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Alicia J. Jenkins
Peter P. Toth *Editors*

Lipoproteins in Diabetes Mellitus

Second Edition

Humana Press

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The **Contemporary Diabetes** series focuses on the clinical aspects of obesity and diabetes and provides the practicing health provider with all the latest information regarding their management. The series also targets both basic and clinical researchers. The audience includes endocrinologists, internists, cardiologists, neurologists, nephrologists, podiatrists, ophthalmologists, family physicians, nurse practitioners, nurse educators, and physician assistants.

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Editors

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This book is dedicated to Professor Richard Louis Klein, Ph.D. (1951–2020). For most of his professional career, Richard (Rick) was an academic and Research Scientist in the Veterans Affairs Department and at the Medical University of South Carolina in Charleston, South Carolina, USA. He was a valued and dear colleague, mentor, and friend to many authors of the book chapters herein. Rick updated his three chapters in this second edition, also involving Andrea Semler, who researched with him in the laboratory for over two decades and whom he supervised for her master's degree. Rick was a highly intelligent man, a skilled basic scientist with excellent knowledge of both the laboratory and clinical aspects of lipoproteins. He was a quiet achiever, whose research and mentoring significantly advanced knowledge in lipoproteins, diabetes, and vascular medicine. Rick's legacy also includes his family, many colleagues, and friends from around the world, and many treasured memories of his and his wife Karen's hospitality, kindness, and capacity for friendship and fun.

Preface

There is an ongoing pandemic of diabetes mellitus. Globally an estimated 537 million adults (1 in 10 adults) and over 1.2 million children live with diabetes, with approximately 80% of affected people being in disadvantaged regions. Almost 1 in 2 (240 million) adults with diabetes are undiagnosed (International Diabetes Federation Atlas, 10th edition, 2021). All people with Type 1 or Type 2 diabetes are at risk of acute and chronic complications, with the latter including micro- and macro-vascular damage. Globally diabetes is the commonest cause of adult-onset vision loss, a common cause of kidney failure, peripheral and autonomic neuropathy, lower limb amputations, and of accelerated atherosclerosis. Both quantitative and qualitative changes in lipoproteins are contributory to these devastating complications. As Elliott P. Joslin (1869–1962), the first doctor in the USA to specialize in diabetes, said in 1928, "People with diabetes die of too much fat: Too much fat in the diet, in the blood, in the body and in the blood vessels." Just over a century after the discovery of insulin, enabling life for people with Type 1 diabetes and improving health outcomes for many people with Type 2 diabetes and women with gestational diabetes, this statement is still relevant. Fortunately, we now have much more knowledge regarding lipoproteins in people with diabetes, more clinical and research laboratory-related tests, many more effective treatments to reduce the adverse effects of lipoproteins, and greater capacity to detect and treat the chronic complications of diabetes. The field continues to advance.

Over 8 years has passed since the first (2014) edition of this book, *Lipoproteins in Diabetes Mellitus*. Further knowledge, new tests, new treatment strategies and therapies, including lifestyle and lipid-related therapies to reduce the burden of diabetes complications are now available. This even more comprehensive second edition includes chapters for clinicians, clinician researchers, and basic scientists. There are chapters on lipoprotein metabolism, relevant cell biology, the pathobiology of lipid-related neurovascular damage, clinical and research tests of lipoproteins, clinical trials, treatment strategies, and existent and emerging lipid-related therapies. An expert group of senior authors from many different countries and fields have voluntarily shared their knowledge and time, often co-authoring with emerging leaders in this important field. Chapters have been updated and many new

chapters added. New topics include: the epidemiology of diabetes and of lipid disorders in diabetes, the roles of lipoproteins and lipid therapies in diabetic peripheral neuropathy, stroke and peripheral vascular disease, the bidirectional links between lipoprotein and glucose metabolism, lipoprotein dysfunction in diabetes, lipid treatment in people with Type 1 diabetes, and detailed chapters on novel therapeutics including PCSK9 inhibitors. Each chapter includes an abstract, summary, key tables and/or figures, suggested research directions, and relevant references.

We hope this book will serve the readership well, helping clinicians provide the best possible care for their patients living with diabetes and helping basic scientists and clinical trialists develop and test the next generation of effective lipoprotein-related therapies. Each of these approaches is key to improving health outcomes for people with diabetes and reducing the great personal and socioeconomic burden of diabetes. We encourage readers to also advocate for equitable access to proven treatments for all people with diabetes who may benefit.

Sydney, NSW, Australia — Alicia J. Jenkins
Baltimore, MD — Peter P. Toth

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Part I
Lipoprotein Metabolism, Qualitative Changes and Measurements

Chapter 1
Laboratory Assessment of Lipoproteins in Type 2 Diabetes

David R. Sullivan

Abbreviations

ApoB	Apolipoprotein B
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein(a)
NHDL-C	Non-high-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglycerides
TRL	Triglyceride-rich lipoproteins

Introduction

Lipids, Lipoproteins, and Other Analytes in Diabetes

Type 1 and type 2 diabetes are often regarded as abnormalities of insulin and glucose metabolism, but it is more appropriate to recognize that they disrupt the pathophysiology of macronutrient metabolism as a whole. Accordingly, it is

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essential to recognize the effects of diabetes on the other major class of macronutrients, namely lipids. The fundamental differences in the pathophysiology and treatment of type 1 and type 2 diabetes are manifest in the changes in lipoprotein metabolism that accompany these two forms of diabetes. Consequently, the role of altered lipoprotein metabolism in the atherosclerotic process that underlies macrovascular complications may differ. Fully treated type 1 diabetes often causes minimal disturbance to the lipoprotein profile, in fact the level of triglycerides may be slightly decreased and that of HDL-C may be slightly increased in insulin-treated patients partly due to activation of lipoprotein lipase due to supraphysiologic levels of insulin [1, 2]. Nevertheless, non-enzymatic glycation of the apolipoprotein component of lipoproteins [3], as well as other modifications, may render lipoproteins dysfunctional in type 1 diabetes. Consequently, the atherogenicity of the diabetic state in type 1, combined with the early age of onset, results in an increased life-long risk of CVD that demands efforts to maintain lipoproteins at target levels or better [4]. This may be difficult to achieve in the face of complications of type 1 diabetes such as renal impairment, obesity, poor glucose control, or the need for immune-suppressive therapy subsequent to organ or islet transplantation. Hypercholesterolemia may occur in type 1 diabetes in association with severe chronic hyperglycaemia [5]. Furthermore, as insulin is required for the action of lipoprotein lipase, in the setting of newly diagnosed type 1 diabetes prior to insulin therapy or with insulin omission or diabetic ketoacidosis, massive hypertriglyceridemia can occur [6].

Type 2 diabetes, on the other hand, is associated with a well characterized disturbance of the lipoprotein profile which features mild to moderate increase in triglyceride-rich lipoproteins (TRL), reduced HDL-C, and modification of LDL particle composition. Type 2 diabetes is becoming increasingly prevalent in the setting of increased dietary energy intakes and reduced physical activity levels in affluent and disadvantaged societies, so it will be the major focus of attention here.

Lipid abnormalities manifest as disturbances of the levels of the lipoproteins that transport lipids in the bloodstream. These disturbances may contribute to the macrovascular complications of diabetes by influencing the processes that underlie atherosclerosis and thrombosis. Less frequently, they may lead to massive increase in TG that greatly increase the risk of acute pancreatitis with associated loss of beta cell function and pancreatic exocrine function. Evidence suggests that disturbances in lipoprotein metabolism may also contribute to the microvascular complications of diabetes such as renal impairment, retinopathy, and neuropathy, which is discussed in other chapters in this book, however the relevant mechanisms are not yet fully elucidated [7].

The laboratory assessment of lipoprotein status in diabetes relies on minimization of the effect of potential confounding factors. Sample collection procedures are designed to reduce pre-analytical sources of variability [8]. One of the most important sources of variability is the presence of intercurrent illness because the associated inflammatory response mediates modifications of the lipid and lipoprotein profile which share many of the features of those associated with type 2 diabetes, as will be described later. The magnitude of modifications associated with an inflammatory response is usually proportional to the severity of the underlying illness [9],

but proportionately smaller responses should also be anticipated in association with minor intercurrent episodes [10].

Routine Lipoprotein Assessment

Clinical evaluation of lipoprotein metabolism in diabetes usually involves the measurement of total cholesterol, HDL-C and TG following a 12-h fast. LDL-C is derived from the fasting results by application of the Friedewald equation [11], but this calculation becomes less reliable in the setting of diabetes [12] and as TG levels increase beyond approximately 4 mmol/L (350 mg/dL). Modified calculations have been developed, but these are complex, relying on computation rather than mental arithmetic [13].

Sustained attention to standardization and quality assurance have established a high level of reliability [8] for routine lipid measurements [14]. Satisfactory analytical performance by clinical laboratories is sustained by well-established systems of internal and external quality assurance [15, 16]. These processes have been extended to include apolipoproteins, most importantly apoB [14] and Lp(a) [17].

Non-fasting plasma or serum has been shown to be a more sensitive marker for the detection of individuals with increased risk of CVD [18], but the un-standardized nature of non-fasting samples makes them unsuitable for the characterization or serial monitoring of lipid status in diabetes. Indeed, even fasting TG levels show considerable within-individual variability [19]. This has implications for the serial measurement of LDL-C which is calculated from the fasting TG. The considerable biological variability of fasting TG increases the proportion by which a serial measurement of fasting TG (and hence LDL-C) must differ in order to indicate a clinically significant alteration [19].

Automated "direct" HDL-C measurements may suffer interference from the cholesterol content of VLDL and remnants, resulting in a positive bias [20]. Method comparison studies prior to 2000 suggested good agreement between "separation" HDL-C methods and the reference method [21], even in the presence of Intralipid [22] or TRL [23]. Where positive bias occurred, it was attributed to incomplete precipitation with the comparator method [24, 25] or the presence of apolipoprotein E-containing HDL [26], but the sources of TG used in these studies had a relatively low cholesterol content. "Direct" HDL methods initially involved the use of α-cyclodextrin, and positive interference from TRL was described in some [27], but not all [24] studies. Since methods involving α-cyclodextrin have been superseded, several recent studies of "direct" HDL methods have reported positive biases which were attributed to TRL [20] or the presence of diabetes [28]. This is an important issue because any overestimation of HDL-C risks underdiagnosis of the metabolic syndrome and insulin resistance, as well as underestimation of LDL-C and NHDL-C. These combined effects could result in a substantial underestimation of absolute risk of CVD, leading to loss of opportunity to effectively identify and treat patients on the basis of their metabolic risk factors. It is possible that TRL may also interfere with "direct" LDL-C assays that utilize a similar strategy based on selective effect of detergents [29].

The accuracy of standard lipid measurements is extremely important because this quantitative information is applied directly to patient management. The atherogenic effects of LDL-C and other apoB-containing lipoproteins such as Lp(a) represent independent risk factors for CVD. Whereas LDL-C (or TC) originally provided thresholds for initiation of treatment and targets for intervention, management decisions are now seen in a wider context that encompasses the overall (absolute) CVD risk of the individual patient. This incorporates the classic modifiable and non-modifiable risk factors to varying extents. The predominance of age is one of several inevitable limitations affecting the performance of the absolute risk calculation algorithms. Diabetes is no longer regarded as a "coronary risk equivalent," but rather the presence or absence of diabetes is treated as a categorical variable, usually without adjustment for severity. The presence of pre-diabetes or impaired glucose tolerance is associated with increased CVD risk relative to the non-diabetic population, but is not associated with microvascular complications. Clinical uncertainty associated with intermediate levels of CVD risk has led to efforts to "re-classify" patients in this category by a variety of methods. Some algorithms allow adjustment for factors such as ethnicity, duration of diabetes, HbAIc level, and the presence or absence of kidney damage such as microalbuminuria or eGFR loss, but most do not consider more than the presence or absence of diabetes, nor do they usually consider pre-diabetes which is associated with high risk of CVD [30]. While the additional CVD risk posed by the presence of diabetes or pre-diabetes often justifies active management of the lipid profile, clinicians need to remember that the presence of massive hypertriglyceridemia (>10 mmol/L, 880 mg/dL) poses a more immediate risk of acute pancreatitis.

LDL Composition and Particle Number

A clinical approach based purely on quantitative assessment of LDL-C and/or TC:HDL-C ratio is inappropriate, particularly in the presence of elevated TG, which often applies in the case in type 2 diabetes. Increased levels of TRL promote the action of cholesteryl ester transfer protein (CETP) which leads to a reduction in HDL-C and a depletion in the amount of cholesterol carried per LDL particle. These changes in LDL composition are proportional to the degree of postprandial lipemia [31, 32] which usually correlates with fasting triglyceride levels.

The relationship between LDL-C and CVD risk [33] can be confounded because the formation of "small dense LDL" may result in an LDL-C level that is low relative to the number of LDL particles. This is illustrated by the superiority of other risk markers such as NHDL-C (calculated as the difference between TC and HDL-C) which reflects the full range of potentially atherogenic lipoproteins [34]. This superiority is thought to reflect the greater atherogenicity of the "small dense LDL" and hence the pre-eminence of particle number as the main determinant of the pro-atherogenic associations of non-HDL lipoproteins. Direct measurement of LDL-C traditionally relied on quantitative ultracentrifuge studies which are too tedious to perform for clinical purposes [35]. Electrophoresis based on sizing gel techniques has attempted to circumvent this problem, leading to designation of

so-called pattern A and pattern B profiles or estimations of LDL diameter. These methods are non-quantitative with respect to the number of atherogenic lipoprotein particles, so their clinical value is only marginal. Vertical ultracentrifugation has introduced another option for quantitative assessment of the spectrum of atherogenic lipoproteins, though this is not widely available as a clinical tool [36]. Detailed analysis from the CARE trial demonstrated that the apoC3 levels in VLDL and LDL were superior to TG for the prediction of CVD risk [37]. Subsequent Mendelian randomization studies have supported the development of anti-apoC3 small interfering RNA therapy as a treatment for elevated TG and the risk of CVD.

An alternative approach to NHDL-C is based on the measurement of serum apoB level [38]. All particles that are capable of transporting cholesterol in a pro-atherogenic manner, such as LDL, Lp(a), and VLDL remnants contain one apoB molecule. As such, apoB provides a direct measurement of the number of atherogenic lipoprotein particles. Human apoB derived from the intestine is the product of post-transcriptional modification (m-RNA editing) that yields a product that consists of the first 48% of the non-intestinal apoB. The two gene products are designated apoB48 and apoB100, respectively. ApoB levels do not change markedly after a meal because the transport of dietary fat is largely accommodated by an increase in TG content per ApoB48 particle, rather than an increase in total apoB. This also reflects the fact that the number of apoB100 particles is large relative to the number of apoB48 particles. Hence apoB measurement need not depend on fasting or the ability to differentiate the apoB100 isoform. Evidence suggests that apoB measurement is superior to LDL-C or NHDL-C for CVD risk assessment [39]. When combined with LDL-C measurement, the LDL-C:ApoB ratio can reflect the degree to which cholesterol depletion of LDL has led to the formation of "small, dense LDL" [40]. Tables 1.1, 1.2, and 1.3 are provided as a means of including apoB measurement as a guide to diagnosis.

Table 1.1 An algorithm for the prediction of the likely class of lipoproteins responsible for dyslipidemia in approximate order of prevalence in type 2 diabetes

Apolipoprotein B level (g/L)	TG >1.5 mmol/L × mg/dL (Y/N)	TG:ApoB ≥10 in mmol/L or >y in mg/dL (Y/N)	TC:ApoB ≥6.2 in mmol/L or ≥ in mg/dL (Y/N)	Lipoprotein accumulation
ApoB <1.2	N	N	N	Normal
ApoB <1.2	Y	N	N	VLDL
ApoB ≥1.2	Y	N/A	N/A	LDL and VLDL
ApoB ≥1.2	N	N/A	N/A	LDL
ApoB ≥0.75–1.2	Y	Y	N	Chylomicron and VLDL
ApoB <1.2	Y	N	Y	IDL or "remnants"
ApoB <0.75	Y	Y	N	Chylomicrons alone

Adapted from de Graaf et al. [23]

Table 1.2 Causes of secondary dyslipidemia, including diabetes

Excess lipoprotein accumulation	Secondary causes
VLDL	Type 2 diabetes Obesity/insulin resistance Chronic renal impairment Hemodialysis Alcohol excess Estrogen use Glucocorticoid use Retinol analogues Other
LDL and VLDL	Type 2 diabetes Obesity/insulin resistance Cholestasis Nephrotic syndrome CAPD Systemic lupus erythematosus Polycystic ovary syndrome Glucocorticoid use HIV and HAART use Antipsychotic drug use Pregnancy Other
LDL	Nephrotic syndrome Hypothyroidism Anabolic steroids Other
Chylomicron and VLDL	Type 2 diabetes Obesity/insulin resistance Chronic renal impairment Alcohol excess Estrogen use Glucocorticoid use Pregnancy Other
IDL or “remnants”	Triggered or exacerbated by Type 2 diabetes Obesity/insulin resistance Chronic renal impairment Alcohol excess Estrogen use Glucocorticoid use Other
Chylomicrons alone	Acquired apoC2 deficiency in systemic lupus erythematosus

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive analytical technique that does not require lipoprotein isolation that may be used to reflect the physical composition of lipoprotein particles, particularly their size and number. Consequently, NMR spectroscopy has been used to provide a more detailed picture of lipoprotein size distributions, including HDL species. Studies suggest that this

Table 1.3 Primary causes of dyslipidemia which may coexist with diabetes

Excess lipoprotein accumulation	Primary causes
VLDL	Polygenic gene/environment interactions Familial hypertriglyceridemia
LDL and VLDL	Polygenic gene/environment interactions Hyperapobetalipoproteinemia preferred instead of "familial combined hyperlipidemia"
LDL	Polygenic gene/environment interactions Familial hypercholesterolemia
Chylomicron and VLDL	Polygenic gene/environment interactions Exacerbation of familial hypertriglyceridemia or familial hyperchylomicronemia
IDL or "remnants"	Dysbetalipoproteinemia
Chylomicron	Familial hyperchylomicronemia

technique may provide additional benefit in terms of the clinical assessment of lipoprotein-associated CVD risk [41], particularly via NMR measurement of LDL particle number [42].

Etiological Assessment

The clinical implications of dyslipidemia depend on the type of lipoprotein responsible for the alteration in lipid levels and the etiological reason for such accumulation. The atherogenic effect of various lipoproteins may differ depending on the pathophysiological context in which they arise, and it should not be assumed that the lipoprotein profile in diabetes is solely and necessarily based on that condition alone. Other secondary causes, such as obesity, renal disease, liver disease, or hypothyroidism, may modify the lipoprotein abnormality while intercurrent primary lipoprotein disorders may influence or even dictate the lipoprotein profile.

Tables 1.1, 1.2, and 1.3 provide a framework for diagnostic considerations that may modify clinical management. The first step in this process is consideration of which lipoprotein class is responsible for any dyslipidemia in a diabetic patient. While this may be inferred from the results of the automated tests, the underlying pattern cannot be guaranteed in all cases. Traditionally, identification of the accumulating lipoproteins was achieved by lipoprotein electrophoresis. The technique provided the basis of the Fredrickson classification, but this non-quantitative approach does little to enhance prognostic assessment. Tables 1.1, 1.2, and 1.3 present an extension of the use of apoB measurement to provide lipoprotein information in a semi-quantitative and potentially more useful format [38]. The different lipoprotein patterns are presented in approximate order of their prevalence in type 2 diabetes, but as will be explained, the first three are somewhat interchangeable. Many patients with diabetes have normal lipoprotein profiles but the most common pattern of diabetic lipoprotein disturbance consists of the overproduction of apoB-containing atherogenic lipoproteins [43].

Lipoprotein Overproduction

Type 2 diabetes infers a tendency toward positive energy balance that favors excess serum levels of markers of macronutrient metabolism, most notably glucose and TG. The overproduction of apoB-containing atherogenic lipoproteins is referred to as hyperbetalipoproteinemia. It involves excessive hepatic synthesis of TG-rich VLDL, which undergo progressive lipolysis to produce IDL and eventually LDL. Historically, this clinical picture was referred to as Familial Combined Hyperlipidemia (FCH). It was thought to be a monogenic disorder which could manifest as predominant hypertriglyceridemia due to increased VLDL, predominant hypercholesterolemia due to conversion to LDL, or a mixed hyperlipidemia due to a combination of both. Even subjects with normal lipid levels could be demonstrated to have elevated apoB on account of increased numbers of small dense LDL. The patterns of lipid disturbances within an individual with FCH are prone to vary with age and obesity [44]. Now it is recognized that hyperbetalipoproteinemia/FCH is polygenic and that it has considerable overlap with insulin resistance. If genetic factors predominate, a pattern consistent with Familial Combined Hyperlipidemia is more likely, while the metabolic pattern associated with insulin resistance may emerge if environmental factors such as central obesity are present. Increased VLDL accompanied by low HDL-C is the most common form of dyslipidemia in type 2 diabetes, but it is by no means static. It may vary with or without episodes of associated increases in cholesterol due to increased LDL, and if these LDL are small and dense, the profile may feature increased apoB rather than increased LDL-C. As a result, the first three profiles listed in Table 1.1 are common in type 2 diabetes. Similarly, type 2 diabetes is a common secondary cause of these lipoprotein patterns in Table 1.2.

It is also important to note that other secondary causes may contribute to these patterns of dyslipidemia, and indeed several, such as renal impairment, and medications, are common accompaniments of type 2 and type 1 diabetes diabetes. Others, such as obesity and corticosteroid use, represent secondary causes of type 2 diabetes itself. Furthermore, the mere presence of diabetes does not exclude the possibility of intercurrent primary causes of dyslipidemia. It has been argued that LDL-C levels in western society are pathologically high due to gene-environment interactions (referred to as "polygenic hypercholesterolaemia") and hence this pattern of primary dyslipidemia may frequently accompany type 2 diabetes (Table 1.3). Indeed, several patterns in Tables 1.1, 1.2, and 1.3 must be regarded as potentially interchangeable.

The tables do not include genetically determined increase in Lp(a) which has emerged as an independent risk factor for macrovascular disease. This lipoprotein is composed of a LDL particle which is covalently linked to apolipoprotein(a). Analysis relies on detection of the apo(a) moiety, but heterogeneity of the number of Kringle IV Type 2 repeats necessitates quantification in terms of molar concentration. The presence of apo(a) endows Lp(a) with additional atherogenicity due to homology with plasminogen, association with oxidized phospholipids and other pro-atherogenic features. It may exert a powerful atherogenic influence at Lp(a)

levels which have only modest, if any, effect on LDL cholesterol levels. Lp(a) level is independent of the type or severity of diabetes, but it may be exacerbated by diabetic renal impairment. Its role as an independent risk factor is evident from Mendelian randomization studies [45]. Lp(a) levels above the 80th percentile in Western populations are capable of contributing to the risk of macrovascular disease, so estimation is recommended as part of risk assessment, particularly in the intermediate risk range. Awareness of Lp(a) levels will be of additional importance if on-going trials of small interfering RNA therapy against this lipoprotein reduce the risk of CVD.

