would, in turn, lead to the body's inability to clear bacteria or viruses and hence, causing a more severe form of infection [35].

Kulcsar and others studied the response of diabetic patients to MERS-CoV using mice models. In the study, the infection was induced by expressing the dipeptidyl peptidase 4 (DPP4) receptor for viral entry, and diabetes was elucidated by administering a diet high in fat contents. The infected mice were seen to have a more severe form of the disease with worse outcomes and slower recovery. They were found to have an abnormal immune response with fewer macrophages and CD4+ T-cells, along with decreased levels of TNF-α, IL-6 but a higher level of IL-17a indicated blunted response [36].

More than a third of 5700 patients with COVID-19 studied in New York were found to have diabetes [37]. Diabetics were found to have worse outcomes with longer lengths of stay, higher chance of ICU admissions, and higher death rates especially in patients with poorly controlled diabetes and elevated HbA1C >8% [38, 39]. COVID-19 itself was also noted to worsen diabetic control inducing diabetic ketoacidosis (DKA), hyperosmolar hyperglycemic state (HHS), or severe insulin resistance [40] (Fig. 36.3).

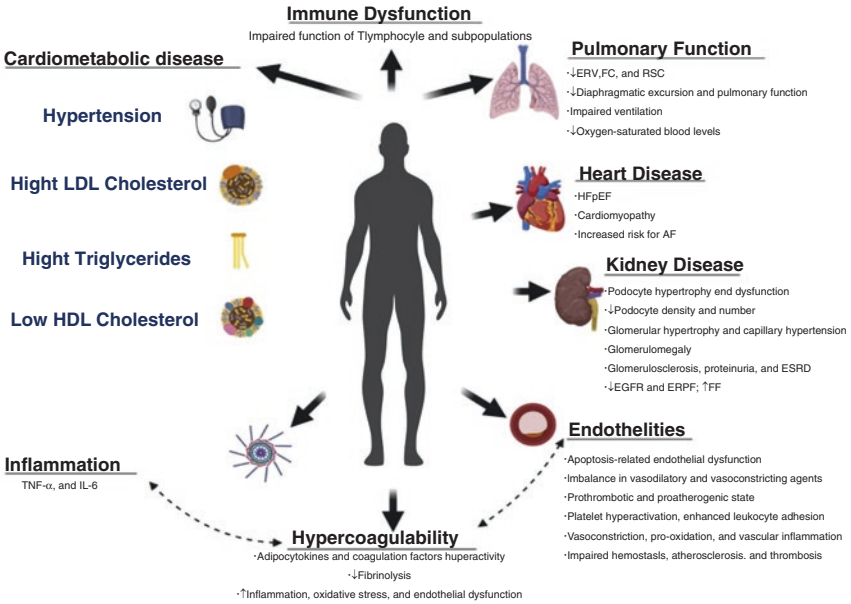

Fig. 36.3 Potential complications of the combination of diabetes in patients with coronavirus disease 2019 (COVID-19) infection. *AF* atrial fibrillation, *eGFR* estimated glomerular filtration rate, *ERPF* effective renal plasma flow, *ERV* expiratory reserve volume, *FC* functional. (Adapted from Sanchis-Gomar F, Lavie CJ, Mehra MR, Henry BM, Lippi G. Obesity and Outcomes in COVID-19: When an Epidemic and Pandemic Collide. Mayo Clin Proc. 2020;95(7):1445–53 [41])

Endothelial Dysfunction

Endothelial cells have antioxidant properties whereby they produce superoxide dismutase that protects against reactive oxygen species, and glutathione peroxidases that decreases effects of oxidative stress [42, 43]. In diabetics, these defense mechanisms are disrupted leaving the endothelium prone to oxidative stress and subsequent damage and endothelial dysfunction [44, 45]. In patients with endothelial dysfunction, the occurrence of hypoxia favors intravascular coagulation. Postmortem studies found changes in the pulmonary vasculature as well as evidence of near total occlusion of the alveolar capillaries and the bronchial vasculature along with severe diffuse thrombosis with near occlusion of the alveolar capillaries [46, 47]. Another study retrospectively assesses diabetic patients who did not survive during hospitalization for COVID-19 and found longer prothrombin times and higher levels of D-dimer than in survivors [48].

The damage would culminate in the loss of integrity of the semipermeable endothelium and lead to capillary leak into the extracellular space. In patients with COVID-19, this damage is augmented with the excessive production of cytokines such as IL-1α and IL-1β, IL-6, and TNF-α, in what is known as the cytokine storm. That same surge of cytokines also tips the coagulation/anticoagulation ecosystem of the endothelial cells toward prothrombotic spectrum. The thrombosis observed was not only in the pulmonary endothelium, but a more global effect on the peripheral veins and arteries of the body ranging from "COVID toes" seen with the affection of microvasculature to cerebrovascular strokes [49].

One hypothesis for the multiorgan disease in patients with COVID-19 suggested by Varga and others was endothelial injury and endotheliitis in several organs, due to effect on the microcirculation various vascular beds. Among patients who were notably more susceptible were diabetics along with patients with hypertension, obesity, and cardiovascular disease [50].

The endothelial dysfunction results in a relative reduction in the anticoagulant factors released, including nitric oxide and prostacyclin. The decrease of nitric oxide level impedes the ability to modulate prothrombotic factors which may be upregulated, notably tissue factor. These prothrombotic factors conspire to increase the likelihood of developing pulmonary arterial thrombosis.

Management of Diabetes in Patients with COVID-19

Diabetes is most often associated with other comorbidities from the metabolic syndrome namely hypertension and dyslipidemia. It is therefore prudent that a discussion of the management of diabetes also involves treatment options of blood pressure and hyperlipidemia, and to discuss the possible interactions in the setting of COVID-19.

In patients with diabetes who are treated with ACE inhibitors (ACEi) and angiotensin II, the expression of ACE2 is increased due to an upregulation of ACE2 [51]. It was therefore suggested that this cohort of patients has a higher risk of acquiring COVID-19 infection by accelerating the viral entry into the host cells [52]. Several studies tested this hypothesis failed to find a relationship of the use of ACEi and in-hospital death or severe disease outcome [53, 54]. It was though the ACE2 is not a target of ACEi due to structure differences with ACE [55]. For these reasons it was recommended by the American College of Cardiology, American Heart Association, and the European Society of Cardiology to continue the use of ACEi and ARBs in patients who were already on the therapy [56–58].

To help establish preliminary guidelines for the management of diabetes in patients with COVID-19, a panel of experts published their recommendations based on literature review and observations from clinical practice [26].

Primary prevention was emphasized in patients with diabetes who did not have signs or symptoms of COVID-19 infection. The aim of primary prevention is to (1) intensify the control of the blood glucose level, (2) tight control of associated blood pressure and lipid profile given the high prevalence of metabolic syndrome, and (3) reduce the risk of infection to SARS-CoV-2 by practicing social distancing and hand hygiene. Patients should be followed closely for symptom development and utilize telemedicine as to reduce exposure.

As in primary prevention, a very strict control of the blood glucose level, blood pressure, and lipid profile control were recommended for diabetic patients who developed COVID-19 and required hospitalizations (Fig. 36.4).

For blood pressure control, patients who were initially on ACE inhibitors or angiotensin II receptor blockers should continue the regimen, as supported by the American College of Cardiology, American Heart Association, and the European Society of Cardiology [58–60]. Statin therapy is to be continued not only for the effect on lowering low-density lipoproteins but also for their anti-inflammatory effect and the risk of rebound increases of IL-6 that subsequently can induce a cytokine storm, where they to be stopped abruptly [61, 62].

Furthermore, the report highlights some major consideration for the use of antidiabetic drugs in patients with COVID-19 (Table 36.2). The occurrence of lactic acidosis or diabetic ketoacidosis in patients on metformin or SGLT-2 (sodium–glucose cotransporter-2) inhibitors, even if was rarely reported, has thus led to discouraging their use especially in patients with severe forms of COVID-19 (Table 36.3). The discontinuation, however, was not recommended in the outpatient setting when there are no clinical signs or symptoms indicating severe COVID-19. Despite the debate of the involvement of the DPP-4 enzyme in the pathogenesis of COVID-19, there is not enough evidence to justify the discontinuation of DPP-4 inhibitors in patients with COVID-19 [32] (Table 36.3).