Reduced Lipoprotein Catabolism

The previous sections highlight the association between type 2 diabetes and the overproduction of serum lipoproteins. Consequently, type 2 diabetes places a strain on the catabolic pathways for TG and apoB-containing lipoproteins, respectively. Most apoB-containing particles undergo final catabolism via the LDL receptor. Competition for this receptor will increase LDL-C, and this is thought to contribute to increases in LDL-C and apoB that are commonly associated with type 2 diabetes [46]. The LDL receptor also mediates the hepatic removal of catabolized TRL, known as "remnants," but in this case, the receptor interacts with apolipoprotein E. The affinity of apoE for the LDL receptor varies according to genetically determined polymorphism. The E2:E2 genotype has the least affinity which causes mild delay in remnant clearance, mild increase in TG, and a mildly reduced LDL-C. The E2:E2 polymorphism has a prevalence of about 1%. If any cause of lipoprotein overproduction is present, this "second hit" may saturate apoE mediated catabolism of "remnants" [47]. This rare form of diabetic dyslipidemia may be inferred from a "broad beta" pattern on lipoprotein EPG and diagnosed by apoE2:E2 genotyping. The resultant massive accumulation of remnants, as reflected by an increase in TG and TC that is out of proportion to accompanying increase in apoB (Table 1.1), is strongly atherogenic, and the severity may even be sufficient to saturate the removal of TG, as discussed below.

The catabolism of TG takes place on the endothelial surface of peripheral tissues due to the action of lipoprotein lipase [48]. There are a number of genetic influences that affect the activity of lipoprotein lipase and rarely, the most severe impairments result in lipoprotein lipase deficiency (the last condition in Table 1.3). Nevertheless, individuals with type 2 diabetes and less severe lipoprotein lipase impairment may accumulate enough TG to fully saturate their lipolytic activity, particularly if they adopt a high fat diet or if their diabetes control lapses (second last condition in Table 1.2). This may rapidly exacerbate TG levels, causing massive hypertriglyceridemia with an attendant risk of acute pancreatitis. As mentioned above, the hypertriglyceridemia associated with accumulation of remnant particles may also saturate the activity of lipoprotein lipase causing progression to the same outcome.

Apolipoprotein Measurement

Other Laboratory Markers

It is inappropriate to assume that lipoprotein status in diabetes is solely due to the diabetic state. Consideration of additional primary and secondary causes provides additional prognostic information. It is difficult, but not impossible to factor these considerations into clinical management decisions that are largely based on quantification and assessment of the absolute CVD (or other) risk of the individual patient. The greatest problem is the need to reclassify patients with intermediate levels of risk, so it is in this category that lipoprotein pattern and underlying etiology can be most helpful. Other forms of laboratory assessment could also play a role in this regard. Detection, quantification, and monitoring of pro-atherogenic diabetic complications, particularly renal impairment warrant the measurement of urinary microalbumin, eGFR via creatinine, and possibly cystatin-C and/or N-gelatinase associated lipocalin in future. The severity of diabetes, as quantified by serum glucose and glycated hemoglobin, also requires consideration. Modification of lipoproteins is not necessarily proportional to the severity of diabetes, so independent measurement of parameters such as oxidized LDL may eventually become relevant. The evidence for their routine use is yet to accumulate. Markers of other potentially atherosclerotic processes such as inflammation may also be relevant. It needs to be remembered that excess central adipose tissue, common in people with type 2 diabetes and increasingly so in type 1 diabetes, may be a source of adipokines that include inflammatory markers such as high sensitivity C-reactive protein (hs-CRP) or lipoprotein-associated phospholipase A2. Specific anti-inflammatory treatments are limited by cost and efficacy, but the use of colchicine in the presence of CVD and increased hs-CRP is under investigation. In this sense, raised levels of inflammatory markers may represent surrogate markers of type 2 diabetes and pre-diabetes. Nevertheless, some guidelines do envisage a role for hs-CRP measurement in the re-classification of intermediate risk patients.

Genetic techniques have enabled studies of the genome-wide association between genetic markers and conditions like diabetes. One of the strongest genetic markers on Chromosome 9 is associated with both vascular disease and type 2 diabetes [49]. Genetic testing in diabetes is not widespread at present, but the development of polygenic risk scores for complex chronic diseases such as diabetes and also cardiovascular disease remains an active area of research.

Several examples cited above demonstrate the technique of Mendelian randomization [50]. This approach has been likened to “Nature’s Clinical Trial.” It relies on randomization of alleles at conception and takes advantage of the persistence of the effect of small genetically determined differences over whole-of-life. Interpretation hinges on the associations of genotype and phenotype with the outcome of interest. If they are congruent, it suggests that the phenotype (e.g., biomarker) is causative for the clinical outcome and that the metabolic mechanism represented by the genotype may warrant assessment as a treatment target in terms of both benefits and side-effects. The evidence in support of apoC3 and Lp(a) as treatable risk factors for

macrovascular disease has already been presented. Mendelian randomization also supports many of the standard lipoprotein biomarkers and therapies such as those related to TG. On the other hand, it challenges the roles of HDL-C [51] and hs-CRP [52] as causative risk factors for CVD.

Summary

Clinical management of diabetes mellitus requires effective laboratory assessment of lipoprotein abnormalities. Diabetes can cause or exacerbate both quantitative and qualitative changes in lipoproteins. Furthermore, diabetic complications may cause secondary dyslipidemia, while important primary dyslipidemia may coexist with diabetes. The risk of macrovascular complications of diabetes can be anticipated by consideration of major CVD risk factors including atherogenic fractions of cholesterol and triglyceride. Quantification of risk is facilitated by derived indicators, particularly LDL-C and NHDL-C. Like diabetes, dyslipidemia is a complex chronic condition that requires on-going assessment and long-term surveillance.

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Chapter 2
Tools for Assessing Lipoprotein Metabolism in Diabetes Mellitus

Richard L. Klein, Andrea J. Semler, and Alicia J. Jenkins

Introduction

Both quantitative and qualitative changes in lipoproteins contribute to the macrovascular and microvascular complications of type 1 and type 2 diabetes [1, 2]. Understanding the whole body (systemic) and cellular metabolism of lipoproteins, including that of the modified lipoproteins that occur in diabetes mellitus, has potential to facilitate development of novel therapeutics that can improve clinical outcomes. We will give several examples of how understanding lipoprotein metabolism has improved clinical outcomes for millions of people around the world. Understanding of the LDL receptor, intracellular cholesterol metabolism, and the central role of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in cellular synthesis led to the development of HMG-CoA reductase inhibitors (statins), usually taken orally daily, which substantially reduce cardiovascular events and premature mortality in both diabetic and non-diabetic people [3–5].

Richard L. Klein has died before the publication of this book.

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Furthermore, understanding of cellular handling of the LDL receptor and of the key role of PCSK9 in its recycling led to the development of humanized monoclonal antibodies that inhibit PCSK9 activity [6]. PCSK9 inhibitors given by two to four weekly subcutaneous injections are currently the most powerful LDL and Lp(a) lowering drug currently available, which can significantly reduce lipid-related cardiovascular events and mortality [7].

Cell culture, animal, and human kinetic lipoprotein studies can contribute knowledge as to lipoprotein metabolism and the effects of clinical factors such as genetics, diabetes, kidney damage, and drugs. In this chapter, we will describe the general principles of commonly used techniques for in vivo studies of lipoprotein kinetics which can be applied to human subjects and to animals and also for the assessment of lipoprotein metabolism in cultured cells, using examples from our research.

In recent years, advances in science have made available many fields of "omics," including lipidomics, proteomics, and metabolomics. Lipidomics and proteomics can analyze hundreds of lipid species or proteins (not whole lipoprotein particles) from small (microliter) volumes of plasma or serum and are complementary to the field. Several techniques including NMR and density gradient ultracentrifugation can provide more detailed characterization of major lipoprotein classes. We will briefly comment on these tools and give several examples, including from our own research.

Lipoprotein Kinetic Studies

In clinical practice and in many clinical research studies, lipid or apolipoprotein levels are commonly measured (as described in the chapter by Dr. David Sullivan), including often as the study endpoint. While these static measures are very useful and are valuable in clinical practice, studies of lipoprotein metabolism are important research tools, akin to looking at a movie (a lipoprotein kinetics study) rather than at a photo or an individual frame of the movie (a traditional lipid panel). Such kinetic studies are complex and require specific skill sets and instrumentation and, due to their high cost, are also usually conducted in small numbers of subjects.

Alterations in lipid levels may relate to differences in lipoprotein production or lipoprotein clearance, or both, and even in the absence of altered lipid levels, there may be changes in lipoprotein production and the rates and pathways of lipoprotein clearance.

While kinetic studies have been undertaken in animals, apart from non-human primates [8], the lipoprotein metabolic pathways of animals, particularly rodents, differ substantially from that of humans. For example, in man most circulating cholesterol is present in Low Density Lipoproteins (LDL), while in rodents most circulating cholesterol is carried in High Density Lipoproteins (HDL) [9].

As LDL is the major circulating lipoprotein and as apoB100 is present in Very Low Density Lipoprotein (VLDL) and its metabolic products of Intermediate Density Lipoprotein (IDL) and LDL, most kinetic studies relate to apoB-containing lipoproteins. ApoB is also present in Lp(a). ApoB and apo(a) are the only

apolipoproteins that do not exchange between lipoprotein classes, as does, for example, ApoC which exchanges between chylomicrons, VLDL and HDL [10].

Apolipoprotein B Turnover Studies

Two general approaches to apoB kinetic studies are taken. The first and earlier technique utilizes radiation, and the more modern technique uses stable isotopes.

Radiation-Based Studies

The study of apoB metabolism has been approached in a number of ways in both normal and hyperlipidemic states in human subjects. The most widely used technique to measure clearance of apoB employs radioiodination of purified VLDL or LDL, usually obtained by ultracentrifugation, which is then reinjected into the study subjects. The decrease in lipoprotein radioactivity is monitored in sequential blood samples collected from each subject.

Another approach has utilized intravenous administration of a radioactively labeled amino acid precursor, such as ^{75}Se-labeled methionine, or [^{3}H]leucine, with subsequent determination of its appearance in, and disappearance from, the lipoprotein fraction(s) of interest. Both approaches use radioactive isotopes and require extensive computerized mathematical modeling to properly estimate lipoprotein residence time in plasma using stochastic or multicompartmental analysis of plasma radioactivity decay curves.

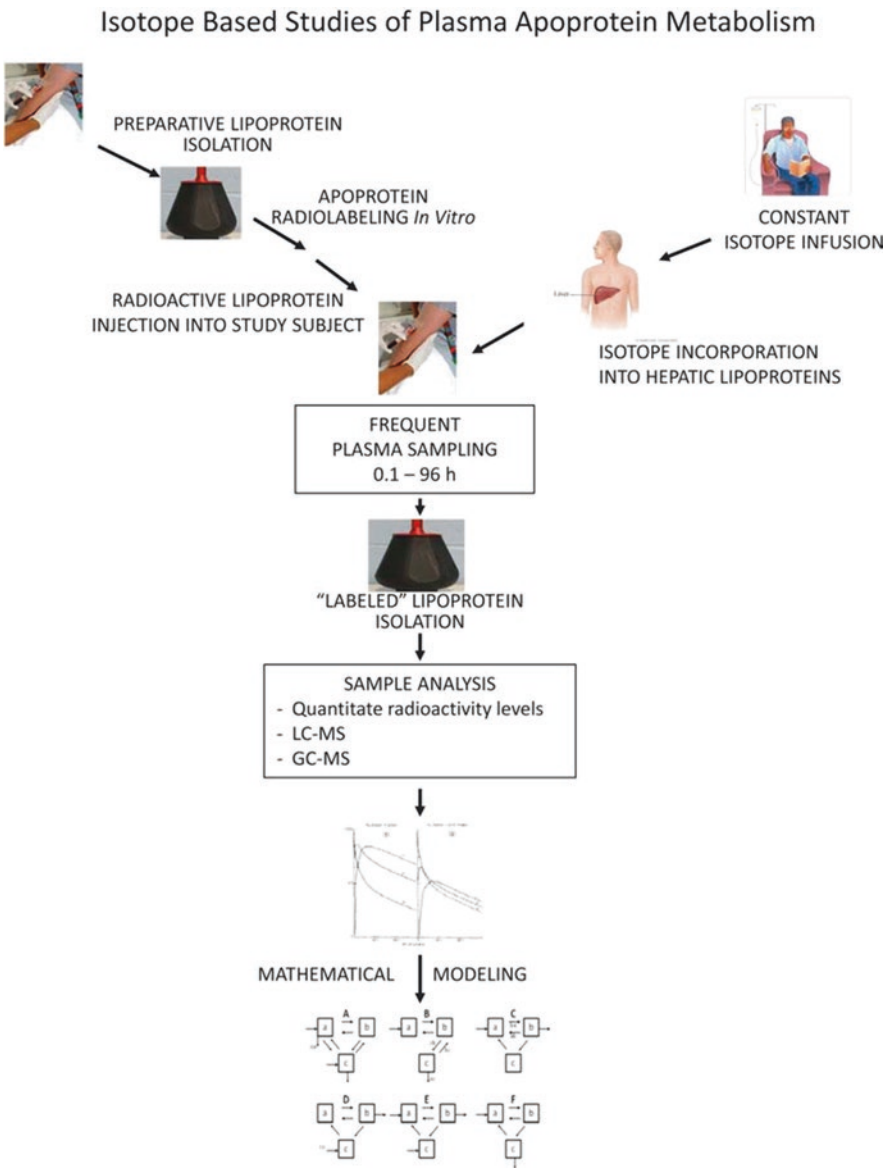
Radioiodination of LDL has been used successfully to monitor LDL turnover because it has been extensively documented that apolipoprotein B (apoB) is unique among the apoproteins in that it is not exchangeable between lipoprotein classes [10, 11] while it is also the major protein component of LDL. In contrast, apoB in VLDL represents only approximately 40% of the total protein mass, and radioiodination of VLDL results in labeling of other apoprotein components. In addition, a larger proportion of the radiolabel may be found in the lipid moiety of the particle than with LDL and may result in less than 50% of total radioactivity being localized in apoB [12]. Therefore, studies of apoB metabolism after injection of radiolabeled VLDL require the isolation of apoB from the other labeled components to permit accurate specific activity measurements. This led to the development of methods to rapidly and quantitatively isolate apoB from other radiolabeled apolipoproteins and lipids, and which permit multiple apoB specific activity determinations on lipoproteins isolated from limited volume plasma samples. One frequently utilized method uses 1,1′,3,3′-tetramethylurea (TMU) to solubilize VLDL apoproteins and leave behind precipitated apoB [13], while another uses butanol-isopropyl ether [14].

To circumvent lipid contamination, other investigators have endogenously labeled VLDL with ^{75}Se-labeled methionine or ^{3}H-labeled lysine. However, interpretation of data from this type of experiments conducted in humans is difficult to analyze mathematically because of the inherent complexity of endogenous labeling. These experiments also do not allow complete analysis of the source of apoB input into the higher density lipoprotein classes; thus, precursor–product relationships between VLDL apoB and apoB in the other lipoprotein density classes cannot be easily studied [15–17]. A general organization and method of conduct of these types of investigations are shown in the schematic presented in Fig. 2.1.

The methods described above rely on radioactively labeled lipoproteins or infusions of radioactivity. This approach, however, is considered by some to be nonideal for several reasons:

(a) Lipoproteins and apolipoproteins potentially can be modified, such as by oxidation or aggregation, during isolation and radioiodination which may influence their metabolic behavior in vivo.
(b) A steady-state condition where production and clearance rates are taken to be constant is difficult or impossible to document and, therefore, must be assumed, an assumption that may not always be physiologically accurate.
(c) Studies cannot be undertaken in children or in pregnant women, nor can multiple studies be undertaken in the same volunteer due to exposure to potentially hazardous levels of radioactivity.

Fig. 2.1 The design and general method of conduct of investigations of lipoprotein and apoprotein metabolism which employ isotopes. Studies investigating lipoprotein metabolism in plasma frequently employ isotopic labeling of the apoprotein moiety of the particles. Both radioisotopes and nonradioactive, stable nuclides (e.g., ^{2}H, ^{15}N) are frequently used to label lipoprotein protein. Studies of lipoprotein metabolism fall into one of two general classes: studies in which lipoproteins are initially isolated and the apoprotein radiolabeled before the lipoprotein is injected back into the study subject and studies in which isotopes are infused at a constant rate until they are absorbed into the liver and subsequently incorporated into lipoprotein proteins. In the latter type of study, the lipoprotein particles which are secreted de novo by the liver contain the stable nuclides which permit these lipoproteins to be distinguished from those already present in the circulation. Both approaches require frequent sampling of plasma from the study subject. Lipoprotein metabolism may be studied using whole plasma, but more often individual lipoprotein classes are separated and purified using ultracentrifugation or other methodology. Lipoprotein metabolism or "turnover" is quantitated as the appearance/disappearance of radioactivity in plasma and isolated lipoprotein fractions when radioactive tracers have been employed. When stable nuclides are infused, plasma and lipoprotein samples must be extensively processed to enable detection and quantitation of the stable isotopes using gas chromatography (GC) or liquid chromatography (LC) in combination with mass spectroscopy (MS). Both approaches ultimately require sophisticated, computer-aided, mathematical modeling to convert the patterns of isotope appearance/disappearance in plasma/lipoproteins into intuitive metabolic pathways


Isotope Based Studies of Plasma Apoprotein Metabolism
PREPARATIVE LIPOPROTEIN ISOLATION
APOPROTEIN RADIOLABELING *In Vitro*
CONSTANT ISOTOPE INFUSION
RADIOACTIVE LIPOPROTEIN INJECTION INTO STUDY SUBJECT
ISOTOPE INCORPORATION INTO HEPATIC LIPOPROTEINS
FREQUENT PLASMA SAMPLING 0.1 – 96 h
"LABELED" LIPOPROTEIN ISOLATION
SAMPLE ANALYSIS
- Quantitate radioactivity levels
- LC-MS
- GC-MS
MATHEMATICAL MODELING

Stable Isotope-Based Studies

A new experimental approach to investigations of apoB metabolism has been developed which relies on modern instrumentation and which eliminates complications associated with the administration of radioactivity to humans. This method uses intravenous infusion of stable isotope-labeled amino acids after a priming dose that achieves and maintains an isotopic steady state [18, 19] that is necessary for the successful conduct of this type of study. The intricacies and advantages or disadvantages associated with each of these three types of lipoproteins, or lipoprotein precursor, tracer infusion studies have been discussed at length in other excellent articles [20].

Dual Radiolabel Studies

As discussed in the chapter on lipoprotein glycation, the incubation of human LDL with glucose results in a non-enzymatic formation of a Schiff base between the monosaccharide and lysyl residues in apoB. As a greater percentage of the lysyl residues of apoB in LDL become modified by glycation, the fractional catabolic rate of the glycated LDL decreases in in vivo studies [21]. The rates of catabolism of glycated LDL by cultured human skin fibroblasts are also decreased suggesting that glycated LDL is catabolized primarily via a receptor-independent process. Thus, radiolabeled LDL which has been extensively glycated is frequently injected concomitantly with native LDL radiolabeled using another isotope, and the rates of LDL metabolism via receptor-independent and receptor-dependent pathways, respectively, estimated from the ratio of the fractional catabolic rates determined using each uniquely radiolabeled lipoprotein preparation [21].

HDL-Related Turnover Studies

Studies investigating the synthesis and catabolism of HDL apolipoproteins, primarily apoA-I and apoA-II, are conducted in a manner similar to those described above for apoB. Lipoproteins containing radiolabeled apolipoprotein(s) are injected into each study subject, and the lipoprotein fraction of interest is isolated from serially collected blood samples to monitor radioactivity decay patterns. Investigating the metabolism of HDL apolipoproteins using this type of study technique is inherently more difficult because it has been documented that protein in HDL is freely exchangeable between HDL particles and lipoproteins in other density classes [22, 23].

It is these types of kinetic studies that have led to the knowledge regarding changes in VLDL, LDL, and HDL production and clearance in people with vs. without diabetes, as are described in other chapters herein on lipoprotein metabolism in diabetes by Drs. Tomkin and Owens and by Drs. Dayspring and Toth.

Lipoprotein Metabolism in Cultured Cells

Investigations of lipoprotein metabolism in cultured cells generally focus on three distinct stages of cell–lipoprotein interaction:

(a) Lipoprotein binding to cell surface lipoprotein receptors.
(b) Internalization of the lipoprotein from the cell surface in preparation for subsequent metabolism by the cell (if binding of the lipoprotein to the receptor results in receptor internalization).
(c) Degradation of the internalized lipoprotein in the cell lysosomal compartment.

Lipoprotein Binding to Cells

The Nobel Prize in Physiology or Medicine in 1985 was awarded to Joseph L. Goldstein and Michael S. Brown. Their Nobel prize-winning research elucidated the molecular mechanism whereby exogenous cholesterol in VLDL, and LDL downregulates cellular 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) enzymatic activity, the rate-limiting step of cellular endogenous cholesterol biosynthesis. Their research also developed many of the techniques necessary for the study of lipoprotein metabolism in cultured cells. Many of these research techniques remain in use today. Their earliest studies demonstrated that LDL radiolabeled with tracer ^{125}I-iodine can be taken up by cultured fibroblasts from normal subjects in a temperature dependent process that is highly specific, reaches equilibrium with time, and is saturable at low levels of LDL [24].

These studies clearly demonstrated for the first time that the amount of ^{125}I-LDL bound to the cells was reduced by the addition of a 50-fold excess of non-radiolabeled, native LDL, which suggested that the radiolabeled LDL and native LDL were competing for a limited number of specific binding sites. Furthermore, these studies introduced the concept of "specific" lipoprotein binding to cells, which would be reported from this juncture as the difference between the lipoprotein radioactivity bound to cells in the absence and presence of excess native LDL. The development of techniques to measure both "specific" lipoprotein binding and "non-specific" binding (defined as the level of ^{125}I-LDL radioactivity bound in the presence of a 50-fold excess of native LDL) enabled these investigators to conduct Scatchard analyses of the LDL binding to fibroblast receptors. These study results suggested the presence of a specific LDL binding site of high affinity which could be saturated at relatively low LDL concentrations (20 μg/mL). Most importantly, these studies demonstrated that specific LDL binding appeared to be required in the process by which LDL normally suppressed HMG-CoA reductase activity and further, the binding of LDL to fibroblasts from patients homozygous for familial hypercholesterolemia was defective and appeared to explain the previously reported

failure of LDL to suppress the synthesis of this enzyme in fibroblasts isolated from these patients [24].