SGLT-2i have received high praise as antidiabetic drugs for their action in reducing the risk of death and worsening heart failure in patients with and without diabetes, as well as the progression of kidney disease and onset of AKI (acute kidney injury). The DARE-19 trial was designed as to study the potential impact for the protection of patients with COVID-19 against cardiovascular and renal

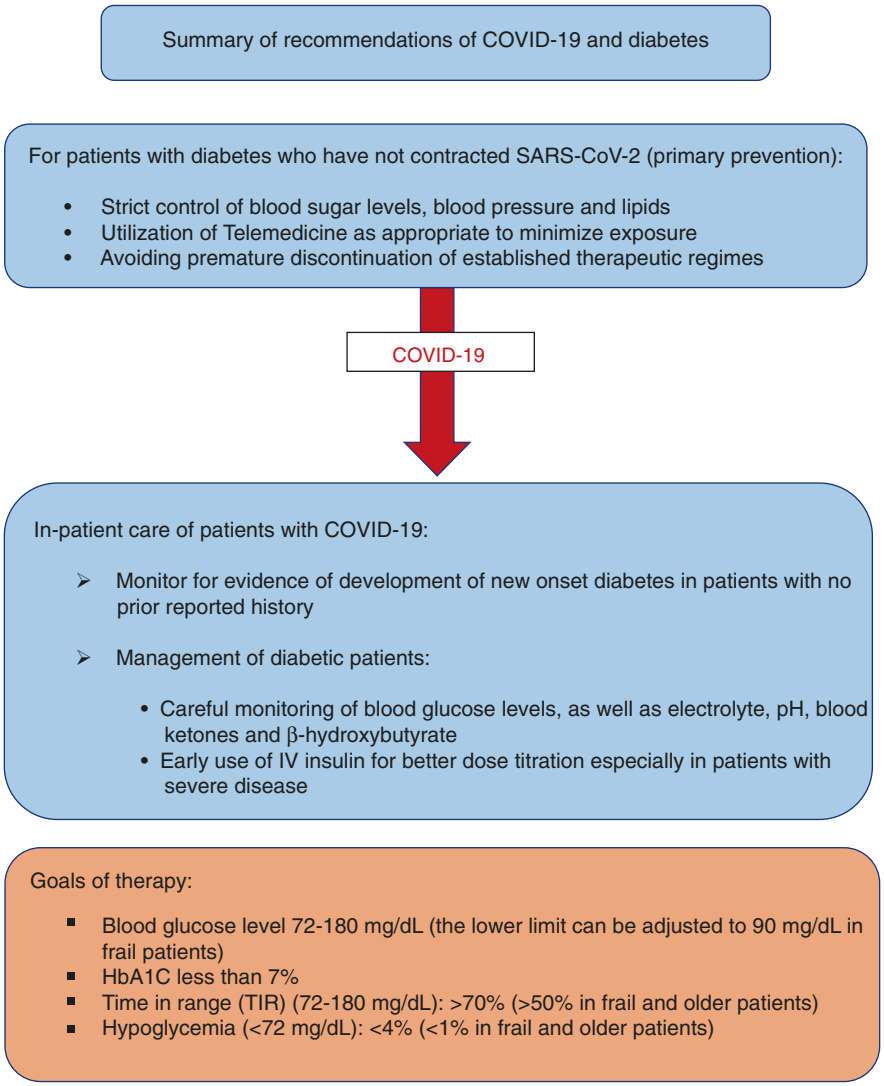

Fig. 36.4 Summary of recommendations on the management of diabetes in patients with COVID-19. (Adapted from Cariou B, Hadjadj S, Wargny M, Pichelin M, Al-Salameh A, Allix I, et al. Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study. Diabetologia. 2020;63(8):1500–15 [26])

complications. The trial, however, did not achieve statistical significance for the prevention of organ dysfunction and all-cause mortality, or recovery at 30 days.

Argawal and others studied over 1100 patients assessed for a relationship between prehospitalization HbA1C and insulin use with mortality. The study showed that HbA1C was nonpredictive of mortality but interestingly outpatient

Table 36.2 Summary of antidiabetic drugs use in patients with COVID-19

	Advantages	Disadvantage
Metformin	• No risk of developing hypoglycemia	• Risk of dehydration and lactic acidosis in critically ill
		• Risk of alteration of renal function especially in patients with acute kidney injury or chronic kidney disease
		• Risk of heart failure
DPP-4 inhibitors	• No risk of developing hypoglycemia	
	• Can used in in patients with kidney disease	
	• Anti-inflammatory effects	
	• Can potentially modify SARS-CoV-2 binding sites	
SGLT2-inhibitors	• No risk of developing hypoglycemia	• Risk of dehydration and euglycemic DKA during the disease
		• Electrolyte disturbances
GLP-1 receptor agonist	• No risk of developing hypoglycemia	• Risk of dehydration
	• Anti-inflammatory effects	• Gastrointestinal side effects
		• Risk of aspiration
Sulphonylurea		• Risk of hypoglycemia especially if co-administered with other oral anti-diabetic drugs
Pioglitazone	• Anti-inflammatory effects	• Risk of volume overload and heart failure
Insulin	• Drug of choice for managing critically ill patients	• Risk of hypoglycemia
		• High doses may be needed
		• Close and frequent monitoring especially if IV administration

DPP-4 dipeptidyl peptidase 4, *SGLT2* sodium–glucose cotransporter-2, *GLP-1* glucose-dependent insulinotropic peptide

Adapted from Apicella M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes Endocrinol. 2020;8(9):782–92 [63]

insulin use was associated with higher rates of mortality [64]. The same study also observed the highest rates of mortality in patients who were dependent on insulin alone versus patients who took a combination of insulin and non-insulin regimens. Another study of 904 patients from Wuhan included 120 patients who were diabetic on treatment. The patients who used insulin for glucose control had higher infection indices, with high CRP, procalcitonin, and erythrocyte sedimentation rate (ESR).

Table 36.3 Summary of recommendations on use of antidiabetic medications in patients with diabetes and COVID-19 based on severity and setting

	No disease/ ambulatory	Mild disease/ ambulatory	Moderate disease/ hospitalized	Severe disease/ hospitalized
Recommended use	Insulin	Insulin	Insulin	Insulin
	Metformin	DPP4i	DPP4i	DPP4i
	TZD	Metformin	Metformin	
	DPP4i	GLP1 analogues	GLP1 analogues	
	GLP1 analogues			
	α-Glucosidase inhibitors			
Cautious use	SGLT2i	SGLT2i	α-Glucosidase inhibitors	Metformin
	Sulfonylurea	TZD	Sulfonylurea	GLP1 analogues
		α-Glucosidase inhibitors		α-Glucosidase inhibitors
		Sulfonylurea		
Use not recommended			TZD	Sulfonylurea
			SGLT2i	TZD
				SGLT2i

Adapted from Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nat Rev Endocrinol. 2021;17(1):11–30

Insulin users were found to have a greater risk for poor prognosis when multivariable regression models were made to compare different glucose-lowering drugs [65].

It is still unclear whether insulin itself was responsible for the observation, or if there were other confounding characteristics that were contributing. On the other hand, the use of continuous intravenous insulin infusion improved glucose control, especially in the ICU and in patients who require high-dose steroids. To reduce the risk of healthcare personnel exposure, it was recommended to start an NPH-regular insulin regimen, but if the prime target in management is to reduce the variability in the levels of measures blood glucose, intravenous insulin infusion is recommended [66].

The potassium balance must be carefully adjusted given the potential for hypokalemia that is a common feature in patients with COVID-19, due to high levels of angiotensin II and hyperaldosteronism. COVID-19 was also found to worsen hyperglycemia, by affecting the β-cells of the pancreas [67]. The increased insulin requirement would thus predispose to worse hypokalemia. Special attention must be paid to the fluid balance of patients with COVID-19 as to avoid volume overload and pulmonary edema in a lung tissue that already sustained significant injury. Fluid balance must be tailored as such to avoid development of pulmonary edema in severely inflamed lung tissue.

Various anti-inflammatory agents have been tried to treat COVID-19. They have had differing effects on blood glucose and insulin levels (Table 36.4). Recently,

Table 36.4 The mechanism of action and effect of some drugs used for treatment of COVID-19 on blood glucose, insulin, and β-cell

Drug	Mechanism of action	Effect on blood glucose	Effect on insulin	Effect on β-cells
Camostat mesylate	Serine protease (TMPRSS2) inhibitor	↓ In patients with new-onset DM and in patients with chronic pancreatitis	–	–
Hydroxychloroquine	Blockade of viral entry and modulation of immune system	↓ HbA_{1c} as well as fasting and postprandial glucose	↑ Insulin sensitivity and ↑ hepatic sensitivity to insulin	↑ β-cell activity
Protease inhibitor	Blockade of proteolytic enzyme activity	↑ Fasting and postprandial glucose	↑ Insulin levels, ↓ insulin sensitivity and ↓ glucose clearance	↓ β-cell activity and insulin release
IL-6 receptor inhibitors	Blockade of IL-6 effect of activation of inflammatory mediators that produce B- and T-cells	↓ HbA_{1c}	↓ Insulin level ↓ Insulin-to-glucose ratio ↑ insulin sensitivity and ↓ insulin resistance	–
IL-1 receptor inhibitors	Blockade of IL-1 effects	↓ HbA_{1c}, ↓ fasting blood glucose. Both effects are not seen in patients with new onset type 1 DM	↑ C-peptide secretion and ↑ proinsulin-to-insulin ratio	–
IL-1β receptor inhibitors	Blockade of IL-1β effects	No effects are seen in patients with new onset type 1 DM	No effects on C-peptide secretion or proinsulin-to-insulin ratio in patients with new onset type 1 DM	–
TNF inhibitors	Blockade of TNF effect	↓ HbA_{1c}, ↓ fasting blood glucose	↓ Insulin resistance ↑ Insulin sensitivity	↑ β-cell activity
Corticosteroids	Anti-inflammatory effects	↑ HbA_{1c}, ↑ blood glucose especially postprandial	↑ Insulin resistance ↓ Insulin sensitivity	↓ β-cell activity and insulin release

dexamethasone has been shown to be effective in the treatment of COVID-19 [68]. However, this same drug may worsen the glucose control in diabetic patients, and adjustments in insulin treatment to maintain glucose control will become increasingly difficult.

Aside from its anticoagulation effect, heparinoids also have anti-inflammatory effects [69]. In a study by Yin and others, patients with severe pneumonia caused by COVID-19 associated with endothelial dysfunction and coagulopathy and elevated D-dimer more than 3.0 μg/mL were found to have better prognosis and lower mortality as compared to patients who also had severe pneumonia, however, not implicated to SARS-CoV-2 and elevated d-dimer of more than 3.0 μg/mL when started on heparin [70]. Low-molecular weight heparin or fondaparinux were preferred as agents for anticoagulation over unfractionated heparin as per an expert panel report [71].

A careful screening of patients with COVID-19 and diabetes would aim as to identify patients who are at risk of developing worsening disease and help improve outcome. An increasing ferritin, C-reactive protein, erythrocyte sedimentation rate (ESR), or decreasing platelet count are markers of a heightened immune response that can contribute to worse outcome and in whom immunomodulator therapy can be warranted.

Conclusion

While the highest mortality rates in patients with COVID-19 are seen in the older age population, diabetes and metabolic disease are very strong risk factors and predictors of disease outcome. Patients with diabetes have weakened immune system and endothelial dysfunction at baseline, and in the setting of COVID-19 infection, a fulmination of both processes would lead to ARDS and multiorgan failure.

Treatment of COVID-19 remains in the most part empirical, and even though a number of promising therapeutic options are currently used on a compassionate basis, large-scale clinical trials are yet to validate their efficacy, with over 2300 trials being planned for or currently launched as per ClinicalTrials.gov website [72].

Besides tailoring therapy to address COVID-19 based on signs of end organ damage/failure, a very tight control of blood glucose level is warranted in patients with diabetes with COVID-19. We also discussed some challenges that face clinicians when treating diabetics with COVID-19, as well as some differences noted between COVID-19 and other pandemics in relationship to management, and possible solutions to overcome some clinical scenarios in daily practice. The ability to

maintain and/or restore endothelial function is important to treating patients and may provide a source of treatment of COVID-19.

A year since the news of the first case, COVID-19 has claimed the lives of over 1.6 million patients. With genome isolation and the subsequent development and introduction of different vaccines, comes hope for improved control of the pandemic. We do, however, need to continue to tackle another pandemic that has outlived COVID-19 and that according to the WHO has affected 8.5% of the world's population in 2014, diabetes [73].

With the administration of around 300 million doses of COVID-19 vaccines worldwide, we have begun to see a silver lining as the numbers of new daily cases and deaths have since plateaued and decreased. States across the US have relaxed their mask mandates and are largely back to what it was pre-COVID. International travel is now allowed for fully vaccinated individuals. We are, however, not entirely out of the woods as COVID continues to infect many though now having decreased rates of mortality. We need to continue to better understand this disease process, in both nondiabetics and diabetics to further reduce the seriousness of further COVID infections especially as new strains of the virus.

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Chapter 37
Tailoring the Treatment of Type 2 Diabetes Mellitus to the Individual

Patricia R. Peter and Silvio E. Inzucchi

The Impact of Cardiovascular Disease in Patients with Diabetes

Cardiovascular disease (CVD) is a major source of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) [1]. These patients are more likely to develop and die of atherosclerotic cardiovascular disease when compared to their non-diabetic counterparts [2]. People with diabetes also have a 2–5 times higher risk of developing heart failure in their lifetime with a 50% greater risk of mortality after hospitalization for heart failure (HHF) when compared to those without diabetes [3–5]. Fortunately, there have been promising signs of improving cardiovascular outcomes in this population and decreasing burden of disease over time due to an increased focus on aggressive risk factor reduction as well as early and more efficacious cardiovascular interventions [6]. There is also new hope that the burden of cardiovascular disease will continue to decline as some of the newer classes of diabetes medications appear to not only reduce hyperglycemia but also improve cardiovascular outcomes.

Setting a Glycemic Target

The hemoglobin A1c (HbA1c) test has been used for decades to assess the overall quality of glycemic control and has formally been part of the American Diabetes Association's (ADA) diagnostic criteria for diabetes mellitus since 2010 [7]. Given

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the clear link between improving glycemic control and a reduction in rates of microvascular complications, an A1c goal of <7% has generally been accepted as striking the appropriate balance between reducing the risk of retinopathy, nephropathy, and neuropathy while avoiding the dangers of hypoglycemia (mainly a concern in those using insulin or insulin secretagogues) [8, 9]. However, the 7% threshold should be viewed as a general goal that is then adjusted based on the circumstances of each individual patient. In particular, factors that should be taken into account when setting a glycemic target include duration of disease, life expectancy, comorbid conditions, established complications, resources and support at home, patient motivation and preference, and the risks of adverse effects related to therapy, especially with regard to hypoglycemia [10]. For example, a more stringent goal of 6–6.5% might be appropriate for a motivated young patient with newly diagnosed diabetes, while a target of <8% (or even slightly higher) would be reasonable for an older individual with advanced comorbidities in whom hypoglycemia risk and quality of life considerations are of more pressing concern than the long-term sequela of hyperglycemia.

With regard to the impact of glucose control on cardiovascular complications in this population, older landmark trials in the field were largely disappointing in that they found no consistent link between tight glycemic control and improved cardiovascular outcomes [11–13]. In fact, the ACCORD trial showed increased cardiovascular mortality in those randomized to more intensive glucose control, possibly (but certainly not conclusively) due to the increased burden of hypoglycemia in that group [11]. Subsequent follow-up studies did demonstrate modestly improved cardiovascular outcomes in the groups whose HbA1c had previously been more stringently controlled, leading to a hypothesis that there was a "legacy" effect that was conferring some protective effect even though their glucose control was at present no different from their counterparts [14, 15]. Given the absence of clear-cut data linking lower glucose levels to a reduced risk of cardiovascular disease, there has recently been a new focus on prioritizing the use of glucose-lowering pharmacological agents with demonstrated cardiovascular benefits—rather than merely lowering A1c to a particular target.

Cardiovascular Implications of Glucose-Lowering Drug Classes

Managing type 2 diabetes in patients with cardiovascular disease typically starts with metformin, an agent that has been used for decades with excellent glucose-lowering efficacy and a clearly established safety profile. Supporting the early use of this agent in patients with cardiovascular disease are the results of some older, small studies that have indicated a potential cardiovascular benefit of this agent [8].

However, if hyperglycemia is inadequately controlled after metformin, the choice of what agent to use next has become increasingly complex as the number of available glucose-lowering drugs has multiplied greatly over the years. Since 2008, the Food and Drug Administration (FDA)-mandated cardiovascular outcomes trials (CVOTs) have proved essential to making these decisions as they have provided us with a wealth of information regarding the safety and potential benefits of many of the available glucose-lowering agents (Table 37.1).