Lipoprotein Degradation by Cells

While studying the binding of ^{125}I-LDL to normal fibroblasts, Brown and Goldstein noted that the ^{125}I-LDL bound to cells was ultimately degraded to form a product that was dialyzable, and which could no longer be precipitated with trichloroacetic acid (TCA) [25]. In subsequent studies [26], they refined this technique to include an additional step in which the acid-soluble material appearing in the culture media harvested after cells were incubated with ^{125}I-LDL at 37 °C for several hours was oxidized with hydrogen peroxide and then extracted with chloroform. This step eliminated the artifactual contamination of the small amount of radioactive free iodide that persisted in the ^{125}I-LDL preparation despite extensive dialysis. Control studies conducted by incubating the ^{125}I-LDL preparation at 37 °C in culture media without cells revealed that the formation of this iodide-free, acid-soluble degradative product of LDL was absolutely dependent on the presence of cells and, furthermore, was linear with time up to at least 30 h. The use of this cell-free, spontaneous degradation control became routine for all subsequent studies of lipoprotein metabolism by cells. Most of the TCA-soluble material secreted into the culture medium was identified to be ^{125}I-tyrosine. Uptake of LDL by both the specific, high-affinity process and also the nonspecific, lower-affinity process results in degradation of the lipoprotein, and the degradation processes appear to be similar [25].

Lipoprotein Accumulation by Cells

To demonstrate the conversion of bound ^{125}I-LDL to acid-soluble material, cultured human fibroblast cells were first preincubated at 4 °C with ^{125}I-LDL. These conditions permit the LDL to bind to cell surface receptors as demonstrated by continued LDL susceptibility to protease degradation [24, 25] even after 4-h incubation, but without appearance of ^{125}I-acid-soluble material in the media. Cells which had been preincubated at 4 °C with ^{125}I-LDL were then transferred to medium without ^{125}I-LDL and were additionally incubated at either 4 °C or 37 °C. At the beginning of this second incubation, all radioactivity bound to the cells was precipitable with TCA. When the cells were incubated at 37 °C, nearly all this bound radioactivity was released into the cell culture medium within 3-h, and approximately two-thirds had been converted to acid-soluble material. In contrast, when the cells were incubated at 4 °C, only about one-third of the ^{125}I bound to the cells was released, and all the radioactivity was precipitable by TCA. The difference in the amounts of radioactivity localized to cells incubated at 37 °C (bound and internalized, but not degraded LDL) compared to that found in

cells incubated at 4 °C (bound LDL only) is considered to be accumulated LDL. These types of studies are not conducted as often as those measuring lipoprotein binding or degradation.

Studies of Glycated LDL Metabolism by Human Macrophages

We have used the techniques described above to investigate the metabolism of in vivo glycated LDL by cultured human macrophages. Using sequential ultracentrifugation we isolated LDL ($1.019 < d < 1.063$ g/mL) from 10 adults with type 1 diabetes and from 10 age-, sex-, and race-matched non-diabetic subjects to serve as controls [27]. The HbA1c level in the diabetic subjects and in the non-diabetic control subjects averaged 8.2 ± 0.6%, 5.6 ± 0.1% (66 vs. 38 mmol/mol), respectively. We incubated human monocyte-derived macrophages with increasing concentrations of ^{125}I-LDL from each diabetic subject and their matched control subject for 20 h at 37 °C and then determined the amount of ^{125}I-TCA-soluble (non-iodide) material formed by the cells and secreted into the culture medium. We calculated the rates of high-affinity, receptor-mediated degradation of the LDL as the difference between LDL degradation levels in cells incubated with only ^{125}I-LDL and parallel incubations containing ^{125}I-LDL plus a 25-fold excess of non-radiolabeled LDL. Corrections were made for the small amounts of ^{125}I-acid-soluble material that was found in parallel incubations without cells. We determined there was no significant difference between the receptor-mediated degradation of LDL isolated from control subjects and diabetic patients.

Unexpectedly, we observed a significant increase ($P < 0.05$) in the rates of total and non-high-affinity receptor-mediated degradation of LDL from diabetic vs. non-diabetic subjects. We determined that there were no statistically significant differences in the lipid composition of LDL isolated from the two groups, and therefore, we investigated whether abnormalities in apoB could be responsible for the altered pattern of degradation.

ApoB can also be covalently modified by incubation with glucose in vitro and is similar to the LDL from diabetic patients (modified in vivo). We determined that the level of glycation in LDL from the people with diabetes was increased four-fold over that determined in LDL from the control subjects. This was a critical observation because these results suggested the presence of an abnormality in LDL apoproteins that could alter LDL metabolism by macrophages even in people with diabetes with moderately good glycemic control (HbA1c 8.2 ± 0.6%, mean 66 mmol/mol) and whose LDL lipid composition was normal. We pursued additional studies to determine the mechanism responsible for the enhanced degradation of LDL from people with diabetes by human macrophages.

To further investigate the interaction of glycated LDL with human macrophages, we modified LDL in vitro by incubating LDL isolated from plasma pooled from young, healthy, euglycemic donors with glucose for 7 days at 37 °C, which we have shown will increase the fructoselysine content of the LDL to levels observed in

LDL isolated from people with diabetes [28]. As reviewed in our chapter on lipoprotein glycation herein, fructoselysine is an early glycation product. We incubated ^{125}I-labeled native and in vitro glycated LDL (glc-LDL) with human macrophages and determined the rates of LDL degradation [29]. We determined that the rates of total degradation of glc-LDL were greater than those of control LDL, particularly at high LDL concentrations. More significantly, the degradation of glc-LDL by the classic LDL receptor pathway was slightly less than that of control LDL. This suggested that the increase in degradation of glc-LDL was mediated by a pathway independent of the classic LDL receptor [29]. Additional studies in our laboratory demonstrated that the increased degradation of glc-LDL by human macrophages was not mediated by the scavenger receptor or by carbohydrate receptors known to be expressed on human macrophages. This series of studies suggested that in human macrophages, there exists a low-affinity, high-capacity pathway that enhances the uptake and degradation of glc-LDL.

Cellular Metabolism of Lipoprotein Cholesterol

The early seminal studies conducted by Brown and Goldstein [26] clearly demonstrated that LDL, but not VLDL or HDL, could significantly reduce the activity of HMG-CoA reductase in fibroblasts [30]. Subsequent studies further revealed that when LDL was incubated with cultured fibroblasts, there was a 30–40-fold increase in the rate of incorporation of ^{14}C-oleate into the fatty acid fraction of cellular cholesteryl esters [31]. Most importantly, the stimulation of cholesteryl ester formation by LDL occurred despite the fact that endogenous synthesis of unesterified cholesterol was completely suppressed by the lipoprotein. That is, exogenous cholesterol in the LDL rather than endogenous cholesterol synthesized by the cell appeared to provide the cholesterol substrate for cellular cholesterol esterification.

Using this same approach, we determined that LDL isolated from hyperglycemic, normolipidemic adults with diabetes stimulated cholesteryl ester synthesis rates in human macrophages significantly more than LDL isolated from nondiabetic, control subjects [27]. We further determined that the increase in cellular cholesteryl ester synthesis in cells incubated with LDL from diabetic patients did not result from increases in cholesterol content in the LDL compared to LDL from the control subjects but rather resulted from enhanced catabolism of the LDL particles by the glycated LDL receptor present on human macrophages, as detailed above. We further demonstrated that the enhancement in cholesteryl ester synthesis by macrophages exposed to glc-LDL was paralleled by intracellular accumulation of cholesteryl ester [29].

Lastly, these findings clearly demonstrate the importance of glycation of apoprotein B in LDL on inducing abnormal LDL-macrophage interaction. They are also of interest because they suggest the presence of abnormal lipoprotein metabolism in diabetes even in patients with relatively good glycemic control and whose plasma lipid and lipoprotein levels are “normal.”

Lipidomics and Lipoprotein Subclass Analyses

In recent years, many fields of "omics" have emerged, including lipidomics, proteomics, genomics, metabolomics, and transcriptomics. These currently predominantly research tools are relevant to lipoprotein metabolism. Lipidomics and proteomics usually also function like snapshots, albeit a very detailed snapshot, of lipoproteins, rather than a film, such as the kinetic analyses described above. The laboratory methodologies used are usually mass spectroscopy (MS) based and sometimes NMR. Using just a few microliters of plasma or serum, which can be analyzed fresh or after frozen storage (preferably at −80 °C), lipidomics can quantify hundreds of species of individual lipid species, such as phospholipids, sphingolipids, and ceramides [32–35], and similarly proteomics can detect hundreds of circulating proteins, including larger proteins such as apolipoproteins, immunoglobulins, albumin, and haptoglobin. Such outputs may assist in the better prediction of diabetes complication risk and in the monitoring of and response to various therapies. These tools will likely increasingly enter clinical practice, provided accuracy and precision standards are met, with externally monitored quality control programs, evidence of cost efficiency, health benefit gain from such knowledge and regulatory body approvals.

Two examples of using lipidomic analyses in cardiovascular disease trials relate to the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) trial, which monitored cardiovascular outcomes in 3779 adults with type 2 diabetes [33], and the Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) trial including stable patients with a prior acute coronary syndrome [34].

In the ADVANCE trial, 310 plasma lipids were quantified using liquid chromatography electrospray ionization-tandem mass spectrometry in a case-control substudy of 3779 participants. All subjects (mean age 67 years, 61% male) had type 2 diabetes and at least one other cardiovascular risk factor, and 35% had a history of macrovascular disease. The goal was to identify individual lipid species associated with future non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death during a 5-year follow-up. Multivariable models combining traditional risk factors with lipid species were evaluated. Sphingolipids, phospholipids (including lyso- and ether-species), cholesteryl esters, and glycerolipids were associated with future cardiovascular events and with cardiovascular death. The addition of seven lipid species to a panel of 14 traditional risk factors and medications to predict cardiovascular events increased the C statistic from 0.680 (95% confidence interval [CI], 0.678–0.682) to 0.700 (95% CI, 0.698–0.702; $P < 0.0001$) with a continuous net reclassification index (NRI) of 0.227 (95% CI, 0.219–0.235). The addition of four lipid species to the panel of 14 traditional risk factors and medications increased the C statistic from 0.740 (95% CI, 0.738–0.742) to 0.760 (95% CI, 0.757–0.762; $P < 0.0001$) and the continuous NRI 0.328 (95% CI, 0.317–0.339) [33]. These results were validated in a sub-study of 511 adults with type 2 diabetes in the LIPID trial [34].

In the LIPID trial sub-study ($n = 5991$ subjects), the goal was to identify lipid species from a lipidomics panel of 342 species that predicted the risk reduction from

pravastatin. Pravastatin significantly altered most lipids, with the ratio of two lipid species, a phosphatidylinositol (36:2) and a PC (38:4) being predictive of those who did or did not benefit from statin therapy, independent of changes in traditional lipid (total cholesterol, HDL-C, and triglycerides) levels [34]. These studies support the potential use of lipidomics as biomarkers in research and potentially in clinical practice.

There are also NMR-based means of assessing lipoproteins in research, at least one of which was FDA approved and used in clinical practice. While reporting size-based lipoprotein measures and particle concentrations rather than individual lipid species, they still are akin to a snapshot rather than a movie of lipoprotein metabolism.

NMR spectroscopy is based on the detection of ^{1}H NMR signals from terminal methyl groups in the lipid hydrocarbon chains of lipoproteins, and their shift to lower frequencies with decreasing particular size. NMR can detect these signals in fresh or previously frozen plasma or serum without lipoprotein separation. Using a mathematical deconvolution process, Otvos et al. were able to report up to 16 chylomicron, VLDL, LDL, and HDL subclasses [35, 36]. We applied this technique to retrospective analyses of samples from the Diabetes Control and Complications Trial/Epidemiology of Interventions and Complications (DCCT/EDIC) type 1 diabetes cohort. In a series of publications, NMR analyses provided more detail in the relationships of lipoprotein subclasses with glycemia [37], intensive diabetes therapy [38], diabetic nephropathy [39], retinopathy [40], and carotid intima media thickness (IMT) [41–43] than was evident with traditional lipids alone. NMR also enabled insights into lipoprotein immune complex formation in this type 1 diabetes cohort [44].

Similar detailed lipoprotein "snapshots" can also be obtained by density gradient ultracentrifugation, such as the Vertical Auto Profile (VAP) analysis, which requires lipoprotein separation. VAP analysis has also been used as a research tool and in clinical practice. As an example, the effect of the PCSK9 inhibitor, alirocumab, on lipoprotein subfractions was evaluated using stored samples from three phase II trials, all of which included background statin therapy. Relative to placebo, alirocumab significantly reduced triglycerides, LDL-C, and the cholesterol content of LDL subfractions 1, 2 and 3 + 4 and of VLDL-, IDL-, and remnant lipoproteins [45].

Conclusions and Future Directions

Greater knowledge of lipoprotein metabolism in humans and by cells has been key to the development of lipid modulating therapy that has already substantially improved cardiometabolic health outcomes for people with and without diabetes. It is expected that these or related tools including "omics" will continue to be of use in the assessment of systemic and cellular lipoprotein metabolism. They have contributed to our understanding of lipoprotein metabolism in health and in diabetes and other disease states and of the effects of lipoprotein modifications, such as non-enzymatic glycation. Other clinical, animal, and cell culture research has demonstrated that changes in lipoproteins levels and composition can promote atherosclerosis and the retinal and kidney complications of diabetes. Based on such knowledge increasingly effective lipid lowering treatments have been developed to

reduce such lipid-related vascular damage. These laboratory techniques can also contribute to the development of emerging drug classes such as targeted RNA silencing therapies. Despite modern means for glucose, blood pressure, and lipid control, chronic complications still often occur in people with diabetes and good traditional risk factor control. Residual risk may reside within alterations in lipoprotein metabolism and composition and in the cellular handling (and responses to) lipoproteins. The tools described herein, most likely used with other cell biology, molecular techniques, and drug development tools, may facilitate the development of treatments to tightly control dyslipoproteinemia and reduce the vascular complications of diabetes.

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Chapter 3
Links Between Glucose and Lipoproteins

Alicia J. Jenkins

Introduction

While hyperglycemia is the hallmark of diabetes mellitus, there are many associated abnormalities of lipoprotein metabolism, including quantitative and qualitative changes in lipoprotein classes and subclasses. There are also multiple associations between hyperglycemia and its treatment and lipoproteins and vice versa, which may also impact complication susceptibility.

Lipoprotein Functions

The primary function of lipoproteins is the transportation of fatty acids, triglycerides, and cholesterol from the gut to the liver (via chylomicrons and chylomicron remnants) and from the liver to the periphery (via Very Low Density Lipoproteins (VLDL), VLDL remnants, and Low Density Lipoprotein (LDL)) to target cells where it is used for cellular structures, such as cell membranes, for energy use or storage (e.g., in adipocytes) and for cellular functions, such as steroid hormone synthesis. Reverse cholesterol transport, enacted predominantly by High Density Lipoprotein (HDL), in particular the smaller, denser, lipid poor protein-rich HDL particles, removes excess cholesterol from peripheral tissues, delivering it to the liver, from where it is redistributed to other tissues or removed via the gallbladder and intestine [1, 2]. HDL is also involved in defenses against viruses and toxins [1,

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3]. The physiologic role of the proatherogenic and pro-thrombotic lipoprotein lipoprotein(a) is not yet fully elucidated, and people without Lp(a) are healthy. It has been speculated that with its pro-thrombotic and lipid delivery capability it may be a means of assisting with blood clotting and with wound repair [4]. As reviewed in other chapters in this book, lipoproteins have many roles in cell survival, thrombosis, inflammation, oxidative stress, defense against infection, vascular dysfunction, atherosclerosis, and in modulating insulin section and action, hence glycemia. Lipoproteins act via many receptors, cell signaling and molecular pathways [1–4].

As well as the same general roles of lipoproteins in the non-diabetic population, in people with diabetes quantitative and qualitative changes in lipoproteins also impact the development and progression of the macrovascular and microvascular complications of diabetes, including cardiovascular disease, diabetic retinopathy, kidney disease, and neuropathy [1]. Relationships between lipoproteins and these chronic complications are discussed in more detail in other chapters in this book. Effects are mediated directly by lipoprotein effects and indirectly by lipoprotein effects on glycemia and other pleiotropic effects.

Contributors to Lipoprotein Levels in Diabetes

There are many factors contributing to lipoprotein levels in people with diabetes, including *glycemia* [2], which are summarized in Table 3.1. Many factors, such as adiposity, physical fitness, and diet, impact both lipoproteins and glycemia, hence quantifying the exact contributions of glycemia on lipoproteins can be challenging in free-living people with diabetes, especially as changes in lipoproteins and in some glycemia measures, such as HbA1c, fructosamine, and 1,5-anhydroglucitol [5] take weeks to months.

HbA1c levels are usually, but not always, correlated with fasting triglyceride levels and inversely with HDL-cholesterol (HDL-C) levels. Worsening hyperglycemia is usually associated with increasing total triglyceride, VLDL and ApoB levels, and with lowering of HDL-cholesterol (HDL-C) and ApoA1 levels. Hyperglycemia is usually associated with similar or somewhat elevated Low Density Lipoprotein Cholesterol (LDL-C) levels than in normoglycemic people, with a shift toward more pathogenic small, dense, lipid poor, protein-rich LDL particles. This pattern of "diabetic dyslipidemia," is common in people with Type 2 diabetes, and also in people with Type 1 diabetes in the setting of poor glucose control, obesity, and/or renal impairment. In people with Type 1 diabetes with good metabolic control, a healthy body mass index (BMI) and normal kidney function traditional lipid levels are similar to that of non-diabetic subjects, often with lower triglyceride and higher HDL-C levels due to the activating effects of supraphysiologic insulin levels on lipoprotein lipase (LPL). Lipoprotein(a) levels are usually higher with poor glucose control in people with Type 1 diabetes, but not with Type 2 diabetes, in which levels

Table 3.1 Contributors to lipoprotein levels and composition in diabetes

Diabetes related
Hyperglycemia
Insulin levels
Insulin resistance
Glucose variability
Lifestyle
Poor diet
Adiposity
Physical inactivity/high sedentary time
Smoking
Stress
Diabetes complications and comorbidities
Renal disease
Liver disease, e.g., non-alcoholic fatty liver disease
Inflammation
Others, e.g., hypothyroidism
Genetics
Drugs
Examples
For glucose control
For lipid control
Diuretics
Beta blockers
Sex steroids
For infections, e.g., HIV

may even be lower than in the non-diabetic population, perhaps related to other, e.g., genetic effects [1–4]. Lp(a) is discussed in more detail herein in the book chapter by Drs' K. and G. Kostner.

As for people without diabetes, lipid levels in people with diabetes are impacted by *lifestyle factors*, such as diet quality, alcohol intake, obesity, physical activity or inactivity, and smoking. These effects are discussed in another chapter in this book, by Dr. Peter Clifton.

Insulin levels and insulin resistance also modulate lipoprotein levels and metabolism, with peripheral insulin resistance being associated with diabetic dyslipidemia (usually defined as high triglycerides and low HDL-C levels). In people with Type 1 diabetes endogenous insulin production is low, though even very low level residual insulin production, reflected by detectable C-peptide using high sensitivity assays, is associated with better glycemic control, including less hypoglycemia and lower glucose variability, and lower risk of micro- and macrovascular complications [6, 7]. In people with Type 1 diabetes, their essential for life exogenous insulin therapy is injected into their subcutaneous tissue rather than via the portal system,

hence circulating insulin levels are supraphysiologic, which can induce insulin resistance, which can also exacerbated by obesity, growth spurts, puberty, and intercurrent illnesses [8].

Renal disease also changes lipoproteins. Even very early kidney disease, such as low level albuminuria (microalbuminuria) and normal or high (hyper-filtering) glomerular filtration rates (GFR), is associated with dyslipidemia and elevated lipoprotein(a) levels. More severe kidney disease with proteinuria and/or GFR loss are associated with worsening lipoprotein profiles (higher triglycerides, VLDL and apolipoprotein B (ApoB), and lower HDL and ApoA1 and a shift to small dense LDL subclasses). With proteinuria, ApoA1 levels can be lost via urine, contributing to HDL-lowering and altering lipoprotein metabolism (discussed in the chapter by Dr. Per-Henrik Groop et al.). Renal dysfunction is also associated with increases in Lp(a) levels, and higher Lp(a) levels may be a risk factor for diabetic nephropathy as well as cardiovascular disease [1, 9–11].

Similarly *liver disease*, such as due to alcohol excess, obesity, and hypertriglyceridemia or non-alcoholic fatty liver disease (NAFLD) which can also be due to poor glucose control (in both Type 1 and Type 2 diabetes) also promote dyslipidemia [1, 2, 12].

Common comorbidities such as *hypothyroidism* can also aggravate dyslipidemia and, until treated, can reduce benefit and tolerance of some lipid lowering drugs, such as increasing the risk of myalgia and elevated creatinine kinase levels from statin or fibrate drug classes [13, 14].

Genetics can impact lipoprotein metabolism in both people with and without diabetes. There are many polygenic and some monogenic conditions which modulate lipoprotein and lipid levels, which may even modulate Type 2 diabetes risk. For example, heterozygous (autosomal dominant) familial hypercholesterolemia (FH), which causes very high LDL-C levels, is associated with lower risk of Type 2 diabetes than in people without FH [15]. Lp(a) levels and genes modulating Lp(a) levels may also be associated with Type 2 diabetes risk [16].

Drugs. In general, all oral and injectable agents for glucose control in people with diabetes impact lipid levels, mostly by improving glycemia, though there may also be other glucose independent effects.

In general, people with and without diabetes respond similarly to lipid drugs regarding effects on lipid levels and cardiovascular risk reduction, as discussed in other chapters herein.

Other drugs such as hormone therapies (such as the oral contraceptive pill, hormone replacement therapy, testosterone, corticosteroids, thiazide diuretics, and some anti-retroviral drugs for HIV) can affect lipoproteins [2].

Thus, as well as glucose and insulin levels and insulin resistance, there are many aspects of the diabetes milieu, including inherited and acquired factors that can impact lipoprotein levels, composition, and function in diabetes. Lipoproteins can also impact glucose levels in people with diabetes, and this may even begin prior to diabetes onset [1–3]. The effects of glucose levels on lipoproteins will now be briefly overviewed. More details are found in other chapters in this book.

Table 3.2 Effects of glycemia on lipoproteins and lipoprotein metabolism

Altered lipid levels and metabolism
Altered lipoprotein size, density, and composition
Non-enzymatic glycation, which alters metabolism and cellular handling
Impact on lipoprotein oxidation and glyco-oxidation
Altered immunogenicity
Altered lipoprotein function

Effects of Glycemia on Lipoproteins and Lipids

There are multiple effects of glucose on lipoproteins and lipoprotein metabolism, summarized in Table 3.2, and discussed briefly below. This area is also expanded upon in other book chapters herein.

Hyperglycemia and high free fatty acid levels (both of which occur with suboptimal insulinization and/or marked insulin resistance) increase hepatic VLDL production and reduce VLDL clearance rates via reduced activity of vascular endothelial lipoprotein lipase (LPL) activity and by non-enzymatic glycation of apolipoproteins. Hyperglycemia also reduces the rate of HDL production and impairs HDL maturation, partly related to increased non-enzymatic glycation of HDL which increases HDL turnover rates [1–3]. Insulin resistance, common in people with Type 2 diabetes and present in some with Type 1 diabetes [8, 17], is associated with an increase in the ratio of ApoCIII/ApoCII, which reduces LPL activity the function of which is to enhance triglyceride transfer from triglyceride-rich lipoproteins to HDL, which are more rapidly removed by hepatic lipase. The rate of LDL clearance is also slowed by non-enzymatic glycation of LDL [18–20]. The level of non-enzymatic glycation of apolipoproteins in lipoproteins is influenced by both ambient glucose levels, the duration of lipoprotein exposure, lipoprotein subclasses [20], and other lipoproteins, such as HDL [21]. Hence, fasting triglycerides and HDL-C levels are often correlated with concurrent HbA1c levels (positive for triglycerides and negatively for HDL-C) and will usually improve somewhat with better glucose control.

Lipoprotein glycation may also promote lipoprotein oxidation and glycoxidation, which require research laboratory assays for their quantification. As well as altering lipoprotein turnover, these changes can sometimes also adversely impact cellular interactions with receptors, immunogenicity and cellular responses, including worsening the pathogenicity of lipoproteins, such as by promoting foam cell formation and reducing HDL's vasoprotective actions [18–26]. Examples include HDL glycation reducing HDL-associated paraoxonase activity, and its anti-oxidant and anti-inflammatory efficacy [27]. Increased glycation of lipoproteins enhances their immunogenicity, and the resultant lipoprotein and antibody immune complexes can increase foam cell formation, promoting the macrovascular and microvascular complications of diabetes [24, 26]. These changes are discussed in more detail elsewhere in this book, including in chapters on lipoprotein glycation and lipoprotein immune complexes.

Effects of Lipoproteins on Glycemia and Insulin

HDL Effects on Glucose Metabolism

HDL can lower glucose by both insulin-dependent and insulin-independent methods, with HDL effects in the pancreas, liver, skeletal muscle, adipose tissue, and myocardium. HDL can protect against cellular apoptosis, including that of insulin producing pancreatic beta cells, and HDL and ApoA1 can also promote pancreatic islet cell release of insulin. In the liver, HDL activates (phosphorylates) AMPK, increases expression of insulin receptors, and suppresses enzymes for gluconeogenesis. In skeletal muscle, HDL and ApoA1 can also activate AMPK, increasing glucose uptake. In adipose tissue, it enhances adiponectin, an insulin sensitizer. In the myocardium, HDL can decrease glycogen content, which is greatly increased in the hearts of people and animals with diabetes relative to non-diabetic subjects, and that glycogen overload may contribute to diabetic cardiomyopathy [3, 28].

In basic science experiments, HDL has been shown to inhibit endoplasmic reticulum stress-induced apoptosis of pancreatic β-cells [29]. In a double-blind, placebo controlled cross-over study in 13 adults with Type 2 diabetes, a single intravenous infusion of rHDL transiently increased their HDL levels and reduced their blood glucose levels and increased insulin levels [30]. Large scale clinical trials of CETP inhibitors which substantially increase HDL-C and ApoA-1 levels also support HDL roles in glycemia. In the "Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events" (ILLUMINATE) trial, the CETP inhibitor, torcetrapib, improved glycemic control in statin-treated patients with Type 2 diabetes [31]. Another CETP inhibitor trial, the "Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib" in Patients at a High Risk for Vascular Outcomes' (ACCELERATE) trial, glycemia was also improved in Type 2 diabetes participants [32].

Hypertriglyceridemia

High triglycerides can induce insulin resistance and hyperglycemia. The body's level of insulin resistance has been positively correlated with the level of skeletal muscle triglycerides, which usually correlated with circulating triglyceride and free fatty acid levels [33, 34]. The intravenous infusion of lipids can worse insulin resistance and inflammation, even in lean non-diabetic subjects [35]. Lowering triglyceride levels such as in people with severe hypertriglyceridemia can also improve insulin sensitivity and glycemia. Some lipid drugs in development, such as a monoclonal antibody targeting ANGPTL3, lowered triglyceride levels by about two-thirds, LDL-C levels by one-third, and also reduced hepatic fat and improved insulin resistance in early phase human trials [36].

Severe hyperglycemia, often associated with inherited forms of hypertriglyceridemia and an environmental trigger, such as obesity or drugs such as the pill or thiazides, can trigger acute pancreatitis, which can lead to destruction of both the endocrine and exocrine pancreas, inducing insulin requiring diabetes and need for digestive enzyme replacement [2, 37].

Associations Between Lipid Levels and Diabetes Onset

Type 2 Diabetes

HDL: Evidence is mixed as to whether low HDL levels are a risk factor for Type 2 diabetes. Many observational studies in humans support inverse associations between HDL-C levels, HDL particle numbers, and apoA1 levels even years before the development of Type 2 diabetes [38–42]. In the PREVEND study, both lower HDL-C and HDL-C/ApoA1 were independent predictors of Type 2 diabetes onset [38] and in the Diabetes Presentation Program (DPP) on-study rises in HDL-C were associated with lower rates of progression from pre-diabetes to Type 2 diabetes in the control, intensive lifestyle and metformin arms [43].

Lipid variability has also been associated with increased risk of Type 2 diabetes [44]. In 45,911 Chinese patients with three TG and HDL-C measures during 2006–2011, average real variability (ARV) was calculated and participants subdivided into tertiles of TG/HDL-ARV. There were 3724 cases of incident diabetes during follow-up. The 7-year cumulative incidences of diabetes in TG/HDL-ARV tertiles 1, 2, and 3 were 6.13%, 8.09%, and 11.77%, respectively. Results remained significant after adjustment for mean TG/HDL-C ratio, TG/HDL-C ratio change slope, fasting plasma glucose variability (ARV), and other traditional risk factors for diabetes. The HR for new-onset diabetes was 1.38 (1.25–1.50) for the highest tertile, with risk of diabetes increasing by 4% per 1 standard deviation (SD) increase in TG/HDL-C ratio variability [44].

HDL-related genetics: In contrast, a very large Mendelian randomization study in the general population ($n = 47{,}627$) evaluating genes associated with HDL levels does not support associations between low HDL levels and subsequent Type 2 diabetes [45].

ApoCIII: High levels of serum apoCIII, an inhibitor of LPL, which leads to elevated triglycerides, and the ratio apoCIII/apoA1, have also been found to be independent predictors of subsequent Type 2 diabetes [46].

Lipoprotein(a): While higher levels of Lp(a) are associated with increased risk of cardiovascular disease, some studies support that high levels of Lp(a) are associated with lower risk of Type 2 diabetes [47]. In a prospective study of 26,746 healthy US women (mean age 54.6 years), baseline Lp(a) concentrations were related to incident type 2 diabetes ($n = 1670$) over a 13-year follow-up. Analyses were adjusted for risk factors including age, ethnicity, smoking, hormone use, family history, blood pressure, CRP, BMI, HbA1c, and lipids. Baseline Lp(a)

levels were inversely associated with incident diabetes, with fully adjusted hazard ratios (HRs) and 95% CIs for quintiles 2–5 versus quintile 1: 0.87 (0.75–1.01), 0.80 (0.68–0.93), 0.88 (0.76–1.02), and 0.78 (0.67–0.91); p for trend 0.002. Results were confirmed in a study in 9652 Danish men and women with prevalent diabetes ($n = 419$) [47].

Type 1 Diabetes

Lipids have also been implicated in the development of Type 1 diabetes in humans. While the clinical onset of Type 1 diabetes is quite acute, often with symptoms (polyuria, polydipsia, weight loss) for only days to weeks pre-diagnosis, autoantibodies to insulin producing cells in the pancreatic islets of Langerhans are usually present for many years pre-diagnosis. The AMORIS cohort followed 591,239 people in Sweden from 1985–1996 up until 2012, during which time 1122 people developed Type 1 diabetes. Levels of triglycerides and ApoB/ApoA1 were positively associated with Type 1 diabetes risk, and higher ApoA1 levels were associated with lower Type 1 diabetes incidence. Even 15 years pre-diagnosis triglycerides, uric acid (which can reflect insulin resistance) [48], and glycemia (reflected by glucose and fructosamine levels) were higher in subsequent Type 1 diabetes cases vs. non-cases [48]. These changes may relate to lipotoxicity and glucotoxicity effects on islets, which are relevant to the pathogenesis of both Type 1 and Type 2 diabetes.

HDL and Glycemic Progression in Type 2 Diabetes

There are associations between HDL levels, insulin resistance, and the progression of Type 2 diabetes to need for pharmacologic glucose control [49, 50]. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial in adults with Type 2 diabetes HDL-C and HDL-C/ApoA1 at baseline predicted glycemic progression in adults with Type 2 diabetes over a median of 5 years follow-up. To be eligible for the FIELD trial, people had to have Type 2 diabetes with a lipid profile that did not merit lipid lowering drugs by treatment standards then. Total cholesterol levels had to be 3.0–6.5 mmol/L, triglycerides ≥1.0 mmol/L, HDL-C <1.5 mmol/L, or TG/HDL-C ratio ≥4.0. People with severe hypertriglyceridemia or with marked renal or liver dysfunction were excluded from the trial, reducing the generalizability of results.

In the FIELD trial, HDL-C levels did not correlate significantly with concurrent HbA1c levels, but in the 13,900 subjects screened, with a wider range of HDL-C levels than those 9795 who progressed to the trial, HDL-C correlated weakly with HbA1c levels ($r = -0.030$, $p < 0.001$). For the 9795 participants, HbA1c was not correlated with HDL-C, ApoA1, or HDL-C/ApoA1; but these three HDL-related measures were weakly correlated with HOMA-IR and HOMA-B (all $p < 0.001$) and HOMA-B adjusted for HOMA-IR correlated weakly with ApoA-1 ($r = -0.041$, $p < 0.001$) [50].

At baseline 2698 of the 9795 participants had their glycemia managed by lifestyle measures alone: mean age 62 years, 38% female, mean known diabetes duration 2 years, fasting triglycerides 1.68 mmol/L, HDL 1.11 mmol/L, Apo-A1 1.3 g/L, and HDL-C/Apo A1 0.33. On age and sex-adjusted analyses, HDL-C, ApoA1, and HDL-C/ApoA1 levels did not correlate significantly with concurrent HbA1c levels. However, in these 2608 subjects, baseline HDL-related measures were significantly inversely correlated with insulin resistance: HOMA-IR (HDL-C, $r = -0.245$; ApoA1, $r = -0.169$; and HDL-C/ApoA1 $r = -0.254$, all $p < 0.05$). Only ApoA1 significantly, albeit weakly, correlated with HOMA-Beta ($r = -0.063$, $p < 0.05$). Thus, HDL levels were more strongly associated with measures of insulin resistance and secretion than with concurrent glycemia, however baseline HDL-related measures were strongly associated with progression to need for glucose control drugs [50].

In the FIELD trial glucose management was not part of the trial and was left to the usual treating doctors, with national guidelines recommending up-titration of glucose management at HbA1c levels over 7% (53 mmol/mol). Of the 2608 subjects on lifestyle measures for glucose control at baseline 1520 subjects (58%) progressed to needing oral glucose drugs and/or insulin injections. At the time of the FIELD trial, this was usually sulfonylureas, metformin, and insulin. Incretin based drugs and SGLT2 inhibitors were not routinely available. Even so glucose management was up-titrated (by the usual care doctors, not the trialists) during the FIELD trial at a mean HbA1c of 7.1% (54 mmol/mol) and achieved a mean HbA1c level of about 7% (53 mmol/mol) during the (median 5-years) trial. There was no significant difference in changes in HbA1c or drug up-titration by fenofibrate or placebo allocation in the FIELD trial. Higher HDL-C and higher HDL-C/ApoA1 levels, but not ApoA1 levels, at baseline were associated with significantly longer periods of glucose management by lifestyle only measures. Comparing the first vs. fourth quartile of HDL-C/ApoA1 and of HDL-C levels, there were a mean of 24- and 13-months delay, respectively, in need for glucose control drugs (HR 1.51, $p < 0.01$ for HDL-C/ApoA1 and HR 1.26, $p = 0.02$ for HDL-C). Analyses were adjusted for age and sex. There were no significant differences by fenofibrate or placebo allocation [50]. Significance was retained for HDL-C and HDL-C/ApoA1 retarding need for glucose control drugs even when adjusted for HOMA-IR, BMI and HbA1c, WHR, renal function, liver function test, smoking and alcohol use, exercise, female menopause status, and statin or RAAS drug use. Significance of HDL alone but not that of HDL/ApoA1 as a predictor of glycemic progression was lost when adjusted for baseline triglycerides. As HDL-C/ApoA1 reflects smaller HDL particles, we speculate that smaller HDL particles may be particularly relevant to protection against the glycemic progression of Type 2 diabetes [50].

Glucose and Lipid Variability and Diabetes Complications

Associations between glycemia and traditional lipid levels are recognized with higher HbA1c levels usually being associated with higher triglycerides and lower HDL-C levels and with more adverse VLDL, LDL, and HDL subclass profiles.

Correlations usually positive between HbA1c and calculated or measured LDL-C levels are less strong than between HbA1c and triglycerides and LDL, but associations with a shift to more pathogenic small dense LDL are recognized [1–3]. More recently, variations in glucose and in traditional lipid levels [51–56] have been associated with chronic complications and with mortality, though there are relatively fewer studies of lipid variability and diabetes complications than of glucose variability. As a number of measures of glycemia and its variability are now available and they cover different time frames, we now briefly review glycemia measures which have been or could be related to lipids and lipid variability and to health outcomes in clinical studies.

Aspects of Glycemia

Longer term measures: There are several aspects of glucose metabolism to consider, summarized in Table 3.3. Most measured in clinical practice are *HbA1c* levels, a longer term measure of non-enzymatic glycation of hemoglobin which reflects mean blood glucose levels over the previous 2–3 months. Higher HbA1c levels are exponentially associated with increased risk of microvascular complications of diabetes (retinopathy, nephropathy, and neuropathy) and also with risk of macrovascular complications (cardiovascular, cerebrovascular, and peripheral vascular disease) [52].

Intermediate-term measures of glycemia are fructosamine, glycated albumin, and 1,5 anhydroglucitol reflect mean glucose levels over shorter time frames than

Table 3.3 Clinical and research measures of glucose metabolism

Hyperglycemia: Glucose (by blood test or interstitial fluid Continuous Glucose Monitor (CGM) time above range (TAR), HbA1c, fructosamine, 1,5 anhydroglucitol, glycated albumin)
Hypoglycemia: Glucose (by blood test or interstitial fluid CGM time below range (TBR))
Glucose variability
HbA1c CV or SD
Blood glucose CV or SD
Continuous Glucose Monitor (CGM)-based glucose CV, or MAGE or CONGA
Insulin or C-peptide levels
Insulin sensitivity/insulin resistance
Hyperinsulinemic, euglycemic clamp studies
Estimated glucose disposal rate (eGDR)
Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)
HOMA-beta (both HOMA-IR and HOMA-beta are calculated from fasting insulin and glucose levels in non-insulin treated subjects)
Insulin secretion in response to oral or intravenous glucose
Pancreatic islet beta cell mass
Markers of pancreatic islet cell death, e.g., microRNA signatures
Adipokines, e.g., adiponectin, an insulin sensitizer
Glycogen stores, e.g., in heart, liver, or skeletal muscle

HbA1c. *Fructosamine*, which is widely available for clinical use, and *glycated albumin*, a research tool, are non-enzymatically glycated plasma proteins which reflect mean blood glucose over about 2 weeks. Plasma or serum *1,5 anhydroglucitol* (1,5AG) levels, which are used clinically in some countries and as a research tool in others, reflects levels of a naturally occurring metabolically inert monosaccharide present in almost all foods, that competes with glucose for reabsorption in the kidneys. Blood levels fall with glycosuria, which usually occurs with blood glucose levels over 10 mmol/L (180 mg/dL) and reflects glucose control over the previous 2 weeks. Unlike HbA1c, fructosamine, and glycated albumin levels, 1,5-AG levels more accurately reflect glucose variability and post-prandial glucose levels [5].

Short-term measures of glycemia are reflected by changes in glucose levels of days to a week or two. Increasingly commonly used *Continuous Glucose Monitoring* (CGM) systems enable the measurement of interstitial fluid glucose levels every five to 15 min for six to 14–180 days, depending on which commercial system is used. Many CGM systems can alarm to alert the wearer and sometimes a carer to high or low glucose levels, and some when linked with a compatible insulin pump can increase or decrease insulin delivery [57]. International consensus groups now recommend glucose targets for CGM metrics for clinical and research use with glucose CV recommended to be <36% [58].

Hypoglycemia: While links between hyperglycemia and chronic diabetes complications are well-recognized, hypoglycemia has also been associated with increased risk of vascular complications. Hypoglycemia can prolong the cardiac QT interval and induce cardiac arrhythmias, which can cause sudden cardiac death [59]. An episode of hypoglycemia can also cause vascular endothelial dysfunction, inflammation, and oxidative stress which can last for several days [60]. CGM are reliable systems to detect low glucose levels [57].

Glucose variability has also been associated with increased vascular dysfunction, inflammation, oxidative stress, and risk of macrovascular and microvascular complications and of mortality [52, 54]. While relevant data are available in many existent and planned trials, there are few reports of the role of lipids and lipid variability in the pathogenesis of diabetes complications and of their mitigation by therapeutic interventions. Most of those available to date are positive.

Lipids and Lipid Variability in Diabetes and Effects on Chronic Complications

Both lipid and HbA1c levels have been associated with chronic diabetes complications and mortality, often with non-linear (U or J-shaped associations between HbA1c and lipids and complications) [61–63]. The time course, magnitude, and causes of variations in lipid levels are not as well-studied as those in glycemia. Associations between lipid variability, chronic complications, mortality, and other modulators are less well-studied. It is yet unclear as to the links between lipid and

glucose variability on the development of events, such as microvascular complications, macrovascular events, and mortality.

In most, but not all, major cohort studies variability in lipid levels has been associated with chronic diabetes complications and with death. Similar to HbA1c, in a very large (n = 25,186) study of Asians with diabetes, there was heterogeneity in the associations of HDL-C variability with adverse outcomes. Some studies report higher risk of adverse events with greater HDL-C variability, while others do not find statistically significant associations [64–68]. Factors contributing to variations in findings may be differences in study size, study duration, laboratory measures, the number of lipid measures, underlying genetics, and interactions by lifestyle factors such as smoking, diet, and statistical analyses, including whether adjustment for mean levels is performed and whether the standard deviation (SD) or coefficient of variability (CV) is used. Standardization of variability measures between studies can assist with meta-analyses and the use of lipid variability measures in clinical practice.

In the Italian AMD Annals database, 7560 Type 2 diabetic patients with at least five measures of traditional lipid levels (total cholesterol, triglycerides, LDL-C, HDL-C), HbA1c, systolic and diastolic blood pressure, and uric acid over 3-years were followed for up to 5-years. The impact of risk factor variability on the risk of diabetic kidney disease was assessed. Lipid variability was not significantly associated with risk of developing albuminuria in 4231 subjects, but prediction was strong when considering variability in both HDL-C and HbA1c (HR = 1.47; 95% CI 1.17–1.84). Variability in HDL-C and in LDL-C significantly predicted loss of eGFR to <60 mL/min/1.73^2 [69].

In a large Asian study of 25,186 people with Type 1 diabetes or insulin-treated Type 2 diabetes (mean age 63 years, 50% male) attending Hong Kong public hospitals during 2009, the variability of total cholesterol, LDL-C, HDL-C and triglycerides and HbA1c was related to all-cause mortality (primary endpoint) and to (secondary outcomes) diabetes complications. Lipid and HbA1c variability were significant predictors of all-cause mortality, and for incident cardiovascular, cerebrovascular, peripheral vascular disease, heart failure, and atrial fibrillation ($p < 0.05$). There were also significant correlations between lipid variability, like HbA1c, and the baseline blood neutrophil-lymphocyte ratio (reflecting inflammation) [56].

Another large multicenter study in Asia found similar results for mortality and also for non-fatal CVD [70]. In a retrospective cohort study of 125,047 Type 2 diabetes patients aged 45–84 years, managed in primary care during 2009–2012 variability (SD) of LDL-C, TC/HDL-C, and TG (in mmol/L) was related to a composite endpoint of CVD events (n = 19,913) and death (n = 15,329) over a median follow-up of 77.5 months, including 0.8 million person-years. Positive linear relationships between lipid variability and the clinical endpoint (CVD and death) were identified. Each unit increase in the variability of LDL-C, TC/HDL-C, and TG was associated with increased risk of CVD or death: LDL-C, 27% (HR: 1.27 [95% CI: 1.20–1.34]); TC/HDL-C, 31% (HR: 1.31 [95% CI: 1.25–1.38]); and TG 9% (HR: 1.09 [95% CI: 1.04–1.15]). Age-specific effects for 45–54 y.o. subjects were found for LDL

variability (HR: 1.70 [95% CI: 1.42–2.02]) with a 53% increased risk for the composite endpoint than those aged 75–84 y.o. (HR: 1.11 [95% CI: 1.01–1.23]). Similar age effects were observed for TC/HDL-C and TG variability [70].

Lipid variability has also been demonstrated to be a risk factor for kidney disease in people with Type 2 diabetes [71]. LDL-C, TC/HDL-C, and TG variability were evaluated in a retrospective cohort study of 105,552 Type 2 diabetes patients aged 45–84 with normal urine albumin to creatinine ratio and eGFR >60 mL/min/1.73m^2 who attended Hong Kong public primary care clinics during 2008–2012. Variabilities of LDL-C, total cholesterol to HDL-C ratio, and triglyceride were determined using the standard deviation of the respective parameter obtained from a mixed effects model to minimize regression dilution bias. The associations between LDL-C, TC/HDL-C, and TG variability and incident kidney disease, ≥30% reduction in estimated glomerular filtration rate (eGFR) since baseline, and end-stage renal disease (ESRD: eGFR <15 mL/min/1.73 m^2) were evaluated with a median follow-up of 66.5 months (0.5 million person-years in total), during which 49,653 new-onset kidney disease cases, 29,358 with renal function decline, and 1765 with ESRD developed. There were no associations with TGs, but positive linear associations with LDL-C and TC/HDL-C were found. Each mmol/L increase in LDL-C variability was associated with 20% (HR 1.20 [95% CI 1.15–1.25]), 38% (HR 1.37 [95% CI 1.30–1.45]), and 108% (HR 2.08 [95% CI 1.74–2.50]) higher risk in incident kidney disease, renal function decline, and ESRD, respectively. Similarly, each unit increase in TC/HDL-C was associated with 35% (HR 1.15 [95% CI 1.10–1.20]), 33% (HR 1.33 [95% CI 1.26–1.40]), and 75% (HR 1.75 [95% CI 1.46–2.09]) heightened risk in incident kidney disease, eGFR loss, and ESRD, respectively.

Such data for diabetic retinopathy, and for Type 1 diabetes and for other ethnic groups are of interest. Potential medicators of lipid variability may be alternations in glycemia, in diet, exercise, smoking, and use of lipid modulating drugs. The effects of interventions that reduce lipid variability on clinical events including the development and progression of microvascular and macrovascular complications and of mortality are merited. As lipotoxicity, as well as glucotoxicity, is implicated in the progression of Type 2 diabetes, the effects of lipid variability on the need for pharmacologic agents for glucose control in people with Type 2 diabetes are merited.