Table 37.1 Summary of major cardiovascular outcomes trial data

Study (drug)	Patient population (*n*)	Mean duration of follow-up (years)	Baseline prevalence of CVD (%)	Significant CV outcomes	Other findings
SGLT2i					
EMPA-REG OUTCOME [1] (empagliflozin)	7020	3.1	99	• 14% RRR in MACE • 34% RRR in HHF or CV death • 38% RRR in CV death • 32% RRR in all-cause mortality • 35% RRR in HHF	• 46% RRR in the renal composite endpoint
CANVAS [2] (canagliflozin)	10,142	3.6	65.6	• 14% RRR in MACE • 33% RRR in HHF	• 40% RRR in the renal composite endpoint • Significantly higher rates of fracture and amputation in the treatment group
DECLARE-TIMI 58 [3] (dapagliflozin)	17,160	4.2	40	• 17% RRR in CV death or HHF • 27% RRR in HHF	• 24% RRR in the renal composite endpoint

(continued)

Table 37.1 (continued)

Study (drug)	Patient population (*n*)	Mean duration of follow-up (years)	Baseline prevalence of CVD (%)	Significant CV outcomes	Other findings
CREDENCE [4] (canagliflozin)	4401	2.6	50.4	• 20% RRR in MACE • 39% RRR in HHF • Borderline significant 22% RRR in CV death	• 30% RRR in primary renal composite outcome • No significant increase in fracture or amputation in the treatment group
VERTIS CV [5] (ertugliflozin)	8238	3.5	>99	• 30% RRR in HHF	
SCORED [6] (sotagliflozin)	10,584	1.3	48.6	• 26% RRR in composite of CV death, HHF, and urgent visits for HF	• Trial ended early due to loss of funding
GLP-1 RA					
LEADER [7] (liraglutide)	9340	3.8	81	• 13% RRR in MACE • 22% RRR in CV death • 15% RRR in all-cause mortality • Borderline significant 14% RRR in MI	• 36% RRR in the renal composite endpoint
SUSTAIN 6 [8] (semaglutide)	3297	2.1	60	• 26% RRR in MACE • 39% RRR in stroke	• 36% RRR in the renal composite endpoint • Higher rates of retinopathy in the treatment group
REWIND [9] (dulaglutide)	9901	5.4	32	• 12% RRR in MACE • 24% RRR in stroke	• 15% RRR in the renal composite endpoint

Table 37.1 (continued)

Study (drug)	Patient population (*n*)	Mean duration of follow-up (years)	Baseline prevalence of CVD (%)	Significant CV outcomes	Other findings
TZD					
PROactive [10] (pioglitazone)	5238	2.9	98	• 16% RRR in the secondary composite outcome of all-cause mortality, non-fatal MI, or stroke • Reports of nonfatal heart failure (unadjudicated) were more common in the treatment group	
IRIS[a] [11] (pioglitazone)	3895	4.8	100	• 24% RRR in stroke or MI • No increase in serous heart failure events (adjudicated) in the treatment group	• 52% RRR in progression to diabetes • Increased bone fractures in the treatment arm
DPP4i					
SAVOR-TIMI 53 [12] (saxagliptin)	16,492	2.1	78	• 27% Relative increased risk in HHF in treatment group	
CAROLINA[b] [13] (linagliptin)	6042	6.3	34.5	• No difference between linagliptin and SU with respect to any CV endpoint	

SGLT2i sodium–glucose cotransporter 2 inhibitors, *CV* cardiovascular, *CVD* cardiovascular disease, *HF* heart failure, *HHF* hospitalization for heart failure, *MACE* major adverse cardiac events, *MI* myocardial infarction, *RRR* relative risk reduction

[a]Insulin resistant, non-diabetic population

[b]Compared to glimepiride

Sodium–Glucose Cotransporter 2 Inhibitors

The sodium–glucose cotransporter SGLT 2 inhibitors (SGLT2i) are the newest class of anti-hyperglycemic agents and some of the first to show positive cardiovascular outcomes in patients with diabetes. These agents lower blood glucose by blocking renal glucose reabsorption in the proximal nephron and by increasing glucose excretion in the urine.

Several agents in this class have demonstrated improvements in the rates of major adverse cardiovascular events (MACE), which is a composite outcome that includes death from cardiovascular causes, non-fatal myocardial infarction (MI), or non-fatal stroke. In the EMPA-REG OUTCOME trial, patients with T2DM and cardiovascular disease who received empagliflozin experienced lower rates of MACE (HR = 0.86 [95% CI 0.74–0.99]; $p = 0.04$) driven primarily by a 38% relative reduction in the risk of CV (cardiovascular) death (HR = 0.62, 95% CI 0.49–0.77; $p < 0.001$) with no significant differences in the rates of MI or stroke between the two groups [16]. In both the CANVAS and the CREDENCE trials, use of canagliflozin was also associated with a lower risk of MACE, with the CREDENCE trial demonstrating a strong trend toward a significant 22% reduction in CV death (HR = 0.78, 95% CI 0.61–1.00; $p = 0.05$) [17, 18]. Dapagliflozin and ertugliflozin were both non-inferior to placebo with respect to MACE in the DECLARE TIMI 58 trial and the VERTIS CV trial, respectively [19, 20]. Thus, the reduction in CV death associated with empagliflozin has not yet been fully reproduced by other agents in this class and it remains to be seen if this is due to a unique property of this agent or more a function of differences in the study populations and trial designs. A meta-analysis by Zelniker et al. found that SGLT2i use reduced the risk of MACE by 11% (HR 0.89 [95% CI 0.83–0.96], $p = 0.0014$), but this effect was only found in patients with preexisting atherosclerotic cardiovascular disease [21]. By contrast, an updated meta-analysis by Arnott et al. including data from the CREDENCE trial with its large number of patients without established CVD found comparable reductions in MACE for those in the primary or secondary prevention setting [22]. In both of these meta-analyses, SGLT2i use was associated with a significant reduction in CV death, but there was a moderate to high level of heterogeneity among the included studies, and this risk reduction was only noted in those with established cardiovascular disease [21, 22]. Importantly, a multinational real-world observational study found that SGLT2i use was associated with reduced risk of death, myocardial infarction, and stroke in those with and without established CVD [23, 24].

The data supporting the use of these agents in patients with regard to heart failure outcomes is even more striking and consistent across all members of this class. Empagliflozin, canagliflozin, dapagliflozin, and ertugliflozin have all been associated with significant reductions in heart failure hospitalizations in patients with T2DM, ranging from a 27 to 39% relative risk reduction across their respective CVOTs [16, 17, 19, 20]. These findings have been consistent and robust across two subsequent meta-analyses and in a large study of SGLT2i use in a real-world clinical setting [21, 22, 25]. With regard to empagliflozin specifically, the Empagliflozin

Comparative Effectiveness and Safety (EMPRISE) study assessed the efficacy and safety of this agent in a real-world setting using several large insurance claims data sources. In their first interim analysis, the investigators identified a 50% reduced risk in hospitalizations for heart failure (HR = 0.50 [95% CI 0.28–0.91]) among patients with T2DM with or without cardiovascular disease who were treated with empagliflozin versus sitagliptin [26].

The improvements in heart failure outcomes in these CVOTs have been so sufficiently compelling as to prompt several investigations into the impact of these agents in the general heart failure population, regardless of diabetes status. For example, the DAPA-HF trial demonstrated a 26% relative risk reduction (HR = 0.74 [95% CI 0.65–0.85]) of the composite outcome of worsening heart failure and death from CV causes in patients with heart failure with reduced ejection fraction (HFrEF) who received dapagliflozin when compared to placebo [27]. The majority of patients in this study who derived benefit from dapagliflozin did not have diabetes, leading to its approval by the FDA for use in HFrEF [27]. Similarly, the EMPEROR-Reduced trial investigated the use of empagliflozin in a population with HFrEF who, on average, had lower EFs and higher levels of natriuretic peptides at baseline than those in the DAPA-HF trial [28]. Echoing the results of the DAPA-HF trial, patients treated with empagliflozin experienced a 25% relative risk reduction (HR = 0.75 [95% CI 0.65–0.86]) in the composite outcome of hospitalization for heart failure and CV death, again regardless of diabetes status [28]. In terms of improving outcomes in patients with HFpEF, 21% of patients in the SOLOIST-WHF trial with EF >50% appeared to experience improved CV outcomes similar to those with reduced EF, but the authors could not draw definitive conclusions about this population given the early termination of the trial and resultant small size of this subgroup [29]. The ongoing DELIVER and EMPEROR-Preserved trials will continue to investigate the potential therapeutic impact of these agents in patients with heart failure with preserved ejection fraction (HFpEF), perhaps further expanding the indications for the use of these agents [30].