While the underlying mechanisms linking lipid variability are unclear, hypotheses include increased oxidative stress and inflammation, and that large fluctuations in LDL and HDL can induce plaque instability and proatherogenic substances.

Lipid Drugs and Effects on Glycemia

Some lipid drugs have been associated with changes in glycemia, with divergent effects, predominantly between classes, summarized in Table 3.4. Both direct and indirect effects may be involved. Effects on glycemia and may differ between non-diabetic and diabetic subjects and between humans and animals.

Table 3.4 Effects of lipid drug classes on glycemia

Drug class	Example(s)	Effects on glycemia
Predominantly LDL-lowering drugs		
Statins	Atorvastatin Rosuvastatin Pravastatin Simvastatin Pitavastatin	May worsen glucose and HbA1c ↑ Risk of new-onset diabetes (NOD)
Bempedoic acid		No worsening of glycemia or NOD May improve glycemia
PCSK9 inhibitors	Evolucmab	No change or perhaps mild worsening of glycemia. No NOD
Ezetimibe		No change or mild improvement
Resins	Cholestyramine Colesevelam Colestimide	Moderate improvements in glucose and HbA1c, with no risk of hypoglycemia Colesevelam approved by FDA for glucose (and lipid) lowering in T2D
Predominantly TG-lowering drugs		
Fibrates	Fenofibrate Pemafibrate Bezafibrate	No substantial effect of PPARα agonists (e.g., fenofibrate) Fibrates with PPAR alpha and gamma activity (e.g., bezafibrate) improve glucose and HbA1c
Omega-3 fatty acids	Omacor	Inconsistent results due to different sources, types, and doses
Fish oils		Overall—no major benefit or harm re glycemia and diabetes
Niacin/nicotinic acid		Worsens glucose and HbA1c, more so in diabetes subjects Less marked with low dose, slow titration, and slow-release preparations Reversible with cessation
Predominantly HDL-elevating drugs (currently research only)		
rHDL	Lower glucose, increase insulin	
CETP inhibitors	Lower glucose and HbA1c	

LDL-Lowering Drugs

The effect of *statins* on glycemia is most well-studied. While HMG CoA reductase inhibitors (statins) have major primary and secondary cardioprotective effects in diabetes [72], this class has been associated with mild elevations in glucose (in non-diabetic and diabetic subjects) and HbA1c and increased risk (by about 9%) of new-onset (Type 2) diabetes, (NOD) [73–78]. Most statins are thought not to worsen insulin sensitivity, and pravastatin may even improve insulin sensitivity in non-diabetic subjects [74]. The risk of NOD is higher with older age, higher statin doses, more potent statins, greater (>50%) LDL-C reductions, longer statin use and the presence of pre-diabetes or the metabolic syndrome. Mechanisms may relate to HMGCoA reductase inhibition, direct drug effects, and altered intracellular lipids, which decrease insulin sensitivity and/or beta cell function [79]. While new-onset diabetes would increase the risk of diabetes complications and of CVD, the overall cardiovascular and death risk-benefit ratio for most people being offered statin therapy is deemed favorable [79].

Bempedoic acid is the first drug in a relatively new drug class of lipid lowering drugs, the adenosine triphosphate-citrate lyase (ACL) inhibitors, which blocks hepatic cholesterol production at a different site than HMG CoA reductase inhibitors. In a meta-analysis of 11 trials ($n = 4391$ general population subjects), the drug significantly reduced LDL-C (median 22.9%, 95% CI 27.3–18.5), CRP (median 24.7%, 95% CI 32.1–17.3), composite cardiovascular events (RR 0.75, 95% CI 0.56–0.99) and significantly reduced rates of NOD or worsening of glucose levels (RR 0.65, 95% CI 0.44–0.96) [80]. Another meta-analysis of five bempedoic acid trials with at least 4-weeks follow-up and 2419 bempedoic acid treated subjects and 1210 control arm subjects specifically addressed glycemia and NOD and was confirmatory. Bempedoic acid allocation was associated with a significant reduction in new-onset or worsening diabetes [Odds Ratio: 0.66, 95% CI: 0.48–0.90; $I2$: 0%]. It is speculated that the drug may reduce gluconeogenesis [81].

PCSK9-inhibitors. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors given by subcutaneous injections every 2–4 weeks are even more potent LDL-C lowering drugs than statins, with similar efficacy for lipid and cardiovascular benefit in people with and without diabetes. In a recent meta-analysis (38 trials, $n = 68{,}123$ subjects), there was no significant effect on glucose control reflected by fasting glucose, HbA1c or NOD [82]. Longer term follow-up will be of interest.

Ezetimibe, a once daily selective inhibitor of cholesterol absorption from the intestine, acts predominantly via inhibition of the Niemann-Pick C1-Like 1 transporter on the brush border of enterocytes. In animal studies, ezetimibe has improved glucose tolerance, insulin sensitivity, and insulin production and may also have incretin effects. In humans, ezetimibe effects range from no changes in glycemia or insulin sensitivity [83] to small (about 0.3%) HbA1c reductions (even with concomitant statins) and lower fasting insulin levels, with no significant difference in glucose levels [79].

Resins/bile acid sequestrants. While often not as potent or as well-tolerated as other LDL-lowering drugs, and sometimes increasing triglyceride levels, this drug class has been associated with small to modest reductions in glucose and HbA1c levels, including in adults with existent Type 2 diabetes. In a meta-analysis of bile acid sequestrants in adults with Type 2 diabetes in 17 trials, with 2950 subjects randomized to colesevelam or colestimide or to placebo resin allocation were associated with statistically and clinically significant lower HbA1c % levels (mean difference 0.55%, 95% CI 0.64–0.46%) [84]. Indeed, in 2012 the USA FDA approved colesevelam for use in Type 2 diabetes patients as an adjunct to improve glycemia. Suggested mechanisms related to the resin effects on removing bile acids which also interact with various membrane and nuclear receptors [79, 84].

Triglyceride Lowering Drugs

PPAR subtypes alpha, gamma, and beta/delta exist. Fenofibrate is a PPAR alpha agonist. Despite lowering triglycerides and free fatty acids, in the fenofibrate based FIELD, ACCORD and DAIS trials, there were no significant changes in glycemia in their Type 2 diabetes participants [79]. However, as discussed above, in the

FIELD trial, higher HDL and HDL/ApoA1 levels were associated with retarding the need for glucose lowering drugs in adults with Type 2 diabetes [50], but there was no difference between those allocated fenofibrate or placebo.

PPAR gamma activation increases glucose uptake by skeletal muscle and decreases hepatic glucose production, hence as expected, fibrates with PPAR gamma activity, such as bezafibrate (which activates all three PPAR subtypes) have been associated with improvements in glycemia and insulin sensitivity [79]. In a Japanese study 6-months of bezafibrate was associated with lower glucose and HbA1c (% units) reductions of 0.47% in all subjects and by 0.76% in those with baseline HbA1c levels >7% (53 mmol/mol), with HbA1c reductions correlating with triglyceride reductions [85].

Fish oils: Results vary depending on whether fish oils are taken by diet or supplement, and the dose, duration of treatment, and type of fish oils. A Cochrane systematic review of 23 randomized controlled trials of *n*−3 PUFA supplements in people with Type 2 diabetes found no significant changes in fasting glucose, fasting insulin, or HbA1c levels [86]. Several meta-analyses report no significant deterioration in glucose or HbA1c levels in people with Type 2 diabetes [86]. Similarly, no effects on insulin sensitivity were identified in a systematic review of *n*−3 PUFA in diabetic and non-diabetic subjects [87].

Niacin and nicotinic acid: In human studies, this drug class worsens glucose control (reflected by glucose and HbA1c levels) likely by worsening insulin resistance. Lower doses and slow-release preparations are usually less adverse [79]. In a meta-analysis of 11 trials with 26,340 non-diabetic participants, 1371 were assigned niacin and 646 were assigned control tablets, with a mean of 3.6 years follow-up. Niacin was associated with a RR of 1.34 (95% CIs 1.21–1.49) for new-onset diabetes, with limited heterogeneity between trials ($I2 = 0.0\%$, $p = 0.87$). The number needed to treat for 5 years to develop one additional case of diabetes was 43 (95% CI 30–70). Results were similar whether subjects also received a statin or laropiprant [88].

Marked HDL-C Elevating Drugs (Research Only)

IV infusion of rHDL on humans with Type 2 diabetes has been associated with improved glucose levels, which may relate to HDL, including its components apoA1 and apoAII, promoting insulin secretion and activating AMPK in skeletal muscle [89].

While not in clinical use, CETP inhibitors, which markedly elevate HDL levels, have been shown to significantly lower glucose and HbA1c levels, likely by enhanced beta cell insulin secretion [79]. In the ILLUMINATE trial, HbA1c (in % units) was reduced by 0.33% after 3-months treatment with the CETP inhibitor torcetrapib and atorvastatin in people with Type 2 diabetes [90]. There was no increase in new-onset Type 2 diabetes.

Future Directions

Other studies of interest include analyses of existent and future trials regarding effects of lipid levels, lipid variability, and lipid drugs on various measures of glycemia, insulin levels, and insulin sensitivity. Relevant subgroups include non-diabetic subjects, those with pre-diabetes, Type 1 diabetes, and Type 2 diabetes. Lipid drug mechanisms of action, potency, and duration of therapy should be considered. The reversibility of any adverse glycemic effects is of interest. The understanding of underlying mechanisms of action and the development of clinically effective drugs that improve both lipids, glycemia and reduce vascular events and mortality rates are desirable.

Conclusions

Abnormalities in lipoproteins and of glycemia and insulin sensitivity often co-exist. There are complex bidirectional relationships between lipoproteins and glucose, some of which are favorable and others adverse. Treatment of glucose levels usually improves lipoprotein levels, but the treatment of lipid levels, particularly with drugs, has variable effects of glucose metabolism depending on drug types. Some drugs are beneficial for both lipids and glycemia. Relatively new concepts are the relationship between lipid and glucose variability and adverse clinical outcomes. Very few studies have evaluated both, nor evaluated the causes of lipid variability and the consequences of its treatment. Consideration of the bidirectional relationships between lipoproteins and glycemia is important in both clinical practice and in research. Drugs that favorably modulate both lipoproteins and glycemia are desirable.

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Chapter 4
Apoproteins and Cell Surface Receptors Regulating Lipoprotein Metabolism in the Setting of Type 2 Diabetes

Thomas D. Dayspring and Peter P. Toth

Introduction

The epidemic of diabetes mellitus that is occurring throughout the world portends a profound increase in the incidence of macrovascular atherosclerotic disease, the leading cause of death among diabetic patients, in addition to its comorbidities, such as myocardial infarction, ischemic stroke, need for revascularization, and ischemic cardiomyopathy. Diabetes, especially type 2 diabetes mellitus (T2DM), is a lipidosis which has classically been described as a secondary lipid disorder. Lipids are broadly defined as hydrophobic, nonpolar molecules that are insoluble in an aqueous phase, but are freely soluble in nonpolar solvents. Physiologically lipids contribute to numerous biological processes, including energy transduction, cell membrane and organelle structure, membrane function, cellular signaling pathways, the activation and resolution of inflammation, as well as steroid hormone and bile acid biosynthesis. Some lipids are amphipathic molecules having unique molecular structures where one end of the molecule is polar, and the other is not: with the polar end having some water solubility and the other end being hydrophobic. Such molecules are critically important for the structure of lipid monolayers (lipoproteins and micelles) and lipid bilayers (cell membranes). Within membranes are specific areas of "functional" lipids termed lipid rafts where protein expression and other actions such as caveolae formation occur.

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Although there are multiple risk factors that are associated with or result in atherosclerotic disease, there is only one *sine qua non* for the disease, namely an accumulation of sterols within arterial wall macrophages (foam cells) [1]. It is crucial to recognize that hydrophobic sterols, noncholesterol sterols, and glycerolipids are trafficked in plasma and potentially into the arterial wall as components of protein enwrapped particles called lipoproteins, making atherogenesis a lipoprotein mediated disease [2, 3]. Lipid and lipoprotein biology and physiology are immensely complex field of investigation, and the purpose of this chapter is to first review basic sterol and glycerolipid biochemistry; lipid homeostasis including the synthesis, absorption, and incorporation of sterols and lipids into lipoproteins; and then examine what changes occur and the consequences of those changes in the T2DM patient. Lipid homeostasis is regulated by several nuclear transcription factors which mediate lipogenesis, cellular membrane proteins involved with lipoprotein lipidation and delipidation, catabolic receptors, lipolytic enzymes, and lipid transfer proteins.

Lipoprotein Structure and Nomenclature

There is a continually ongoing, dynamic remodeling of lipoprotein particles where lipid molecules and surface apoproteins are gained and lost and re-acquired through complex pathways involving neutral lipid interchange between particles, hydrolysis of lipids (lipolysis), as well as particle catabolism [4]. Simply stated, lipoproteins and their lipid content are in a continuous state of flux determined by genetics and metabolic milieu, and such behavior is often not reflected in standard lipid concentration measurements. Lipoproteins were originally separated by their density and were named from most to least buoyant as chylomicrons, very low density (VLDL), intermediate density (IDL), low density (LDL), and high density lipoproteins (HDL). The lipoprotein particles can be further separated into sub-particles of incremental buoyancies and sizes ranging from large (more buoyant) to smaller (less buoyant or dense). Buoyancy and density are determined by the molecular weight of the lipid and protein molecules in a given particle and in general proteins have much higher molecular weights than lipids. Thus, the large, lipid-rich lipoproteins are the most buoyant, and the smaller lipid-poor, protein-rich lipoproteins are denser. Within a specific family of lipoproteins, the smaller subspecies are always denser than the larger ones and the terms “small and dense” or “large (fluffy) and buoyant” is therefore redundant, i.e., a small LDL is by definition a dense LDL.

The glycerolipids, triglycerides or triacylglycerols (TG) and phospholipids (PL), are molecules in which three and two fatty acids (FA), respectively, are esterified to glycerol, a three-carbon carbohydrate. TG serve as a carrier of energy (9 kcal/g) which can be oxidized in muscles or stored in adipocytes. Typically, PL consist of a saturated and a long chain polyunsaturated FA including the omega-6 and omega-3 FA. Plasma PL not present in lipoproteins are bound to phospholipid transfer protein (PLTP) [5], and plasma FA not found in PL or TG are bound to albumin and referred to as free fatty acid or nonesterified fatty acids. Sterols are divided into

zoosterols which include free or unesterified cholesterol (UC) and its precursors such as lathosterol and phytosterols (several exist, with β-sitosterol and campesterol being the most common). Molecules that have a structure that is very similar to cholesterol are termed noncholesterol sterols. A saturated sterol is a stanol and is characterized by the absence of the double bond at the Δ5 position in the B ring. For example, β-sitosterol (a sterol), when reduced, becomes sitostanol (a stanol). One cholesterol metabolite is cholestanol which is the stanol form of cholesterol. UC has a –OH (hydroxy) group at the #3 carbon position of the A ring, whereas cholesteryl ester (CE) has the –OH group replaced via the action of the enzyme acyl-cholesterol acyltransferase (ACAT) of which two isoforms exist ACAT1 and ACAT2, with a long chain fatty acid (typically palmitic or oleic acid) forming cholesteryl palmitate or cholesteryl oleate. Unlike cholesterol, phytosterols are not good substrates for ACAT and are not readily esterified in enterocytes or hepatocytes.

Lipoproteins are protein enwrapped collections of several lipids including UC, CE, noncholesterol sterols, TG and PL whose collective function is to traffic lipids to and from various tissues. All lipoproteins consist of a surface monolayer of amphipathic PL and UC which surrounds the particle core, consisting of a variable mixture of hydrophobic TG and CE molecules (Fig. 4.1). Thermodynamic forces play a large role in lipoprotein organization and structure [6]. The charged amino acids of apoproteins and phospholipid head groups of PLs project into the aqueous phase. Hydrophobic CE and TG are concentrated in the core of the particles away from aqueous plasma, and this biochemical property is the reason that, as lipoproteins lipidate, they become spherical; because the volume of a sphere is a function of the third power of the radius, this vastly expands the number of TG lipid molecules and their 9 kcal/gm of energy that can be trafficked. Providing structure, stability, and aqueous solubility in plasma to the lipids are proteins, termed apoproteins, which intermingle with the surface and core lipids. Once an apoprotein is lipidated, it is an apolipoprotein. Apart from structural functions, they also serve as ligands for various receptors and enzymes that participate in particle formation and catabolism. Specific lipoproteins have very different core lipid concentrations which can dynamically vary particle to particle in the same and different individuals [7]. PL are amphipathic molecules, and this unique property allows their polar end to interact with water in plasma, enhancing lipoprotein plasma solubility.

Although there are many known apolipoproteins with multiple functions (Tables 4.1 and 4.2), the main lipoprotein structural peptides are apolipoprotein B (apoB) and apolipoprotein A-I [8]. ApoB, the only non-exchangeable apolipoprotein exists in two isoforms: the hepatically synthesized $apoB_{100}$ and an intestinally expressed truncated $apoB_{48}$, so named as it has 48% of the molecular weight of $apoB_{100}$. $ApoB_{48}$ is missing the LDL receptor binding domain [9]. VLDL, IDL, and LDL particles contain a single molecule of apolipoprotein B_{100}; HDL particles from one to five molecules of apolipoprotein A-I [10]. Under normal physiologic conditions, the plasma residence time of VLDL is approximately 2–6 h, IDL ~ 1 h, and LDL 1.5–3 days [11]. Therefore, approximately >90% of apoB actually represents LDL particle concentration (LDL-P) and apoB is thus not particularly informative in quantifying VLDL particle concentration (VLDL-P) (Fig. 4.2) [12, 13].

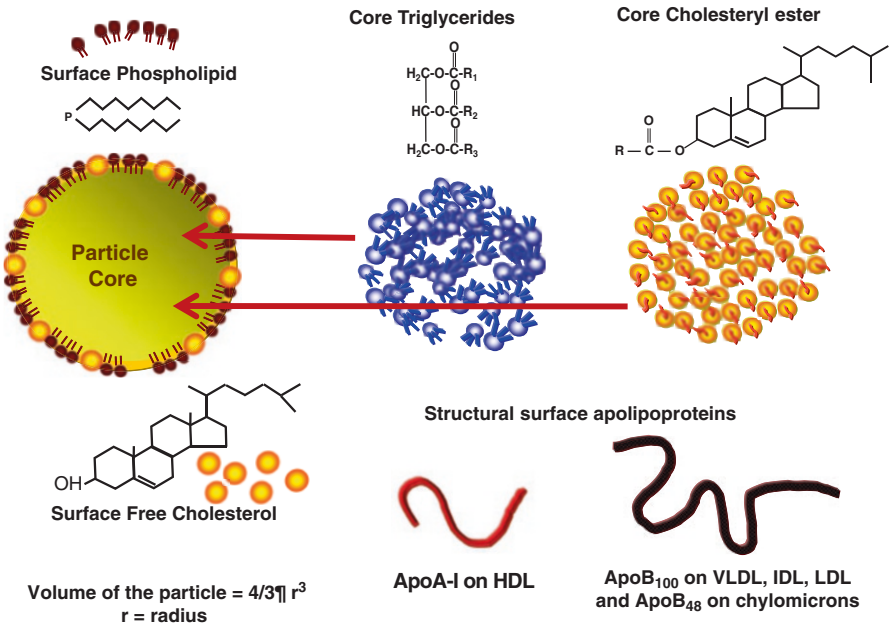

Fig. 4.1 Lipoprotein structure involves amphipathic surface molecules PL and UC, and the core consists of a variable mixture of nonpolar CE and TG. Providing structure and solubility are surface apolipoproteins. Because the volume of a sphere is related to the third power of the radius, even small lipoproteins can traffic significant numbers of lipid molecules per particle

Table 4.1 Human apolipoproteins

Apolipoprotein	Molecular weight	Lipoprotein association	Function
ApoA-I	28,331	HDL, chylomicrons	Activates ACAT, ABCA1
ApoA-II	17,380	HDL, VLDL, Chylos	FFA metabolism, RCT, antiox
ApoA-IV	44,000	Chylomicrons, HDL	Chylo production, RCT, LCAT
ApoA-V	39,000	Chylos, VLDLs, HDLs	TG metabolism
ApoB-48	240,000	Chylomicrons	Structural protein
ApoB-100	513,000	VLDL, IDL, LDL	Binds to LDL receptor
ApoC-I	7000	VLDL, HDL	Inhibits C-II, CETP
ApoC-II	8837	Chylos, VLDL, HDL	Activates lipoprotein lipase
ApoC-III	8751	Chylos, VLDL, HDL	Inhibits lipoprotein lipase
ApoD	32,500	HDL	CETP
ApoE	34,145	Chylos, VLDL, IDL, HDL	Binds to LDLr and LRP

The apoA-I family of lipoproteins are the HDL class, and they remain the most complex, polyfunctional, and enigmatic of all lipoproteins. HDLs not only traffic cholesterol, TG and PL, but a very large collection of other lipids including, fat-soluble vitamins, and a large number of phospholipids, including phosphatidylcholine, sphingomyelin, and ceramide all of which are associated with many biological

Table 4.2 Lipoprotein properties

	Density (kg/L)	Composition by weight (% by weight)				Relative volume[a]	Apolipoproteins
		UC + CE	TG	PL	Protein		
Chylomicron	<0.95	~5	~90	~4	~1	700,000	B-48, A-I, C-I, C-II, C-III, E
VLDL	<1.006	25	55	18	8	360	B-100, A-II, C-I, C-II, C-III, E
IDL	1.006–1.019	Between a VLDL and LDL					B-100, E
LDL	1.019–1.063	55	6	20	~20	32	B-100
HDL_2	1.063–1.125	22	5	33	40	3	A-I, A-II, C-I, C-II, C-III, E
HDL_3	1.125–1.210	17	3	25	55	1	A-I, A-II
Lp(a)	1.04–1.13	48	9	21	22	~LDL	B-100, a