SGLT2i have also demonstrated significant promise in reducing the progression of chronic kidney disease, an important comorbidity in patients with type 2 diabetes and cardiovascular disease. Empagliflozin, canagliflozin, and dapagliflozin have all been associated with improvements in clinically important renal outcomes in their respective CVOTs and in subsequent meta-analyses [16–19, 21, 31]. Specifically, the meta-analysis by Neuen et al. found a 33% reduction in the risk of the renal composite outcome of dialysis, transplantation, or death due to kidney disease (RR = 0.67 [95% CI 0.52–0.86]) with the use of SGLT2i when compared to placebo [31]. This effect was consistent across all studies and demonstrated regardless of baseline eGFR (with most studies allowing baseline eGFR as low as 30 mL/min/1.73 m^2) [31]. These renoprotective effects appear to be glucose-independent as the DAPA-CKD trial demonstrated that dapagliflozin conferred significant improvements in renal outcomes regardless of diabetes status [32]. Notably, DAPA-CKD allowed eGFR down to 25 mL/min/1.73 m^2. The ongoing EMPA-KIDNEY study will look at using empagliflozin in a similar population of patients with CKD with and without T2DM, allowing eGFR down to 20 mL/min/1.73 m^2.

A new addition to this class of agents that has a unique mechanism of action is sotagliflozin which acts on the sodium-glucose cotransporter (SGLT)1 in the gut to block glucose absorption there in addition to inhibiting SGLT2 in the kidney. Though it was terminated early due to loss of funding from the sponsor, the SCORED trial (sotagliflozin's CVOT) enrolled over 10,000 T2DM patients with CKD who were at risk for CVD. This trial found a 26% relative risk reduction in the rates of the composite cardiovascular outcome that included CV death, HHF, and urgent visits for HF (HR = 0.74 [95% CI 0.63–0.88]) [33]. Additionally, SOLOIST-WHF initiated sotagliflozin therapy in patients with T2DM being discharged after an episode of decompensated heart failure and found a 33% reduction in the composite outcome of death from CV causes and hospitalizations and urgent visits for heart failure when compared to placebo (HR = 0.67 [95% CI 0.52–0.85]) [29]. A total number of 6.1–8.5% of patients on sotagliflozin experienced diarrhea compared to 3.4–6% of patients in the placebo groups across these two trials, an adverse effect likely related to sotagliflozin's known actions on the gut. Sotagliflozin is not yet marketed in the USA.

Since the CV benefits of these agents occur irrespective of glucose-lowering, and these effects occur within a few weeks after treatment initiation, their modest positive impact on body weight, blood pressure, and lipids is insufficient to explain their beneficial impact on CV outcomes. Instead, there has been some focus on the diuretic properties of these agents, and how they might confer more benefits than the loop diuretics that are typically used in patients with heart failure. While use of loop diuretics leads to reflexive activation of neurohormonal pathways that attempt to preserve intravascular volume, SGLT2i-induced natriuresis does not appear to lead to this potentially deleterious response [34]. This is perhaps because unlike loop diuretics, SGLT2i acts at the proximal tubule to increase sodium delivery to the macula densa, thereby blunting activation of the sodium-retaining pathways that lead to loop diuretic resistance and may contribute to HF progression [34]. Additionally, SGLT2i may alter energy metabolism at the level of the myocardium by increasing ketone production which could perhaps serve as a more efficient fuel source for the heart, impact myocardial sodium and calcium handling to correct dysregulated whole-body sodium homeostasis, or act on cardiac fibroblasts and adipokines to reduce cardiac fibrosis and inflammation [35]. Using cardiac MRI data, the EMPA-HEART CardioLink-6 and the SUGAR-DM-HF trials found that even a short duration of empagliflozin therapy (i.e., 6–9 months) led to improvements in different parameters of LV function such as LV indexed mass and end-systolic and end-diastolic indexed volumes, suggesting SGLT2 inhibition might promote reversal of deleterious CV remodeling [36, 37].

As would be expected given their mechanism of action, the most common side effect of these agents is polyuria. There is also an increased risk of genital mycotic infections that are typically easily treatable with conventional topical or oral therapies. However, if such infections are recurrent, the drug may need to be stopped. While conceivably linked to urinary tract infections (UTIs) and their complications of pyelonephritis and urosepsis, no imbalance in such events has been observed in most of the large outcomes trials. Of course, those with prior history of severe UTIs,

those with indwelling catheters, or those who retain renal stones could potentially be at higher risk of infections, and so avoiding these agents in these patients may be advisable. Fournier's gangrene has been reported in post-marketing surveys, but these events are too rare to assess in clinical trials and a causative link to SGLT2 inhibitors remains uncertain. However, avoiding the drugs in those at greatest risk for this severe form of fasciitis is logical. Patients treated with these agents are also at increased risk of diabetic ketoacidosis (DKA) despite normal or only mildly elevated serum glucose levels (the so-called euglycemic DKA). This complication was first revealed in the off-label used of SGLT2 inhibitors in patients with type 1 diabetes, but DKA can rarely occur in those with type 2 diabetes as well, especially in sick individuals already on insulin whose insulin dose has been drastically reduced. Since the drugs, as mentioned previously, do increased ketone production, which is further enhanced in the fasted state and when insulin doses are decreased, they should be stopped at least 3 days prior to any surgical procedure. The CANVAS trial demonstrated an association between canagliflozin use and an increased risk of lower extremity amputations, but this association has not been noted with other agents in this class [17]. Subsequent clinical trial and observational data on canagliflozin has shown an inconsistent association with amputation risk, leading the FDA to remove its previous black box warning about this while recommending ongoing monitoring for this potential complication [18, 38, 39]. Although these tend to be costly agents, aside from the beneficial cardiovascular implications, other benefits include their low hypoglycemia risk, promotion of modest weight loss, minor improvements in blood pressure and lipids as well as their previously discussed robust renoprotective effects [21, 31].

GLP-1 Receptor Agonists

The glucagon-like peptide 1 receptor agonists (GLP-1 RA) activate the receptor for the endogenous incretin GLP-1 and improve glucose homeostasis in several ways. These mostly injectable medications stimulate glucose-dependent insulin secretion while indirectly improving insulin sensitivity by decreasing appetite centrally and promoting weight loss. They also inhibit glucagon secretion and thereby suppress endogenous (mainly hepatic) glucose production. Finally, and to a more variable degree, they slow gastric emptying, adding to a sensation of satiety.

Several members of this class have been shown to improve major cardiovascular outcomes. In the first of these, the LEADER trial, liraglutide use was associated with a 13% reduction in MACE (HR = 0.87 [95% CI 0.78–0.97]) and 22% lower risk of CV death (HR = 0.78 [95% CI 0.66–0.93]) in patients with T2DM who were at high risk for CVD [40]. A post hoc analysis of this data found that improvements in MACE were only noted in those with prior CV events or established atherosclerotic cardiovascular disease with essentially neutral effects in those with CV risk factors alone [41]. In the SUSTAIN 6 trial, weekly semaglutide was found to reduce the risk of MACE by 26% (HR = 0.74 [95% CI 0.58–0.95]) compared to placebo

though there was no significant reduction in CV death [42]. Instead, this improvement in MACE was driven largely by a 39% relative risk reduction in non-fatal stroke (HR = 0.61 [95% CI 0.38–0.99]) [42]. The CVOT of the only oral GLP-1 RA, a different formulation of semaglutide, showed non-inferiority to placebo with regard to the primary composite outcome of CV death, non-fatal MI, or non-fatal stroke, but there was a significant risk reduction when the secondary outcomes of CV mortality and all-cause mortality were examined individually when compared to placebo [43]. As a result, when the FDA considered this data combined with that from SUSTAIN 6, it approved injectable (though not oral) semaglutide for reduction of MACE in patients with T2DM in the secondary prevention setting. Following a similar pattern to injectable semaglutide, weekly dulaglutide in the REWIND trial was associated with a 12% relative risk reduction in MACE (HR = 0.88 [95% CI 0.78–0.99]) with no impact on CV death but again driven by a 24% risk reduction in non-fatal stroke (HR = 0.76 [95% CI 0.61–0.95]) [44]. Unlike the other GLP-1 RA CVOTs, only a minority of patients in the REWIND trial had established CVD, so the results of this study suggest the benefits of this drug class extend to a primary prevention population. Albiglutide, another weekly injectable, was also associated with improved cardiovascular outcomes in patients with type 2 diabetes and CVD, but this medication was subsequently withdrawn from the market for financial reasons [45]. Exenatide's weekly formulation and daily lixisenatide demonstrated cardiovascular safety when compared to placebo, but there were no improvements in CV outcomes with use of these members of this drug class [46, 47].