[a] For the purpose of comparison, HDL_3 is assigned a value of 1

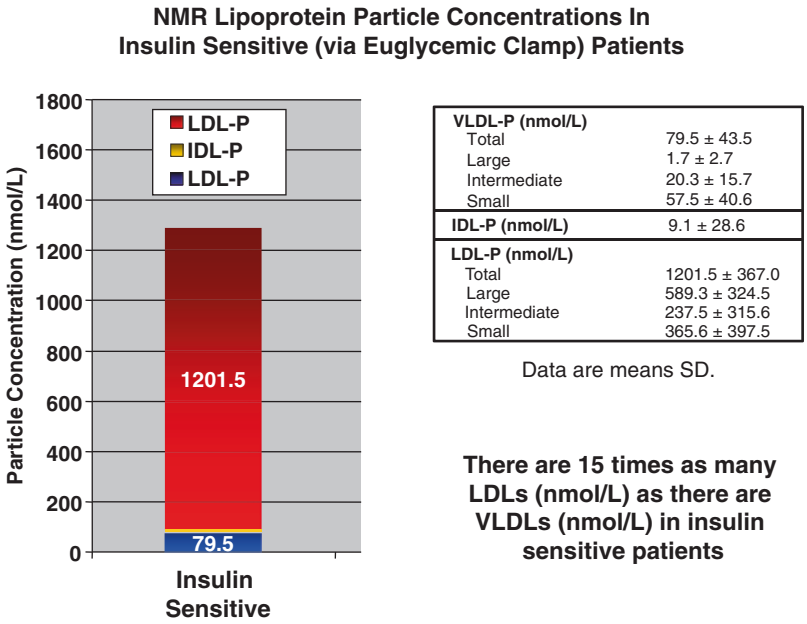

Fig. 4.2 Lipoprotein particle concentrations in insulin sensitive (via euglycemic clamp) patients measured by nuclear magnetic resonance. (Adapted from Garvey WT, et al. Diabetes 2003;52:453–62)

functions [14]. Collectively these are referred to as its lipidome and numerous proteins (over 1000 different proteins have been identified) referred to as its proteome [15, 16]. HDL particles transport many type of proteins, enzymes, globulins,

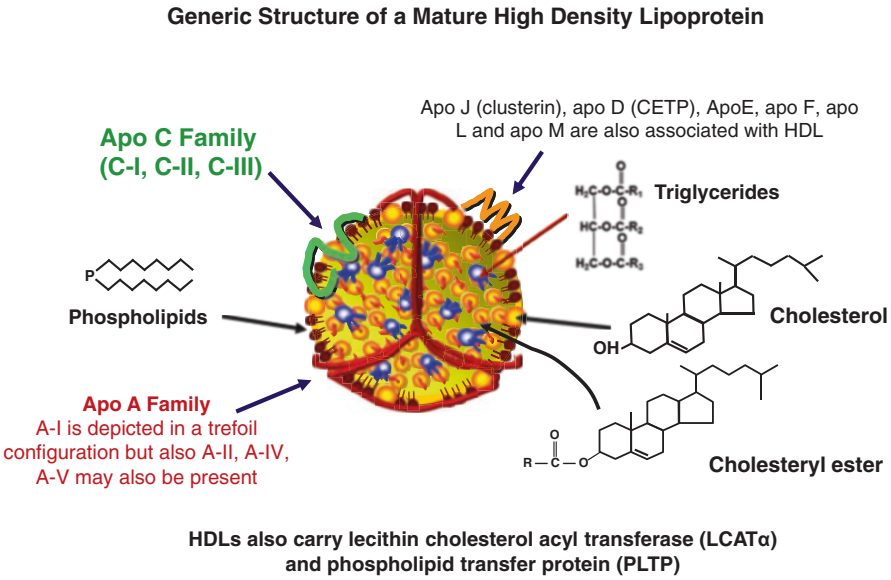

Fig. 4.3 HDL particles are very small lipoproteins with a core of TG and cholesterol ester. The major surface apoproteins are A-I, A-II, or A-IV, members of the apoC family, and apoE. HDL-C refers to the cholesterol mass in all of the HDL particles in a deciliter of plasma, apoA-I to the apoA-I mass on all of the HDLs per deciliter of plasma, and HDL-P to the number of HDL particles per liter of plasma

complement and other components of immunity, coagulation factors, acute phase reactants, and apoproteins [17]. The protein cargo can vary as function of coronary disease status and metabolic milieu [18–20]. HDLs are also an important means by which a large variety of microRNAs are transported in plasma [21, 22]. No doubt the HDL proteome regulates its range of biological activity, collectively referred to as its functionality. With respect to lipid trafficking, HDLs acquire sterols from cells effluxing UC, esterify the acquired UC using lecithin cholesterol acyltransferase (LCAT), and then deliver the UC and CE elsewhere. HDLs are in a constant and dynamic state of lipidation and delipidation or remodeling by virtue of their interactions with a variety of cell membrane sterol transporters, lipid transfer proteins, and lipolytic enzymes [23]. There are many genetic, lifestyle, hormonal, metabolic, and inflammatory influences on HDL's makeup and function and many of these may be manipulated by pharmacologic and lifestyle interventions. Structurally HDLs are similar to all lipoproteins with a surface monolayer of PL and UC and a core of mostly CE, but also a small amount of TG. The main structural protein of HDL is 1–5 copies of apolipoprotein A-I arranged in a "trefoil" configuration (Fig. 4.3) [10].

HDL nomenclature can be very confusing, and some terms are technology dependent. There is a numerical ultracentrifuge classification where super-large

HDLs (not always present) are called HDL_1. As the particles shrink in size, the names change to HDL_{2b}, HDL_{2a} (both large with b being larger than a) and HDL_{3a}, HDL_{3b} and HDL_{3c} (with 3_a being the largest and 3_c the smallest). These terms are also used by labs utilizing ultracentrifugation or gradient gel electrophoretic fractionation. NMR spectroscopic classification of HDLs uses the terms: H1 through 5 (with 5 being largest). Labs using 2D electrophoresis with apoA-I staining report apoA-I, prebeta-HDL, and the α-HDL subspecies. Some labs report how much cholesterol is in various HDL subspecies (i.e., HDL_2-C). The newest attempt from a group of experts to simplify HDL naming is to simply refer to the particles as very small, small, medium, large, and very large. Classically those species have been called HDL_1 (very large), HDL_{2a} and HDL_{2b} (large), HDL_{3a}, HDL_{3b}, HDL3c (medium or small), and unlipidated apoA-I or prebeta (discoidal). Another system differentiates unlipidated apoA-I and prebeta HDLs (1 and 2) from large alpha HDLs (1, 2, 3, and 4 ranging from very large to small). NMR cannot measure unlipidated apoA-I or prebeta HDLs, but because of their transient existence, they only represent about 5% of total HDL-P (Fig. 4.4) [24].

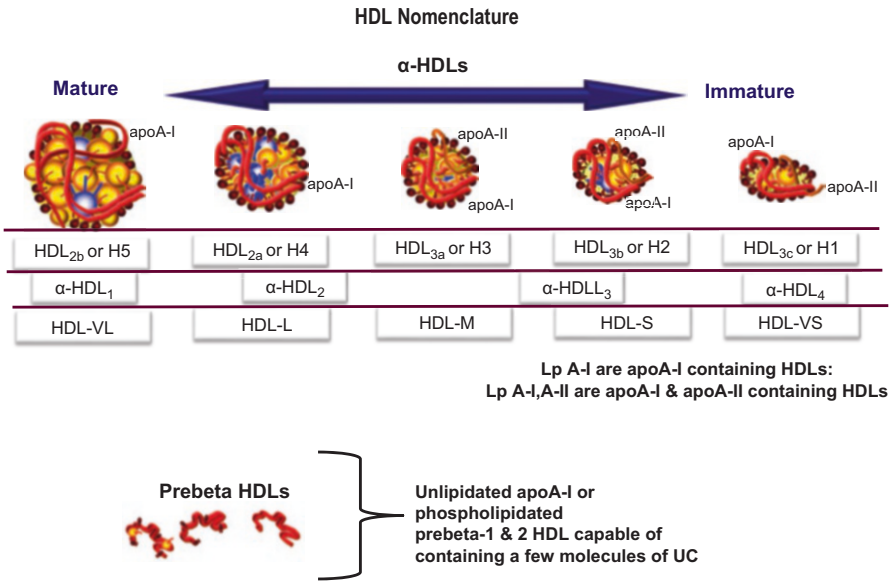

Fig. 4.4 HDL particles can be separated by two-dimensional electrophoresis and apoA-I staining into prebeta and alpha-lipoproteins. The former are unlipidated apoA-I or phospholipidated A-I's. Each HDL particle can have from 1 to 5 molecules of apoA-I. The α-HDLs range in size from small and dense to larger and more buoyant. There can be from one to five molecules of apoA-I per HDL particle, thus apoA-I is only an approximation of the number (concentration) of HDL particles. ApoA-II is present, predominantly on the smaller HDL species

Measurement of Lipids and Lipoproteins

There are many ways of measuring lipoproteins in the laboratory including analyzing their density, their surface apolipoproteins, their core lipid content (expressed as TG or cholesterol mass per deciliter of plasma), or by NMR spectroscopy which determines the number of terminal methyl groups on CE, TG, and PL and translates that to particle numbers [25]. In clinical practice, the majority of clinicians evaluate lipoproteins using lipid concentrations such as total particle cholesterol or TG or subparticle cholesterol mass per unit of plasma volume [26]. With respect to lab nomenclature and lipoprotein particle concentrations, (P) is added to the particle abbreviation and the value is expressed as nanomoles (nm) or micromoles (μmol) per liter. Lipoprotein particle concentrations can be determined using apolipoproteins, nuclear NMR [27], ion mobility transfer technologies [28], or ultracentrifugation with LDL staining.

Total VLDL-P = chylomicron-P + large VLDL-P + medium VLDL-P + small VLDL-P
Total IDL-P (not typically separated into subparticles)
Total LDL-P = Large LDL-P + medium LDL-P + small LDL-P
Total HDL-P = Large HDL-P + medium HDL-P + small HDL-P

With respect to cholesterol mass measurements: (-C) is added to the particle abbreviation and the value is expressed as mg per deciliter (dL) or millimoles per liter (mmol/L).

Total cholesterol = VLDL-C + IDL-C + LDL-C + HDL-C + Lp(a)-C
Calculated VLDL-C = TG/5 using the Friedewald formula [29]
Calculated LDL-C = IDL-C + LDL-C + Lp(a)-C using the formula LDL-C = TC − [HDL-C + VLDL-C]
Non-HDL-C = TC − HDL-C = apoB-C

One should never confuse specific lipoprotein lipid and lipoprotein concentrations such as LDL-C with LDL-P or apoB or HDL-C with HDL-P. Each is a valid way of measuring LDL or HDL and when they correlate highly (r values) with each other as they often do, they are said to be concordant. However, when cholesterol and lipoprotein particle concentrations do not correlate, they are said to be "discordant." With respect to LDLs and HDLs, it is common to have high or low LDL-C and HDL-C with respective low or high LDL-P and HDL-P values. Lipoproteins that are CE-poor will require larger numbers of particles to traffic a given amount of core cholesterol and conversely cholesterol-rich LDL or HDL will require fewer particles to traffic the cholesterol mass. The cholesterol mass or number of cholesterol molecules per particle is a function of both the particle volume and core lipid makeup. Since the volume of a sphere is $4/3\pi(r^2)$, even subtle changes in particle diameter can cause tremendous changes in the number of particles required for lipid trafficking. The same is true of the particle core ratio of TG to CE [30, 31]. Adding to potential discordance between cholesterol mass and particle concentrations is that both calculated and directly assayed cholesterol concentrations often fail to meet accuracy standards [32, 33].

Cellular Lipid Homeostasis

Because cholesterol can crystalize and cause cytotoxicity, cells maintain tight cholesterol homeostasis. All cells can synthesize UC as well as acquire it and FA through a variety of methods, including cell membrane receptors which function as sterol or FA influx transporters or lipoprotein delipidation or internalization receptors (Table 4.3). Cells can also efflux sterols via free diffusion, a family of sterol efflux proteins called ATP binding cassette transporters (ABC) [34] and UC, CE, TG, and PL can be exported by lipoprotein synthesis and secretion. Specialized cells such as enterocytes and hepatocytes can acquire UC through the sterol influx protein Niemann-Pick C1 like-1 protein (NPC1L1), which is expressed at both the jejunal lumen/enterocyte and the hepatobiliary interfaces [35]. NPC1L1 binds cholesterol via a sterol sensing domain and is responsible for the uptake of biliary and dietary sources of cholesterol as well as for counterbalancing hepatobiliary cholesterol excretion [36]. Many cells express the scavenger receptor B1 (SR-B1), a bidirectional transporter which can participate in the efflux or influx of CE [37]. Sterols can also be effluxed from enterocytes and hepatocytes into the gut lumen or bile, respectively, using the sterol efflux transporters ABCG5 and ABCG8 [38]. Mutations in the latter two transporters give rise to β-sitosterolemia, a condition that phenotypically mimics familial hypercholesterolemia and results in the development of premature multivessel coronary disease secondary to large elevations in serum phytosterols [39]. Cells can also acquire UC and CE via receptors that internalize lipoproteins such as LDL receptors (LDLr) [40], LDL receptor related proteins (LRP) [41], or ectopic β-chain of apoA-I synthase [42]. There are also putative receptors yet to be classified that perform these functions including an enterocyte protein involved with transintestinal cholesterol efflux (TICE) [43]. Cellular sterol homeostasis is regulated through synchronized action of all of the above mechanisms.

With respect to lipids, the human diet includes TG, FA, UC, CE, and phytosterols and, to a lesser degree, some stanols. Intestinal esterases and lipases convert some of the ingested CE into UC and TG to FA and monoacylglycerols. However,

Table 4.3 Synthesis, remodeling, and catabolism of circulating HDL particles

Cell surface membrane receptors
Lipoprotein endocytosis
LDL receptor (LDLr)
LDL receptor related protein (LRP)
ApoA-I beta chain synthase or holoparticle receptor
Influx transporters
Niemann-Pick C1 like-1 protein (NPC1L1)
Scavenger receptor B1 (SR-B1)
Fatty acid transport proteins (FATP)
Efflux transporters
ATP binding cassette transporters
ABCA1, ABCG1, ABCG, ABCG8
Scavenger receptor B1
Putative transintestinal cholesterol efflux transporter

after a meal, the majority of the UC in the jejunum is of biliary origin. All of the lipids in the gut lumen are collectively organized and emulsified by lecithin (e.g., phosphatidylcholine) which is a phospholipid in bile. The lipids are then surrounded and organized by amphipathic bile acids into mixed biliary micelles which consist of collections of UC, phytosterols, stanols, phospholipids, monoacylglycerols, and FA. The micelles "ferry" these lipids to the epithelium of the intestinal microvilli. Once there, FA are absorbed into enterocytes by passive diffusion or membrane-located fatty acid transport proteins [34, 44]. The unesterified sterols, but not stanols in the micelles, are internalized by the enterocyte via a sterol permease NPC1L1 protein which utilizes other proteins (AP2-clathrin) to facilitate sterol absorption [45]. NPC1L1 is not involved with FA absorption and in part is regulated by PPAR-α and –Δ and is expressed at both the brush border of the intestinal epithelium and at the hepatobiliary cell junction [46]. Most humans absorb about 50% of the sterols in the gut, but some people are hyperabsorbers (60–80%) and some are hypoabsorbers (~20–40%) [47]. NPC1L1 expressed at the hepatobiliary interface where it facilitates re-entry of biliary UC back into the liver. Only UC, not esterified sterols, can pass through NPC1L1. Once UC enters an enterocyte or hepatocyte, it is subject to esterification catalyzed in enterocytes and hepatocytes by ACAT2 or within lipoproteins catalyzed by LCAT. Unlike UC, phytosterols are poor substrates for human ACAT and LCAT. Thus ACAT2 in the enterocyte is a major regulator of sterol absorption [48]. Upon esterification, UC (the active and amphipathic form of cholesterol) becomes CE (the storage or transportation, hydrophobic form of cholesterol). In hepatocytes, UC can upon exposure to 7α-hydroxylase be converted into the primary bile acids (cholate or chenodeoxycholate) which are effluxed into the biliary system via the bile salt export protein (ABCB11) [49].

The Apolipoprotein B Family of Lipoproteins

In the endoplasmic reticulum of enterocytes and hepatocytes, CE via the action of microsomal TG transfer protein joins with TG and apolipoprotein B_{48} (enterocyte) or B_{100} (hepatocyte) to form nascent chylomicrons or VLDL, respectively. Phospholipidation and additional TG lipidation of the particle occur at the Golgi apparatus resulting in mature TG-rich lipoproteins. Evidence suggests that apolipoprotein A-V (apoA-V) may inhibit this process as apoA-V synthesis modulates VLDL-TG mobilization as well as secretion [50]. In addition, apoA-V suppresses angiopoietin-like 3/8 inhibition of lipoprotein lipase, thereby allowing for greater lipolysis of triglycerides in serum [51]. Also involved as a regulator of chylomicron synthesis and lipid absorption is apolipoprotein A-IV (apoA-IV) which because of its large size functions as a stabilizing, expandable lipid interface, enhancing particle formation. Interestingly, through effects on the hypothalamus and vagus nerve (gastric), apoA-IV also serves as a mediator of satiety and appetite [52]. ApoA-IV also modulates the activation of lipoprotein lipase by apoC2 [51], participates in the

efflux of cholesterol from macrophages driven by ABCA1 [53], and is an activator of LCAT on HDL particles [54].

Under physiologic conditions, the largest lipoproteins, chylomicrons, and VLDLs traffic large amounts of TG and PL, which are released during TG hydrolysis (de-esterification) process called lipolysis. Under fasting conditions, Friedewald noted almost all of the plasma TG are trafficked within VLDL particles and a typical VLDL carries five times more TG than cholesterol and thus VLDL-C can be estimated by dividing TG/5 (Fig. 4.5) [29]. That calculation changed the practice of clinical lipidology as it allowed clinicians to calculate LDL-C using the formula LDL-C = TC − [HDL-C + VLDL-C]. If one assumes a normal TG value is <150 mg/dL, then a desirable VLDL-C is ≤30 mg/dL [55]. The American Heart Association defines an optimal TG to be <100 mg/dL [56]. As hydrolysis of TG occurs during lipase-mediated lipolysis, the TG-rich lipoproteins shrink and shed much of their surface PL which are picked up by PLTP and delivered to cells or maturing HDL particles. As the large TG-rich VLDL loses its core and surface lipids, it becomes smaller and denser. An LDL is basically a VLDL that has lost its TG and is therefore a cholesterol-enriched lipoprotein with a core of four or more times CE than TG. Any LDL independent of its size that has an excess core TG will be necessarily CE poor [57]. Normally HDLs traffic very little TG and their core is 90–95% CE, hence TG-rich HDLs will be cholesterol-poor which can cause a low HDL-C value. The apoB-containing lipoproteins acquire their core lipids during their genesis in

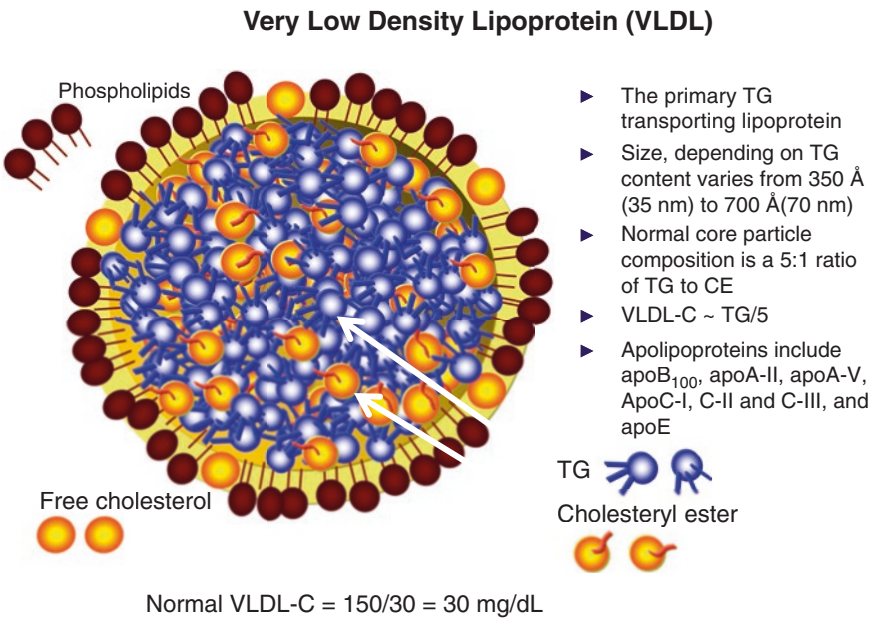

Fig. 4.5 Very low-density lipoprotein is a large TG-rich particle that carries several apoproteins: $apoB_{100}$, apoA-II, apoA-V, apoC-I, apoC-II and apoC-III, and apoE. The primary function of VLDL is to traffic TG to myocytes and adipocytes and PL to the periphery

enterocytes or hepatocytes, whereas the apoA-I particles are sterol lipidated via a variety of cell membrane efflux transporters. Lipoproteins can also undergo additional lipidation or delipidation using a lipid transfer protein called apolipoprotein D (apoD) or cholesteryl ester transfer protein (CETP) which can exchange or swap one molecule of CE for CE, TG for TG, or CE for TG (often referred to as neutral lipids as they do not carry any charged group) [58]. The transfer of lipids between members of the apoB family themselves or the apoA-I family themselves is called a homotypic exchange, whereas the swapping of lipids between apoB and apoA-I particles is termed heterotypic. This lipid exchange is crucial to efficient lipid trafficking and dynamic remodeling of lipoproteins. Any lipoprotein acquiring TG will be subject to the lipolytic action of numerous lipases and thus TG-rich LDLs and HDLs would tend to become smaller and denser.

Other than apoB and apoA-I, there are numerous other apolipoproteins present on lipoproteins which perform multiple functions (Fig. 4.6) [7]. With the exception of apoB, all apoproteins are exchangeable, meaning they can transfer between lipoprotein species. Some function as ligands that direct and bind the lipoproteins to various cell membrane receptors or endothelial surface molecules, some are involved

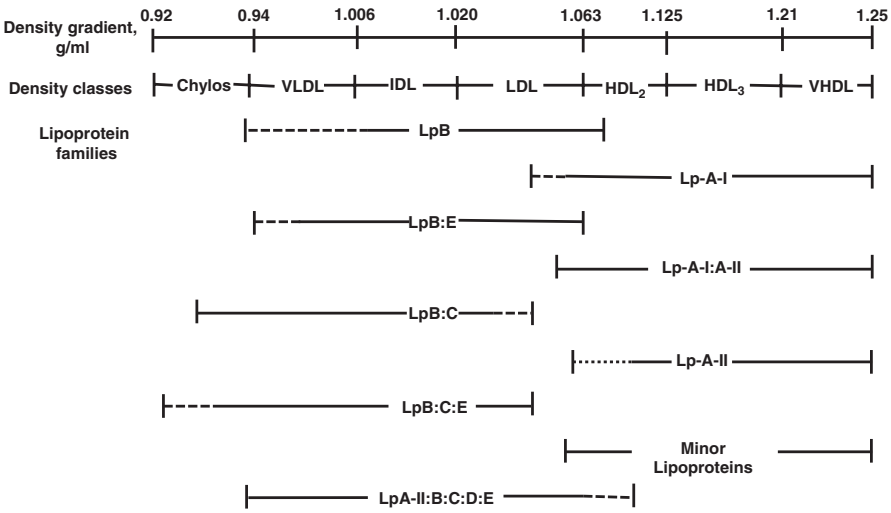

Fig. 4.6 The relationship of individual apolipoprotein A (apoA)- and apoB-containing lipoprotein (Lp) families defined by their unique apolipoprotein composition to major lipoprotein density classes against the density (*d*) gradient background (*d* = 0.92 to 1.25 g/mL). The lines under lipoprotein families designate the approximate density boundaries, with *solid lines* depicting the actual localization of each lipoprotein family and *dotted lines* depicting the possible localization of each lipoprotein family. Lipoprotein families represent polydisperse systems of particles, each of which has a different lipid/protein ratio, but the same qualitative apolipoprotein composition. The polydisperse character of lipoprotein families is the main reason for their overlap within certain density segments. *Chylos* chylomicron, *HDL* high-density lipoprotein, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein, *VHDL* very high-density lipoprotein, *VLDL* very low-density lipoprotein. (Reproduced with permission from Alaupovic P. Curr Atheroscler Rep 2003;5:459–67)

with activation or inactivation of various lipolytic enzymes such as lipoprotein lipase and other enzymes, and some serve as lipid acceptor proteins. Many of the apoproteins exist as genetically determined isoforms, which create individual and population differences in lipoprotein metabolism as well as differences in levels of risk for ASCVD [59].

Under normal physiologic conditions when lipoproteins have the proper core ratios of lipids, the following lipid trafficking pathways are operative. TG-rich chylomicrons are secreted into the lymphatic circulation (chylomicrons) where they make their way into plasma via the thoracic duct and join hepatically synthesized TG-rich-VLDLs [60]. ApoA-V is part of TG-rich lipoprotein formation [50] and traffics with the particle as do multiple copies of apolipoprotein C-II (apoC-II), a ligand for LPL, apolipoprotein C-I (apoC-I) and C-III (apoC-III) (Fig. 4.7) [61–63]. ApoA-I is synthesized in hepatocytes and jejunal enterocytes and is secreted into plasma where it is rapidly lipidated but some apoA-I is also initially carried into plasma on chylomicrons [64]. Delipidation of TG-rich particles or lipolysis occurs as TGs are hydrolyzed by lipoprotein lipase (LPL), a potent triglyceridase upregulated in large part by insulin in muscle and adipocyte vascular beds [65]. ApoA-V is important in the docking of TG-rich lipoproteins to heparan sulfate proteoglycans (HSPGs) in endothelial cell lipid rafts in the area of LPL expression and thus enhances lipolysis. ApoA-V is also involved with docking of lipoproteins to the LDLr and LRP [66]. Chylomicron lipolysis is normally quite rapid due to its large

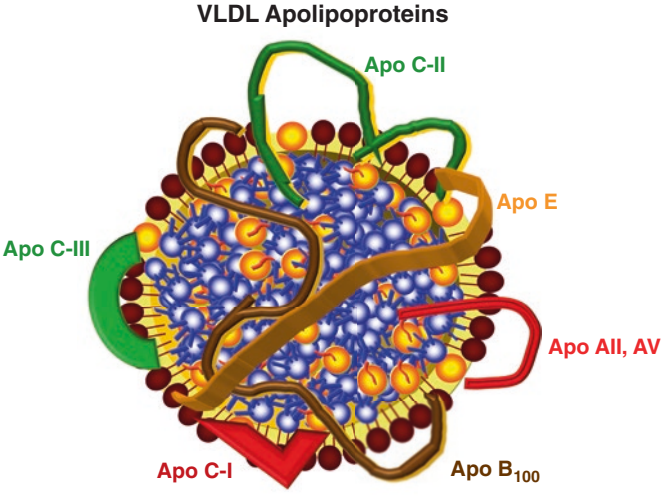

Fig. 4.7 Very low-density lipoprotein is a large TG-rich particle that carries several apolipoproteins. ApoC-II is a ligand for lipoprotein lipase. ApoC-I blocks LPL, HL, CETP, LCAT, VLDLr, and LDLr. ApoC-II binds to and activates LPL. ApoC-III interferes with apoC-II/LPL binding and apoE binding to cell surface lipoprotein receptors. ApoB is a ligand for the LDL receptor. ApoE is a ligand for the LDL, VLDL receptor, and the LDL receptor related protein (LRP). ApoC-III inhibits the action of LPL and the ability of apoE to act with receptors. ApoA-II inhibits VLDL lipolysis, and apoA-V helps bind TG-rich lipoproteins to HSPG in areas of LPL expression

size and multiple copies of apoC-II resulting in smaller TG-poorer particles called chylomicron remnants. Two proteins, lipase maturation factor 1 (LMF1) and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) regulate LPL maturation and binding and thus are important mediators of TG-rich lipoprotein lipolysis [67]. LPL is synthesized in the endoplasmic reticulum of myocytes and adipocytes where LMF 1 plays an essential role in the formation of catalytically active LPL, a process called lipase maturation, which then translocates to the luminal surface of endothelial cells where it binds to HSPGs. GPIHBP1provides a platform for apoC-II binding to LPL. In vascular endothelia where GPIHBP1 is expressed, lipid rafts also express syndecan1 and fatty acid transporters such as CD-36 (Fig. 4.8) [68]. ApoA-V also facilitates interactions between the TG-rich lipoproteins and GPIHBP1 [69].

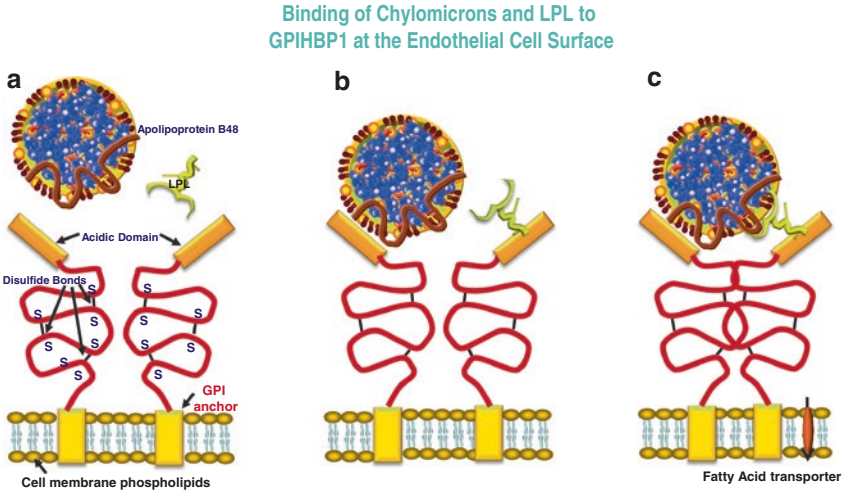

Fig. 4.8 Chylomicrons and lipoprotein lipase (LpL) at the endothelial cell barrier. Model for the binding of chylomicrons and LpL to glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GPIHBP1) at the endothelial cell surface (Adapted from Ory DS. Cell Metab 2007;5:229–31). (**a**) GPIHBP1 is tethered to the endothelial cell surface by a GPI anchor and contains an amino-terminal acidic domain that is proposed to be a specific binding site for both chylomicrons and LpL. (**b**) The acidic domain of GPIHBP1 may bind chylomicrons through interaction with positively charged domains of apolipoproteins exposed on the lipoprotein surface. The acidic domain of GPIHBP1 may also serve as the binding site for LpL, which contains positively charged heparin-binding domains. (**c**) Interaction between GPIHBP1-bound chylomicrons and LpL may involve clustering of the GPI-anchored proteins or homodimerization. Lipolysis of chylomicron-associated triglycerides liberates free fatty acids (FA), which are transported into endothelial cells

ApoA-IV contributes to lipolysis by facilitating efficient release of apoC-II from either HDL or VLDL, and once apoC-II is anchored by GP1HBP1, it binds to and activates LPL [65] resulting in hydrolysis of TG in chylomicrons or VLDL [70]. The resultant remnants are ultimately internalized by VLDL receptors in extrahepatic tissues [71] and by hepatic LDLr and LRP using apolipoprotein E (apoE) as a ligand. Another important regulator of TG-rich lipoprotein lipolysis is apoC-I which inhibits the binding of VLDL by LDLr, LRP, or the VLDL receptor [72]. Such inhibition of TG-rich lipoprotein binding to lipoprotein receptors is thought to be due to the ability of apoC-I to alter or camouflage the conformation of apoE on TG-rich lipoprotein or to displace apoE from these particles. ApoC-I is also known to inhibit LPL, hepatic lipase (HL), phospholipase A2, as well as CETP [73] where it accounts for most of the CETP-inhibitory activity that is associated with human plasma HDL [74]. A chylomicron half-life is normally less than an hour, whereas the duration of VLDL lipolysis ranges from 2 to 6 h.

ApoC-III which is synthesized in hepatocytes and enterocytes is also a potent regulator of lipolysis [75]. It is present in three isoforms related to the number of sialic acid molecules (0, 1, or 2) terminating the oligosaccharides portions of the protein, apoC-III_0, apoC-III_1, and apoC-III_2. In plasma, the isoform makeup is ~10%, 55%, and 35% of the total apoC-III levels. ApoC-III_1 and apoC-III_2 correlate more with TG levels than apoC-III_0 and apoC-III_2 is associated with generation of small LDL. Collectively apoC-III stimulates VLDL assembly and secretion, inhibits LPL, in part by affecting the binding of TG-rich lipoproteins to HSPG, and interferes with VLDLr, LRP, and LDLr uptake of lipoproteins [76, 77].

As TG molecules are hydrolyzed by lipoprotein lipase to FA and monoacylglycerols, the TG-rich lipoproteins shrink resulting in the loss of large amounts of their surface PL as well as some surface apolipoproteins. ApoC-III redistributes from VLDL to HDL and becomes ready for reuse and subsequent retransfer back to newly synthesized VLDL particles [78]. The now smaller particles carrying much less TG and PL still have their CE core: such particles are called VLDL and chylomicron remnants. The particles which were formerly TG-rich are now much less TG-rich and are trafficking primarily CE. The FA released from the TG can enter myocytes to be oxidized for energy or enter adipocytes and be reconverted to and stored as TG or bind to albumin and be trafficked for use elsewhere. The PL can be taken up by the cell membranes or bind to phospholipid transfer proteins (PLTP) and carried to other cells or to maturing (lipidating) HDL particles. Ultimately VLDL size decreases and density increases to the point where they become IDLs which under normal circumstances are rapidly removed by hepatic LDLr to which they dock via their $apoB_{100}$ and apoE. This receptor-attachment is aided by HL, which has both triglyceridase and phospholipase properties resulting in additional particle lipolysis creating apoB-containing LDL particles [79]. LDL is a predominantly cholesterol-rich lipoprotein with a core TG/CE ratio of ≥4:1 [57]. The LDLs typically circulate for 1.5–3 days before most (90%) are cleared by hepatic LDLr [80]. During their plasma residence time, LDLs are subject to homotypic exchange via CETP of their core CE for TG with VLDLs or heterotypic exchange with HDLs. CETP-mediated exchange of neutral lipids can be inhibited by apoC-I and

apolipoprotein F (apoF) [81]. Since every cell in humans can synthesize cholesterol de novo, very little LDL-mediated delivery of cholesterol is necessary. Persons with hypobetalipoproteinemia have very low levels of LDL-C and suffer no cholesterol deficiency consequences [82]. Normally LDLs are cleared by LDLr binding to apoB and the process of LDLs returning their core CE to the liver, much of which originated in HDLs is termed indirect reverse cholesterol transport.

The Apolipoprotein A-I Family of Lipoproteins

Aside from the apoB particles whose main purpose is to traffic TG and phospholipids, are the HDL family of apoA-I lipoproteins. Unlike chylomicrons and VLDLs, HDLs are created not in enterocytes or hepatocytes, but rather in plasma by the lipidation of secreted apoA-I and apolipoprotein A-II (apoA-II). Regulated primarily by PPAR-α, apoA-I is produced and released by hepatocytes and enterocytes. The unique helical structure of apoA-I gives it high affinity for cholesterol. Lipidation occurs at cells with excess UC in which activation of the liver X receptor (LXR) in turn upregulates cell membrane cholesterol efflux proteins including ABCA1. Unlipidated apoA-I accepts effluxed UC and PL, creating the prebeta HDL species. ApoA-I lipidation activates LCAT-α which catalyzes the transfer of fatty acids from the sn2 position of PL to the 3-hydroxy group on UC, changing the amphipathic UC into the hydrophobic CE. The molecule polarity change drives the CE away from the apoA-I particle surface of the HDL to its core explaining why, as the HDL matures, it goes from a discoidal to a spherical particle. ApoA-IV in HDL can activate LCAT and in free form in both lymph and plasma may also play critical roles in mediating ABCA1 cholesterol efflux. Additional HDL lipidation occurs via attachment of larger, more mature HDL species to ABCG1 sterol efflux transporters, SR-B1 or even by free diffusion from cells into larger HDL species [38]. As the HDL matures, it picks up, transfers, and reacquires numerous proteins (its proteome) including several apoproteins involved with lipoprotein catabolism and clearance including CETP, the apoC family, apoE, apolipoprotein A-II (apoA-II), apolipoprotein L (apoL), apolipoprotein M (apoM), and others involved in a multitude of functions [83].

With respect to trafficking UC and CE, the vast majority (>90%) of HDL lipidation occurs via ABCA1 expression at the liver, small intestine, and adipocyte tissue [84]. In effect a serum HDL-C represents cholesterol derived from the gut and the liver and is not a reflection of peripheral cholesterol efflux. This suggests that HDLs evolved not solely to perform RCT but also to engage in the delivery of hepatic and enterocytic UC, primarily to steroidogenic tissues and adipocytes [85]. The likely reason HDLs have a 5-day half-life is to serve as a rapidly available supply of CE for the adrenal cortex under stressful hypercorticoid conditions like inflammation and infection [86] and as a repository for urgently needed immunoproteins. Because of those functions, many refer to HDLs as an innate part of the immune system [87]. A major part of HDL's antiatherogenic potential is the ability to efflux both CE and

UC from sterol-laden macrophages (foam cells) in atherosclerotic plaque referred to as macrophage reverse cholesterol transport (ΩRCT). Compared to total body cholesterol, the amount of cholesterol in plaque is very small, and ΩRCT, although cardioprotective does not contribute significantly to a serum HDL-C value [88]. Other than trafficking cholesterol to the tissues mentioned above, HDLs through numerous pathways can facilitate fecal excretion of cholesterol. HDLs can return UC and CE to the liver where it is delipidated by SR-B1 or endocytosed by LDLr (using apoE as a ligand), or the ectopic beta chain of apoA-I synthase (holoparticle) receptor [42]. HDLs can also be delipidated by a putative enterocyte receptor and the UC exported to the gut lumen via ABCG5 and ABCG8 transporters in a process now termed transintestinal cholesterol efflux or TICE [43]. HDLs returning cholesterol to the liver or gut is called direct RCT [89]. However, a major part of RCT is HDLs heterotypically exchanging their CE for TG with apoB particles (chylomicrons, VLDLs, IDLs, and primarily LDLs). The apoB-particles now carrying a CE load acquired from HDLs are cleared at the liver, in essence returning substantial cholesterol in what is now called indirect RCT. Total RCT is the sum of direct and indirect RCT and it should be clear that a serum HDL-C by itself has no relationship with this complex and dynamic HDL mediated trafficking of cholesterol system [90]. The TG-rich HDLs undergo additional lipolysis utilizing HL and endothelial lipase [91]. During this process, some apoA-I is shed and is cleared via cubilin and megalin in renal tubules [92]. In effect, apoA-I is constantly being synthesized, secreted, lipidated delipidated and ultimately cleared at the liver, gut, or kidney.

In summarizing lipid homeostasis, fatty acids, and cholesterol, derived mostly from the liver and gut but also peripheral cells, are trafficked as components of lipoproteins: FA for energy and cell membranes and cholesterol for cell membranes and steroidogenesis. Unneeded cholesterol in the form of UC and CE is returned to the gut for fecal elimination or to the liver where UC is secreted into bile or converted to a bile acid for potential fecal excretion or become part of a newly formed VLDL or effluxed to a prebeta HDL. The system obviously is complex and mediated by dozens of genes, enzymes, proteins and receptors and pathology in any of those will negatively affect lipid (energy and sterol) homeostasis.

Insulin Resistance and Type 2 Diabetes

In the remaining part of the chapter, lipoprotein pathology related to insulin resistance (IR) and/or T2DM will be reviewed. A normal person is sensitive to insulin, which regulates carbohydrate and fatty acid metabolism, lipogenesis, lipolysis, and hence energy homeostasis. Insulin mediates the uptake of glucose via glycose transport proteins into cells where in muscles and liver it can be converted to and stored as glycogen. In IR there is impaired signaling via the phosphoinositol-3 kinase pathway allowing the buildup of toxic lipid metabolites, such as FA acyl CoA, diacylglycerol, and ceramide in numerous tissues including the liver, pancreatic beta cells and adipocytes [93]. It is the IR-related lipid-mediated macrovascular

complications, in large part related to atherothrombotic events, that results in the high morbidity and mortality risk seen in T2DM.

Cholesterol synthesis and absorption: Major epidemiological trials like the Framingham Offspring [94], PROCAM [95], and Cardiovascular Risk in Young Finns Study [96] have related increased CV risk in patients with increased levels of phytosterols which are measurable markers of sterol absorption. Miettinen showed that in the Scandinavian Simvastatin Survival Study (4S), a high-risk secondary prevention trial of statin/placebo treated patients with high LDL-C, hypoabsorbers did and hyperabsorbers did not have a beneficial effect of simvastatin, theoretically because hyperabsorbers are typically hyposynthesizers of cholesterol and therefore less likely to be responsive to a statin [97]. In the DEBATE [Drugs and Evidence-Based Medicine in the Elderly] study low cholesterol absorption was associated with fewer recurrent cardiovascular events, and with better survival in elderly patients despite frequent abnormalities of glucose metabolism [98]. Intestinal function is abnormal in diabetics and several enterocytic sterol homeostatic regulatory changes occur in IR patient. Conflicting studies have described T2DM as having either reduced [99] or increased cholesterol absorption [100]. A recent study demonstrated cholesterol absorption was highest in the lean insulin sensitive participants, whereas cholesterol synthesis was highest in the lean IR and obese IR participants [101]. In another experiment 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the rate-limiting enzyme for cholesterol synthesis, is increased in animal models of diabetes in both the liver and small intestine [102].

However, rats made diabetic by injection of streptozotosin were made hyperabsorbers of cholesterol which was explained by changes in intestinal absorption-regulating proteins, namely an upregulation of NPC1L1, ACAT2, microsomal triglyceride transfer protein (MTP), and a reduced expression of ABCG5 and ABCG8 [103]. Lally et al. showed that diabetic patients had more NPC1L1 mRNA than the control subjects ($p < 0.02$), expression of ABCG5 and ABCG8 mRNA was lower in the diabetic patients ($p < 0.05$), and MTP expression was increased ($p < 0.05$). There was a positive correlation between NPLC1L1 and MTP mRNA ($p < 0.01$) and a negative correlation between NPC1L1 and ABCG5 mRNA ($p < 0.001$) [104]. In addition an increase in apolipoprotein B_{48} synthesis has been demonstrated in animal models of diabetes and insulin resistance. Generally apoB synthesis and utilization is driven by increased lipid substrate and such intestinal dysfunction, reflective of hyperabsorption will lead to abnormal chylomicron composition which, via the action of CETP, will directly influence other circulating lipoproteins [105].

Experts have speculated on whether knowing one is or is not a hyperabsorber or hyper-synthesizer of cholesterol would be useful in deciding on lifestyle and drug therapies, and there is both null and supporting data on that. Certainly, statins and statins plus cholesterol absorption inhibitors such as ezetimibe improve lipid and lipoprotein abnormalities in T2DM [106]. Of interest is that potent statin monotherapy can significantly increase cholesterol and noncholesterol sterol absorption which has the potential to offset some of the benefit of inhibiting synthesis [107].

The major lipid abnormality in T2DM has been called a TG/HDL axis disorder and is characterized by variable TC and LDL-C but elevations of fasting and often postprandial TG and reductions in HDL-C [108]. Underlying these lipid concentration abnormalities and likely more directly related to macrovascular atherosclerotic disease are significant changes in the number, size, core lipid composition and proteome of lipoproteins. It then becomes crucial to understand what happens to previously described lipoprotein genesis and trafficking of lipids in the IR patient with TG/HDL abnormalities. Clinicians are going to have to respect the pathology related to TG-rich lipoproteins and TG levels which heretofore were not deemed to be of concern (<150 mg/dL). Typically the liver in IR patients has increased pools of retained lipids, especially that of TG which results from an imbalance between the uptake and synthesis of fatty acids and their oxidation and export [109]. Both hyperinsulinemia and hyperglycemia induce the expression of the lipogenic gene-activating hepatic sterol regulatory element binding protein 1c (SREBP1c) and the carbohydrate responsive element binding protein (ChREBP) [110].