Several meta-analyses have further investigated the cardiovascular benefits of this class of agents. In their review of the data from all the available GLP-1 RA CVOTs, Kristensen et al. found that GLP-1 therapy led to a 12% reduction in MACE (HR = 0.88 [95% CI 0.82–0.94]) due to significant reductions in all the component outcomes including cardiovascular death, fatal or non-fatal stroke, and fatal or non-fatal myocardial infarction [48]. There was also a small reduction in heart failure hospitalizations that was surprising as this had not been noted previously with these agents individually [48]. These agents were found to be cardioprotective regardless of baseline cardiovascular status, but the authors caution that the data is not robust enough to strongly recommend the use of these agents in the primary prevention setting [48]. With regard to stroke outcomes, the neuroprotective findings of the SUSTAIN 6 and REWIND trials have been supported by data from subsequent meta-analyses [42, 44]. Kristensen et al. found that treatment with a GLP-1 RA led to a 16% relative risk reduction (HR = 0.84, [95% CI 0.76–0.93]) in fatal or non-fatal stroke [48]. Similarly, another meta-analysis that focused more specifically on the impact of GLP-1 RA on stroke outcomes observed a 15% reduction in the risk of non-fatal stroke (HR = 0.85 [95% CI 0.76–0.94]), 19% reduction in fatal stroke (HR = 0.81 [95% CI 0.62–1.08]), and 16% reduction in total stroke (HR = 0.84 [95% CI 0.76–0.93]) with no heterogeneity across the GLP-1 RA CVOTs [49]. There was no association between the extent of A1c lowering or body weight reduction and these favorable outcomes [49]. Of note, however, an exploratory analysis of the REWIND study found that this stroke-reduction benefit only occurred in

those with ischemic stroke and that A1c reduction accounted statistically for about half of this beneficial effect [50].

The CV benefits that result from GLP-1 RA use are likely due to a variety of mechanisms. Use of GLP-1 receptor agonists leads to amelioration of several traditionally important cardiometabolic risk factors such as hyperglycemia, weight, blood pressure, and lipids which are known to be impactful on long-term CV outcomes. In fact, a mediation analysis of the LEADER trial identified A1c as the primary significant mediator of the improved cardiovascular outcomes associated with liraglutide use, implying that glucose control was an important driver of improved CV outcomes with this agent [51]. However, the cardiovascular benefits observed with this class of agents occur relatively rapidly (i.e., often within 1–2 years) suggesting that risk factor modification alone cannot sufficiently explain the benefits noted with this class. Several other potential mechanisms have been proposed invoking a direct effect of these agents on the cardiovascular system through improvements in endothelial cell function and reduction of vascular inflammation, slowing the progression of atherosclerotic plaque formation in subclinical atherosclerotic disease [52, 53]. With regard to the beneficial impact of these agents on stroke outcomes, pre-clinical trials have observed reductions in infarct volume after treatment with these agents, primarily mediated by decreased neuroinflammation, oxidative stress, and apoptosis, which limits the extent of neuronal damage after an ischemic insult [54, 55].

Of all the currently available glucose-lowering therapies, the GLP-1 RAs are associated with the most significant and consistent weight loss benefit. In fact, liraglutide at a dose of 3.0 mg per day (higher than the recommend anti-hyperglycemic dose) is FDA-approved for the treatment of obesity regardless of diabetes status. Meanwhile, injectable semaglutide appears to be especially promising in this regard and is currently in phase 3 trials as an anti-obesity agent after an earlier dose-finding study found 11.6–13.8% reductions in baseline body weight after 52 weeks of treatment with daily doses of 0.2 mg or higher [56]. These weight reductions are comparable to or greater than that of other currently approved weight loss agents. At these doses of semaglutide, >75% of patients lost more than 5% of their baseline weight with almost 60% losing 10% or more, and the effect of this medication appeared to persist throughout the year-long treatment period rather than plateauing early as other weight loss agents have [56]. The most common side effects with these medications are dose-dependent mild to moderate GI symptoms including nausea, vomiting, and constipation that typically improve over time. These GI effects do not appear to be the primary driver of the weight loss benefits [57, 58]. Rather, these appear to be due to direct actions on the brain to suppress appetite and promote early satiety, reducing overall caloric intake [59].

One drawback to therapy with this class of agents is that most are expensive and are only available as daily or once weekly subcutaneous injections that can be off-putting to those who are leery of self-injections. Semaglutide is also more recently available as a daily oral option but absorption is poor, so current recommendations for taking it on an empty stomach with a small amount of water prior to other oral intake may prove cumbersome to some patients. Although the risk of pancreatitis,

initially a concern with these agents appears to be similar to placebo, there is some data suggesting an increased risk of cholelithiasis with their use [60, 61]. In SUSTAIN 6, treatment with semaglutide was associated with a higher risk of retinopathy complications, but this association has not been redemonstrated in subsequent analyses [62–65]. The worsening noted in this study has therefore been attributed to the rapid tempo of glucose-lowering in these patients, which can result in a transient worsening of disease but which does not translate to long-term progression of retinopathy [63]. These agents appear to have some renoprotective effects as well, but largely through reductions in albuminuria and not on "harder" renal outcomes such as doubling of serum creatinine [40, 42, 44, 48].

Thiazolidinediones

Thiazolidinediones (TZDs) act on the peroxisome proliferator-activated receptors γ (PPAR-γ) nuclear receptor to promote adipocyte differentiation, promote beta cell function, and improve insulin sensitivity in skeletal muscle and adipose tissue (and to a lesser degree in liver).

Pioglitazone has been shown to reduce the risk of cardiovascular events in patients with and without diabetes. The secondary prevention study called the PROactive trial showed that pioglitazone treatment led to a 16% risk reduction in the secondary outcome of MACE (HR = 0.84 [95% CI 0.72–0.98]; $p = 0.027$) in people with T2DM and established CVD [66]. However, because the drug proved neutral for the primary outcome (which included peripheral vascular events), the MACE effect could only be considered hypothesis generating and not conclusive. In further subgroup analyses, PROactive participants with a prior MI experienced a 28% reduction in rates of recurrent MI (HR = 0.72 [95% CI 0.52–0.99]) and those with a history of stroke had a 47% reduction in recurrent stroke (HR = 0.53 [95% CI 0.34–0.85]) [67, 68]. Similarly, in non-diabetic but insulin-resistant patients who recently had a TIA or stroke, the IRIS trial found a 24% reduction in fatal/non-fatal stroke or MI (HR = 0.76 [95% CI 0.62–0.93]) [69]. Planned secondary analyses of this study investigated these component outcomes in more detail and found that treatment with pioglitazone in this insulin-resistant secondary prevention cohort led to a 25% reduction in stroke at 5 years (HR = 0.75 [95% CI 0.60–0.94]), a 29% reduced risk of acute coronary syndrome (ACS) (HR = 0.71 [95% CI 0.54–0.94]), and a 38% reduction in type 1 spontaneous MI (HR = 0.62 [95% CI 0.40–0.96]), effect sizes that are comparable to the benefits seen with more widely used stroke preventative agents such as statins, aspirin, and anti-platelet therapy [70–72]. Supporting the data from these randomized control trials, several meta-analyses have found reductions in MACE associated with pioglitazone use in a broad population of patients, including those with insulin resistance but without overt diabetes [73–75]. Additionally, large-scale studies of pioglitazone use in the real-world setting have demonstrated decreased mortality when compared to alternative treatments such as insulin [76, 77]. Of course, comparing any drug to insulin is

confounded by indication, as those treated with insulin tend to have a more complex medical history and often a longer duration of disease. Adjustments for these factors, including propensity scores, can render the comparisons more balanced but may not fully account for all differences.

By contrast, the cardiovascular safety data has been decidedly less promising with rosiglitazone. In fact, a 2008 meta-analysis by Nissen et al. found that the odds ratio for MI was 1.43 (95% CI 1.03–1.98; $p = 0.03$) and the odds ratio for death from CV causes was 1.64 (95% CI 0.98–2.74; $p = 0.06$) for rosiglitazone when compared to placebo, providing some of the impetus for the FDA's subsequent directive mandating cardiovascular outcome trials prior to approval of future glucose-lowering agents [78]. However, RECORD, an unblinded trial looking at both primary and secondary CV prevention, compared the addition of rosiglitazone or placebo to a background of sulfonylurea/metformin combination therapy and did not demonstrate any increased risk of cardiovascular mortality—but also no benefit [79].

Although pioglitazone has clearly shown some promising potential benefits in secondary prevention of atherosclerotic cardiovascular disease (ASCVD) and stroke, concerns about an increased risk of heart failure with use of these agents have tempered the enthusiasm for this class and somewhat limited their widespread use. Pioglitazone promotes VEGF production by the smooth muscle cells to increase vascular permeability and vasodilation, which when coupled with a reduction in urinary sodium excretion by the kidneys leads to fluid retention [80]. Despite the fact that this edema is unlikely to be the result of a direct deleterious effect of pioglitazone on ventricular function, randomized control trials such as the PROactive and RECORD trials both noted increased risk of heart failure in the TZD arm of their respective studies with the RECORD trial (unlike PROactive) finding excess deaths related to heart failure as well [66, 79]. Although the IRIS study (in which dose reduction was allowed for edema and weight gain) found no increase in heart failure in the pioglitazone group as compared to placebo, several meta-analyses have echoed the findings of PROactive and RECORD by demonstrating a significantly increased risk of heart failure with use of this medication class [73, 74, 81, 82]. Although there very well could be some misattribution of medication-associated edema to true heart failure, patients should be carefully evaluated for heart failure risk prior to starting therapy with these agents.

One potential mechanistic reason why pioglitazone use could be associated with decreased risk of ASCVD and stroke involves its impact on various components of the so-called metabolic syndrome. Improving insulin resistance and preserving beta-cell function ameliorates hyperglycemia, and shifting fat from visceral depots to subcutaneous areas reduces lipotoxicity [72]. Pioglitazone has also been associated with reduced rates of progression of carotid intimal media thickness (a surrogate marker of CV risk) and coronary atherosclerosis likely through direct effects on the vasculature itself [83–85]. These direct effects could be mediated through the PPARγ receptors found in endothelial, smooth muscle and immune cells where pioglitazone can lead to downstream anti-inflammatory and antioxidant effects that can reduce atherosclerotic plaque formation [72].

Pioglitazone is a very low-cost anti-hyperglycemic agent with a durable glucose-lowering effect. The two most common adverse effects are weight gain (typically around 2–3 kg) and peripheral edema which are both dose-dependent and can be difficult for patients to tolerate [72]. As discussed previously, the latter side effect is driven by sodium retention at the level of the distal tubule in the kidney, so medications such as spironolactone, triamterene, and amiloride might ameliorate this effect. However, all TZDs should be avoided in patients with decompensated heart failure [72]. This class of agents has also been associated with an increased risk of fracture, especially in women, and so should be avoided in those at high risk for fracture [69, 79, 86, 87]. An interim analysis of the PROactive trial prompted some concerns after it found a non-significant increase in the number of cases of bladder cancer in the pioglitazone arm of the study, but this association was not redemonstrated in the full 10-year follow-up of the PROactive trial [66, 88]. The data since then has remained mixed with two randomized control trials and at least two other large cohort studies not demonstrating an increased risk of bladder tumors with use of thiazolidinediones, while a number of other studies (particularly several meta-analyses) continue to demonstrate a small increased absolute risk of bladder cancer with these agents [69, 79, 89–94]. Given this ongoing controversy, the potential risk of bladder tumors should be discussed with patients and taken into consideration when using these agents.

Dipeptidyl Peptidase-4 Inhibitors

Drugs in this class inhibit dipeptidyl peptidase-4 (DPP-4) from breaking down endogenous incretins such as GLP-1 thereby augmenting GLP-1's previously noted beneficial downstream effects on insulin secretion. All the CVOTs for this class of agents (i.e., SAVOR-TIMI 53, EXAMINE, TECOS, and CARMELINA) demonstrated CV safety with no improvement in cardiovascular outcomes when compared to placebo [95–98]. SAVOR-TIMI 53 trial found a 27% increased rate of hospitalization for heart failure with saxagliptin when compared to placebo (HR = 1.27 [95% CI 1.07–1.51]) in their mixed primary/secondary prevention population of patients with T2DM, but this association has not been demonstrated in other members of this class [95]. Several meta-analyses have supported the neutral impact on cardiovascular outcomes of these agents [99–101].

These are generally well-tolerated agents associated with minimal hypoglycemia risk but also only have modest glucose-lowering potential. Unlike the related GLP-1 agonist class, these less potent agents are not associated with GI symptoms and are weight-neutral. Given the overlap in mechanism, DPP4i should not be used in conjunction with GLP-1 agonists. Although the potential mechanism is unknown, as mentioned previously, saxagliptin use was associated with an increased rate of heart failure hospitalization and so should be avoided in those with heart failure. Although the association between these medications and the risk of acute pancreatitis is somewhat inconsistent across various studies, there is enough of a safety signal to

recommend avoiding this medication in patients at risk for pancreatitis [102–107]. Lastly, these agents are also quite costly.

Older Agents: Metformin, Sulfonylureas, and Insulin

Cardiovascular safety data are more limited in some of our oldest glucose-lowering therapies as CVOTs were not mandated by the FDA for these agents. However, the data available largely supports the cardiovascular safety of these therapies.

Metformin decreases hepatic gluconeogenesis and improves insulin sensitivity. It is highly efficacious, low cost, has a low hypoglycemia risk, and promotes modest weight loss, making it an attractive first-line therapeutic agent for many practitioners. In a cohort of overweight patients from the UK Prospective Diabetes Study (UKPDS), metformin use was associated with a 32% lower risk of the composite diabetes outcome (which included major macrovascular complications such as MI) (HR = 0.68 [95% CI 0.53–0.87]) and a 36% reduction in all-cause mortality (HR = 0.64 [95% CI 0.45–0.91]) when compared to the "conventional therapy" arm which largely consisted of dietary counseling with the addition of sulfonylurea or insulin therapy if hyperglycemia developed [8]. In the 10-year follow-up study, though glycemic differences between the two groups were lost after 1 year, a significant risk reduction in the composite diabetes outcome, myocardial infarction, and all-cause mortality was retained in the overweight patients who had previously been intensively treated with metformin [14]. Additionally, the results of the meta-analysis by Lamanna et al. also support the cardiovascular safety of metformin, finding potential benefit of metformin when compared to placebo or no treatment and no impact on CV outcomes in active comparator trials [108]. A more recent large-scale retrospective cohort study of US veterans with diabetes and impaired kidney function found a decreased risk of MACE with metformin use when compared to sulfonylurea therapy [109].

Metformin can lead to bothersome diarrhea that does improve over time and can be alleviated by taking the medication with food or as an extended-release formulation. However, in a small minority of patients, this adverse effect, along with associated abdominal pains and gas, is poorly tolerated and a reason for patient non-adherence or discontinuation. There is also a risk of lactic acidosis in decompensated heart failure and advanced CKD, so this agent should be avoided in those populations. B12 Deficiency can also develop after long-term use of this medication and should be monitored periodically.

Sulfonylureas are low-cost agents that are potent in their glucose-lowering ability. They work by increasing insulin secretion from the pancreatic beta cell so as a result they have similar side effects as insulin therapy including weight gain and hypoglycemia risk. There has historically been some concern about the cardiovascular safety of sulfonylureas due to their inhibition of the ATP-sensitive potassium channels that are present on the myocardium as well as in the pancreatic beta cell. These channels play an important role in ischemic preconditioning, a means by

which the myocardium can adapt to an ischemic insult and limit the extent of the resulting damage [110]. Some early data indicating a possible increased cardiovascular risk with an older sulfonylurea was echoed in some subsequent meta-analyses which found an increased risk of cardiovascular mortality with sulfonylurea use when compared to other glucose-lowering agents such as metformin [111, 112]. However, these concerns have largely been assuaged by the results of the CAROLINA trial which included over 6000 adults with T2DM with CV risk factors or a history of CVD and analyzed the outcomes of treatment with linagliptin versus the sulfonylurea glimepiride [113]. There was no difference in rates of MACE, all-cause death, CV death, or HHF between the two groups despite an expected and significant increase in the risk of hypoglycemia with sulfonylurea therapy [113]. Given that the CARMELINA trial found that linagliptin was non-inferior to placebo with regard to CV outcomes, it can reasonably be extrapolated that sulfonylureas (or at least glimepiride) have neutral effects on cardiac outcomes as well [98].

With regard to insulin, information about CV risk is hard to extract from the available data as insulin is often added on to other agents and typically reserved for more advanced stages of diabetes. Mechanistically, there are some data to suggest insulin might have some anti-inflammatory properties that could promote and improve endothelial function [114, 115]. In terms of data from large-scale clinical trials, the UKPDS found that the group treated with sulfonylurea or insulin had similar macrovascular outcomes as those in the diet-control group, and treatment with these agents did not lead to the benefits noted with metformin use in this study [8, 116]. Some more recent data from two large trials has lent credence to the hypothesis that insulin therapy is likely safe from a cardiovascular perspective. The ORIGIN trial, for example, found that the basal insulin glargine had no impact on cardiovascular outcomes when compared to standard care despite increases in weight gain and hypoglycemia [117]. The newer basal insulin degludec was shown to be non-inferior to glargine with respect to CV outcomes and associated with a lower hypoglycemia risk [118]. Insulin is of course essentially limitless in its glucose-lowering ability. In addition to issues with weight gain and hypoglycemia, insulin is an injectable agent that may also contribute to some reluctance from patients when initiating this therapy.

Despite being much less commonly used, meglitinides are similar to sulfonylureas, both in their mechanism of glucose-lowering (albeit with a shorter duration of action) and in their apparent cardiovascular neutrality [119]. Pramlintide is an amylin mimetic that is an injectable agent that can be used as an adjunctive therapy in patients requiring prandial insulin. From a cardiovascular perspective, this is likely to be a safe therapy, but as it cannot be mixed with insulin, the extra injections per day can be difficult to tolerate for most patients [120]. Several other older classes of glucose-lowering agents including alpha-glucosidase inhibitors, bile acid sequestrants, and dopamine agonists are rarely used for glycemic management due to their limited efficacy and/or their side effect profile.

Implementing a Personalized Treatment Strategy in Patients with CVD

The wealth of good-quality cardiovascular safety data for the newest classes of diabetes agents has led to a paradigm shift in the focus of diabetes care. Although glucose control is still important in mitigating the risk of microvascular disease, these trials have diminished the relevance of tight glucose control in addressing the profound impact of macrovascular disease in this population. Given the clear and important cardiovascular advantages afforded by certain classes of glucose-lowering drugs, some of the most recent guidance on pharmacologic therapy in diabetes management has emphasized the importance of early adoption of these agents in the care of patients with T2DM. For example, the 2021 ADA's Standards of Medical Care in Diabetes and the 2019 Update to the ADA-EASD Consensus Report recommend that GLP-1 receptor agonists and/or SGLT2 inhibitors should be used in patients at high risk of CVD events, irrespective of hemoglobin A1c values or targets [121, 122]. High-risk patients include those with established CVD (i.e., those with a history of myocardial infarction, ischemic stroke, unstable angina, abnormal stress test, or any revascularization procedure), CKD, or heart failure and those 55 years or older with >50% stenosis of any artery, left ventricular hypertrophy, eGFR <60 mL/min or albuminuria. Another similar approach put forth by the European Society of Cardiology and the European Association for the Study of Diabetes recommends risk stratifying patients based on the presence of preexisting ASCVD or microvascular complications, diabetes duration, and the burden of traditional metabolic risk factors such as hypertension and hyperlipidemia [123]. In these guidelines, for patients deemed to be highest risk for cardiovascular events, SGLT2i, or GLP-1 RA are recommended as first-line therapy even before metformin. The American College of Cardiology Guidelines Expert Consensus Decision Pathway also recommends early use of these agents in patients with T2DM at high risk for CVD [124].

It is essential to then tailor the treatment approach based on the particular cardiovascular disease process that is of most concern in each individual patient (Fig. 37.1). For example, SGLT2i should be prioritized in patients with heart failure or nephropathy given the robust improvement in these particular outcomes afforded by medications in this class. TZDs and saxagliptin would be best avoided in those with heart failure. By contrast, for those with a history of ASCVD including stroke, GLP-1 RA (particularly liraglutide, dulaglutide, and semaglutide) would be preferred with strong consideration of pioglitazone as well given its clear benefits in the stroke population in particular. This is especially true as the weight gain and fluid retention caused by TZDs might even be ameliorated by dual therapy with GLP-1 RA (weight) or SGLT2i (weight, edema).

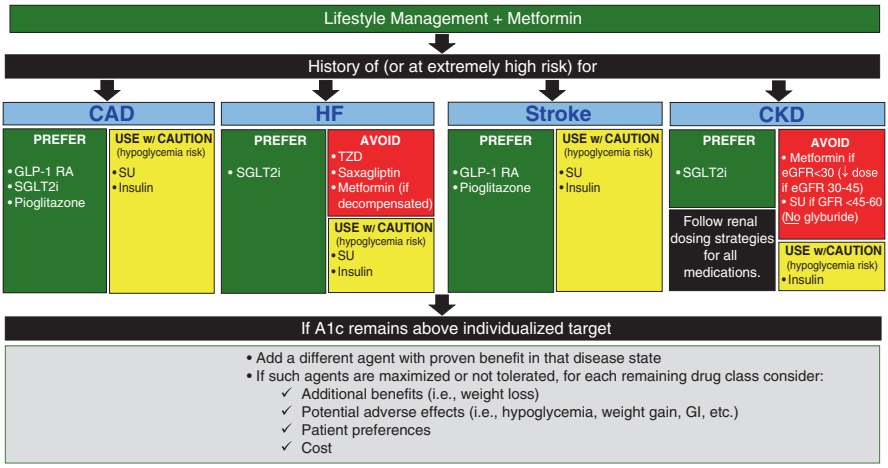

Fig. 37.1 Proposed approach to glucose-lowering in T2DM patients with CVD and/or CKD

Although the emphasis has shifted away from stringent glycemic targets in this population, a glycemic target around 7% if achievable without hypoglycemia remains a reasonable goal, mainly to prevent microvascular disease. Of course, the life expectancy and prevalent comorbidities of the individual patient need to be considered as well. Prevention of hypoglycemia is important in this population to avoid exacerbating the risk of arrhythmias or ischemia. When additional glucose-lowering is needed, the choice of subsequent agents should continue to prioritize the use of agents that have demonstrated cardiovascular benefits while weighing the practicalities surrounding use of the medication as well as the non-cardiovascular risks and benefits (Table 37.2).

Table 37.2 Risks and benefits of most commonly used glucose-lowering drug classes

Class of agent	CV advantages	CV risks	Non-CV benefits and risks
SGLT2i *Canagliflozin* *Dapagliflozin* *Empagliflozin* *Ertugliflozin* *Sotagliflozin* (*SGLT1/SGLT2i*)	Decreased CV mortality (empagliflozin) Decreased MACE (empagliflozin, canagliflozin, sotagliflozin) Decreased HF hospitalizations (all) Low hypoglycemia risk Modest decrease in BP and increase in HDL		*Benefits* Weight loss Decreased nephropathy *Risks* High cost Dehydration Increased risk of GU infections Increased risk of DKA Diarrhea (sotagliflozin) Amputation risk? (canagliflozin)
GLP-1 RA *Dulaglutide* *Exenatide* *Liraglutide* *Lixisenatide* *Semaglutide*	Decreased CV mortality (liraglutide) Decreased MACE (liraglutide, semaglutide, dulaglutide) Decrease in non-fatal strokes (semaglutide, dulaglutide) Low hypoglycemia risk	Increase HR by 2–3 beats/min	*Benefits* Weight loss Decreased nephropathy *Risks* Pancreatitis risk? Cholelithiasis risk? Retinopathy? (semaglutide) High cost Injectable
TZDs *Pioglitazone* *Rosiglitazone*	Decreased MACE (pioglitazone) Decreased stroke (pioglitazone) Low hypoglycemia risk	Increased HF risk? (pioglitazone, rosiglitazone)	*Benefits* Low-cost Improvement in NASH *Risks* Weight gain Edema Bladder cancer?
DPP-4i *Alogliptin* *Linagliptin* *Saxagliptin* *Sitagliptin*	Low hypoglycemia risk	Increased HF risk? (saxagliptin)	*Benefits* Weight neutral *Risks* High cost Pancreatitis risk?
Sulfonylureas *Glimepiride* *Glipizide* *Glyburide*		Increased risk of hypoglycemia	*Benefits* Low cost *Risks* Weight gain

(continued)

Table 37.2 (continued)

Class of agent	CV advantages	CV risks	Non-CV benefits and risks
Metformin	Potential ASCVD benefit Low hypoglycemia risk	Lactic acidosis risk in decompensated HF	*Benefits* Low cost Weight neutral (or loss) *Risks* GI upset B12 deficiency
Insulin		Increased risk of hypoglycemia	*Benefits* Unlimited glucose-lowering effect *Risks* Weight gain Injectable

ASCVD atherosclerotic cardiovascular disease, *BP* blood pressure, *CV* cardiovascular, *DPP-4i* dipeptidyl peptidase-4 inhibitors, *DKA* diabetic ketoacidosis, *GI* gastrointestinal, *GLP-1 RA* GLP-1 receptor agonists, *GU* genitourinary, *HDL* high-density lipoprotein, *HF* heart failure, *HR* heart rate, *MACE* major adverse cardiac events, *NASH* non-alcoholic steatohepatitis, *SGLT1* sodium–glucose cotransporter 1, *SGLT2i*, sodium–glucose cotransporter 2 inhibitors, *TZDs* thiazolidinediones

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Index

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