The more lipid substrate, especially triglycerides in the hepatocyte or enterocyte cytosol that exists, the more apoB will lipidated rather than catabolized (Fig. 4.9). Insulin reduces MTP expression via activation of the mitogen activated protein kinase (MAPK) pathway [111]. Insulin also increases the repression of *apoB* RNA translation [112] and increases expression of ER60, an endoplasmic reticulum associated protease that degrades apoB [113]. Normally lipidation of apoB creates a primordial VLDL that evolves into a normally composed, sized and secretable VLDL2 [114]. Secretion of VLDL2 is the same in IR and insulin-sensitive subjects. When there is a lack of a lipid reservoir, there is improper folding and rapid degradation of apoB and less VLDL is produced [115]. The major cause of hypertriglyceridemia in the HOMA-IR person is the increased availability of free FA substrate causing hepatic overproduction and secretion of larger TG-rich VLDL1 resulting in increased plasma concentrations of apoB and TG (Figs. 4.10 and 4.11) [116]. A recent nutritional study demonstrated apoB production had a strong relation with dietary fructose and especially fructose corn syrup and not glucose [117]. In a kinetic study plasma glucose, insulin, and free fatty acids together explained 55% of the variation in VLDL1 TG production rate [118]. The large VLDL1 seen in T2DM are normally suppressed by insulin, but not in the setting of IR. The $apoB_{100}$-containing VLDL2 are converted to VLDL1 by the addition of a major load of triglycerides in the endoplasmic reticulum (the same is true of enterocyte $apoB_{48}$ and chylomicron formation). VLDL1 creation also is dependent upon ADP ribosylation factor 1 (a small GTP binding protein) which is involved with translocation of nascent lipoprotein from the ER to the Golgi apparatus where final synthesis, including much of the TG acquisition and phospholipidation occurs [119, 120]. The time between $apoB_{100}$ production and lipidation to create large VLDL1 is approximately 15 min [119]. Insulin resistance results in diminished phosphatidylinositol-3-kinase that may add to the increased VLDL secretion [121]. In humans, the mean residence time of VLDL1 apoB is the main determinant of apoB pool size and of plasma TG concentration [11, 122]. There is an increased production of VLDL1, as well as a reduction in the catabolic rate of apoB-containing lipoproteins, in particular IDL and

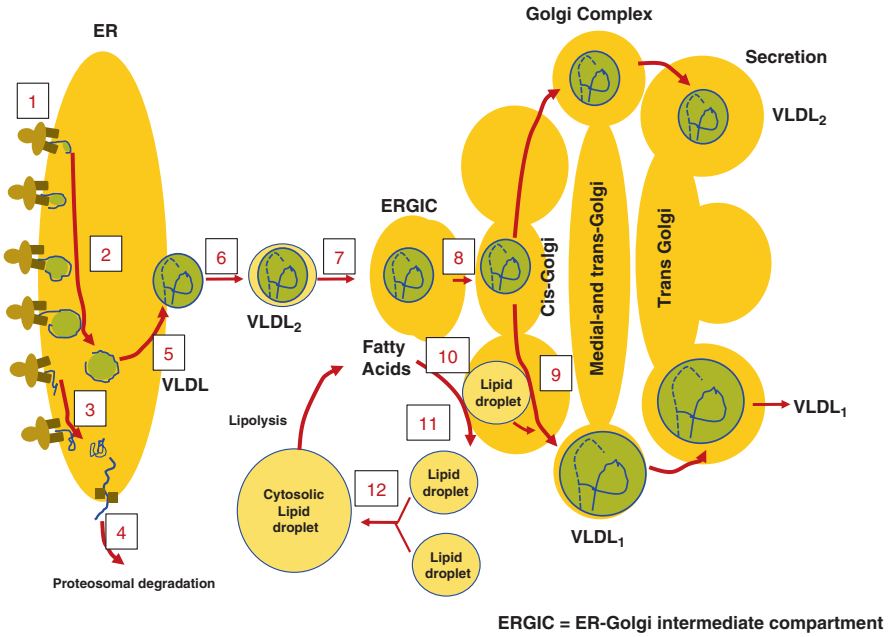

Fig. 4.9 The VLDL assembly process starts in the rough endoplasmic reticulum (ER) with the biosynthesis and concomitant (co-translational) translocation of apolipoprotein B_{100} ($apoB_{100}$) to the lumen of this organelle. $ApoB_{100}$ interacts co-translationally with the microsomal triglyceride transfer protein (MTP) and is thereby lipidated to form a primordial particle (pre-VLDL). Alternatively, $apoB_{100}$ fails to be lipidated and misfolds. This results in a sorting to degradation. Thus, the protein is unfolded and retracted to cytosol, ubiquitinated, and sorted to proteasomal degradation. Pre-VLDL is converted to VLDL2 late in the ER compartment. VLDL2 exits the ER at specific exit sites of this organelle by Sar1/Cop II vesicles, which fuse to become the ER–Golgi intermediate compartment (ERGIC) [7]. ERGIC fuses with Cis-Golgi. In the Golgi apparatus, VLDL2 is converted to VLDL1 by the addition of a bulk load of triglycerides. This lipidation process differs from that which gives rise to pre-VLDL and VLDL2. The formation of VLDL1 may involve the formation of a lipid droplet in the lumen of the secretory pathway. The mechanism behind the formation of lipid droplets in the secretory pathway may follow that of cytosolic lipid droplets. Such droplets are formed from the microsomal membranes under the influence of the enzymes phospholipase D1 and ERK2 as well as of adipocyte differentiation-related protein (also known as adipophilin and caveolin). The formation of the cytosolic droplets also involves a fusion step that is dependent on microtubules and the motor protein dynein. (Reproduced with permission from Adiels M, et al. Arterioscler Thromb Vasc Biol 2008;28:1225–36)

LDL. Collectively this leads to increased levels of apoB related to large VLDL-P but mostly LDL-P. The catabolism of apoA-I, the main apolipoprotein of HDL, is increased by 48% but apoA-I production is increased by 25%, probably because of some compensatory effect. This production/clearance imbalance results in a 16% reduction in the concentration of HDL-apoA-I (Fig. 4.12) [123]. Garvey et al. in an elegant insulin clamp study analyzing NMR derived particle concentrations showed as the patients' status progressed from insulin sensitive to insulin resistance to

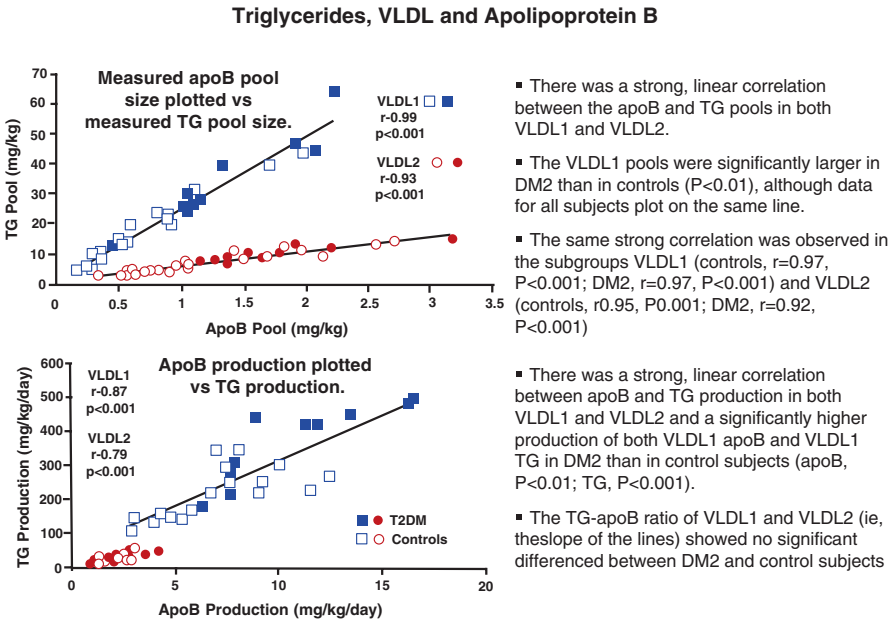

Fig. 4.10 Kinetics of triglyceride, VLDL, and apolipoprotein B production. (Reproduced with permission from Adiels M, et al. Arterioscler Thromb Vasc Biol 2005;25:1697–703)

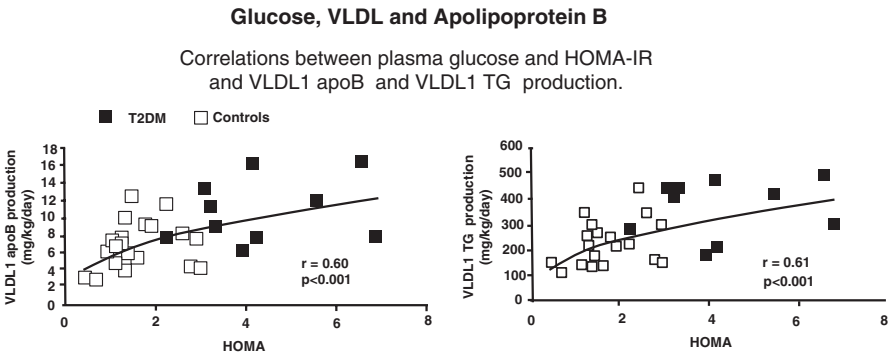

Fig. 4.11 Glucose, VLDL, and apolipoprotein B kinetics in diabetic and non-diabetic patients. (Reproduced with permission from Adiels M, et al. Arterioscler Thromb Vasc Biol 2005;25:1697–703)

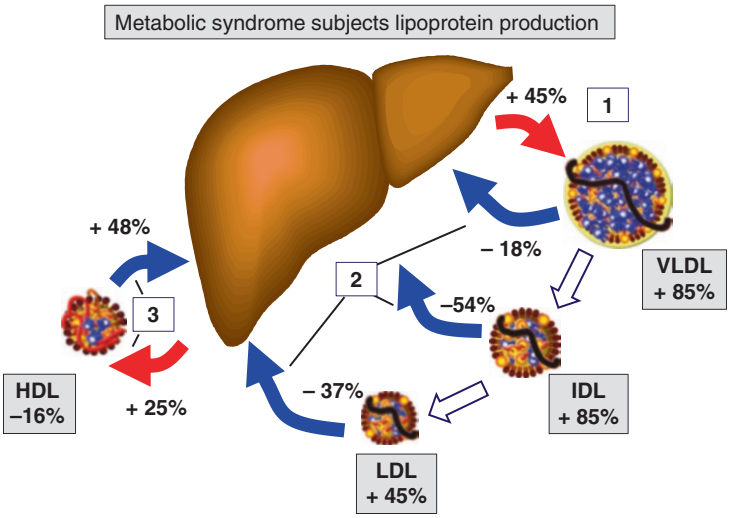

Fig. 4.12 Changes in lipoprotein metabolism in T2DM and the metabolic syndrome. Subjects diagnosed with the metabolic syndrome display, most noticeably, an increased production of VLDL (1), and there is a reduction in the catabolic rate of apoB-containing lipoproteins, in particular IDL and LDL (2). Together, these result in increased concentrations of apoB-containing lipoproteins. The catabolism of apoA-I, the main apolipoprotein of HDL, is increased by 48%, but apoA-I production is increased by 25%, probably because of some compensatory effect (3). This results in a 16% reduction in the concentration of HDL apoA-I. (Reproduced with permission from Adiels M, et al. Arterioscler Thromb Vasc Biol 2008;28:1225–36)

T2DM, there are progressive increases in VLDL-P, IDL-P and, particularly, LDL-P [124].

VLDL lipolysis is delayed in T2DM due to several mechanisms. As previously mentioned, several apolipoproteins are involved with efficient VLDL catabolism including apoE, apoA-II, apoA-IV, apoA-V, apoC-I, apoC-II, apoC-III, apoD, and apoF. Many of those apolipoproteins have altered function in IR and in persons with T2DM. Ultimately, lipolysis of TG-rich lipoproteins requires apoC-II to activate LPL, and thus release of apoC-II from either HDL or VLDL allows for LPL-mediated hydrolysis of TG in nascent chylomicrons and VLDLs [70, 125]. In a small study of diabetic patients vs. normolipemic controls who had TG-tolerance tests, the diabetic patients displayed typical postprandial hypertriglyceridemia, but although apoA-V levels were similar in the two groups, paradoxically the diabetics had increased postprandial apoA-V in non-HDL particles which is suggestive that apoA-V is not involved in the regulation of TG in the postprandial state [126]. In another study the postprandial (after an oral fat load) increase of apoA-V was confirmed and was related not only to plasma TG and VLDL1-TG, but also to apoC-III. It was thought the increase of apoA-V simply reflected the increase of VLDL particles related to apoC-III overproduction [127]. *APOA5* genotypes do not appear to have an impact on risk of development of T2DM [128].

ApoC family members, apoC-II, apoC-III, and apoC-I are crucial to the synthesis of TG-rich lipoproteins as well as their lipolysis and catabolism. High concentrations of apoC-I and apoC-III are associated with increased triglycerides in men with the metabolic syndrome. These findings in humans were first seen in Hyplip2 congenic mouse strain studies which related the elevated TG to delayed catabolism of VLDL, which in turn led to decreased FA delivery to visceral adipose tissue [129]. In obese males with the metabolic syndrome, apoC-I and apoC-III levels were mainly related to the visceral adipose tissue (VAT) compartment (measured using nuclear magnetic resonance). This was related to a higher expression of LPL in VAT versus subcutaneous adipose tissue (SAT). The apoC-I and apoC-III inhibition of LPL therefore contributed to both higher TG and lower VAT area in human subjects. The difference in effect of apoC-I and apoC-III on TG concentrations in this study underlines the stronger inhibition of LPL by apoC-III compared with apoC-I (Figs. 4.13 and 4.14) [130]. Both apoC-I and apoC-III inhibit LPL by blocking its binding to lipoprotein surfaces [131].

All apoCs are distributed in a cycling process between TG-rich apoB-lipoproteins and HDL. In the fasting state, apoC-II and apoC-III are equally distributed between HDL and VLDL, whereas apoC-I is mostly trafficked with HDL (>90%). Thus, in the exchange of apolipoproteins after a meal more apoC-I is transferred than other apoCs. The apoC-I enrichment of TRLs after a meal affects particle catabolism and

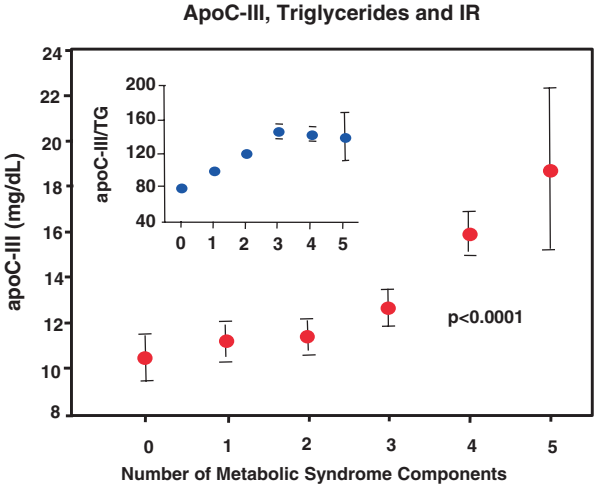

Fig. 4.13 Mean of apoC-III concentration according to the number of metabolic syndrome components. Data normalized for TG values (*figure inset*) show statistically significant trends for the apoC-III/TG ratio ($p < 0.0001$). (Reproduced with permission from Florez H, et al. Atherosclerosis 2006;188:134–41)

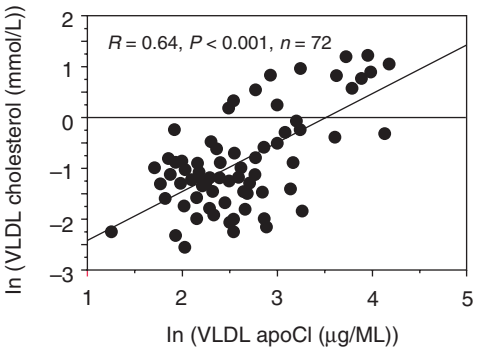

Fig. 4.14 Relationship between VLDL apoC-I and VLDL cholesterol. (Reproduced with permission from Hamsten A, et al. J Am Coll Cardiol 2005;45:1013–7)

is involved with the formation of VLDL and chylomicron CE-rich atherogenic remnants. A paradox is that apoC-I is not known to interfere with TG hydrolysis as studies have shown that apoC-I-enriched TRLs undergo normal hydrolysis forming smaller TRLs and remnants. Because apoC-I is a potent inhibitor of apoE-mediated uptake of TG-rich lipoproteins by LDLr, VLDLr, and LRP, particle clearance is impaired. ApoC-I-enriched particles which have compositional abnormalities (TG-rich) have increased plasma residence time allowing CETP mediated exchange of core TG for CE utilizing heterotypic and homotypic pathways which over time make the remnants even more CE-rich [131]. Numerous studies have demonstrated that CE-rich remnants are atherogenic [132–136] and delayed remnant clearance during the postprandial state is a well-established feature of patients with diabetes and coronary artery disease (CAD) [137]. Remnant lipoproteins are seven-times more proinflammatory than LDL particles [138] and correlate with increased serum leukocyte counts [139]. Unlike LDL particles, remnant lipoproteins do not have to undergo oxidation in order for them to activate macrophage scavenging and foam cell formation [140]. Hence, remnants pose a significant hazard to cardiovascular health.

Interestingly apoC-I is a more potent inhibitor of CETP when it is on HDL but not the apoB particles. Thus, the transfer of apoC-I from HDL to TG-rich lipoproteins facilitate atherogenic remnant formation, suggesting a dual role for apoC-I: (1) preventing remnant formation and premature atherosclerosis if attached to HDL; and (2) promoting remnant formation and atherosclerosis if transferred to TG-rich lipoproteins [141]. The apoC-I content of postprandial TG-rich lipoproteins has been shown to be a risk factor for early atherosclerosis in normolipidemic healthy middle-aged men supporting the conclusion that the enrichment of remnant lipoproteins with cholesterol is not favorable. ApoC-I on TG-rich lipoproteins has been linked to increased CIMT [142]. In healthy normolipidemic men, the number of apoC-I

molecules associated with chylomicron remnants correlated with severity of coronary atherosclerosis [143]. A high molecular weight apoC-1 variant transported by HDL is associated with increased arterial wall smooth muscle cell apoptosis and CAD [144]. There is also evidence that apoC-I participates in arterial wall foam cell development [145]. There are not a lot of published studies evaluating apoC-I, per se in diabetes. In an evaluation of women with PCOS, those with IR were characterized by statistically significantly elevated levels of apoC-I compared with those of non-IR patients. ApoC-I correlated with BMI, TG, HDL-C, apoA1, and HOMA-IR [146].

ApoC-III is perhaps the most complex and enigmatic apolipoprotein. For some time, it has been known that apoC-III enriched particles were a significant CHD risk factor. ApoC-III levels are associated with hypertriglyceridemia and increases in VLDL-P and VLDL-TG and inversely related to the size of LDL particles [147]. In the Cholesterol and Recurrent Events (CARE) trial both the plasma concentrations of VLDL particles and apoC-III in VLDL and LDL were better predictors of coronary heart disease risk than was plasma TG (Fig. 4.15) [148]. In CARE diabetic status compared to non-diabetic status per se was not associated with high concentrations of apoC-III-containing TG-rich lipoprotein particles if their plasma TG levels were similar [149].

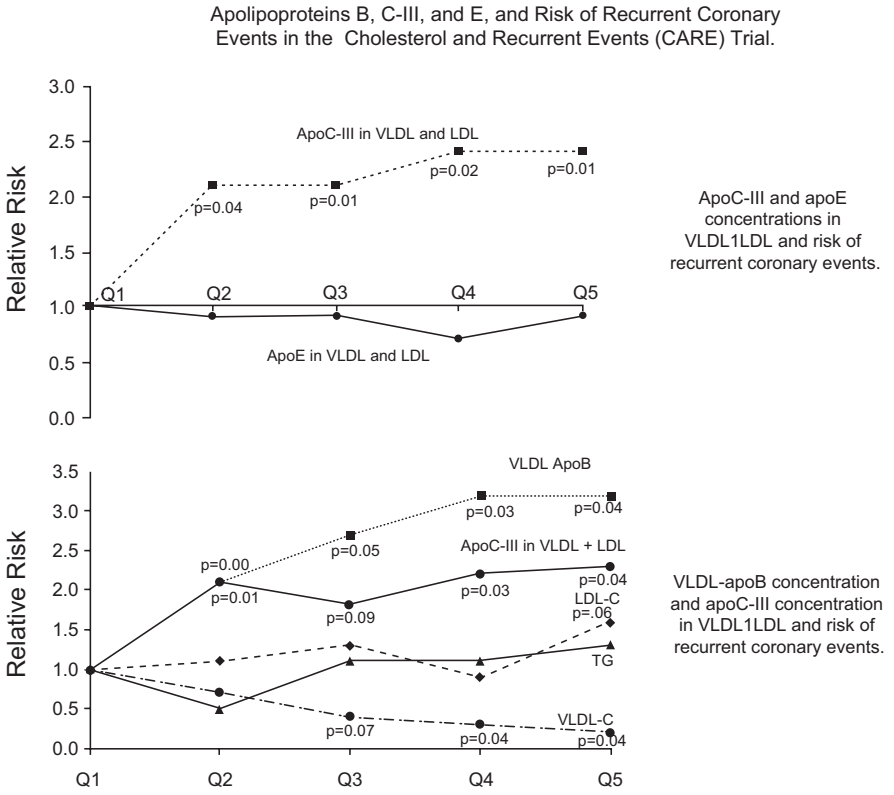

Fig. 4.15 Relationships between apoproteins and risk of recurrent coronary events. (Reproduced with permission from Sacks FM, et al. Circulation 2000;102:1886–92)

APOCIII is located on chromosome 11 and because it is near an insulin response element, a link to diabetes has been suggested [150]. Several nuclear transcription factors (NTF) influence apoC-III expression. One is Foxo1 which provides a molecular link between insulin resistance and the pathogenesis of diabetic hypertriglyceridemia. Foxo1 is a substrate of Akt/protein kinase B and glucocorticoid inducible kinase, which is involved with insulin signaling and in modulating both hepatic and intestinal apoC-III expression. Under both insulin-deficient and insulin-resistant conditions, Foxo1 expression is deregulated, contributing to the increased apoC-III production and impaired plasma TG metabolism [151]. Hepatic nuclear factor 4-alpha (HNF-4α), which regulates LPL, is also a strong positive regulator of apoC-III expression [152]. HNF-4α is stimulated by glucose and the carbohydrate-responsive element binding protein (ChREBP). In individuals with IR and diabetes, there is a loss of insulin-mediated suppression of apoC-III that, coupled with glucose-stimulated apoC-III expression, leads to hypertriglyceridemia (Fig. 4.16)

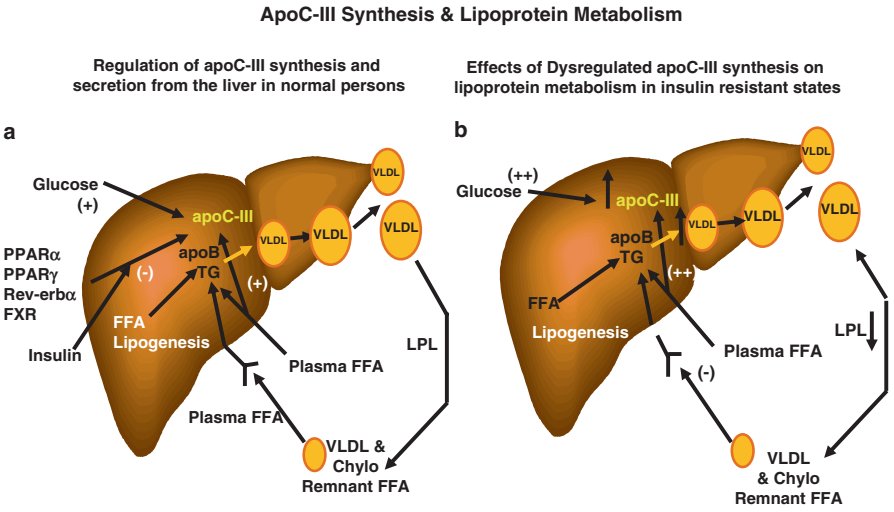

Fig. 4.16 Apoprotein C-III in normal and insulin-resistant states. (**a**) Under normal conditions, apoC-III gene expression and synthesis are regulated by several factors, including nuclear transcription factors PPARα, PPARγ, Rev-erb, farnesoid X receptor, as well as insulin and glucose. All are inhibitory except for glucose, which stimulates apoC-III expression. Plasma free fatty acids (FFA) stimulate apo-CIII secretion, but it is not known whether this occurs at the transcriptional or posttranslational level. ApoC-III in plasma inhibits lipoprotein lipase-mediated catabolism of VLDL (and chylomicrons) and inhibits the uptake of VLDL (and chylomicron) remnants by the liver. In addition, apoC-III may increase VLDL assembly and secretion. (**b**) In states of insulin resistance, any inhibitory role of insulin on apoC-III expression may be lost, whereas higher glucose levels, particularly in patients T2DM, would further stimulate apoC-III expression. Increased plasma FFA delivery to the liver would exacerbate this problem. The results of dysregulated apoC-III synthesis and secretion would be defective LPL–mediated lipolysis of TG-rich lipoproteins and reduced remnant lipoprotein clearance. Thus, dysregulated apoC-III synthesis and secretion could play a major role in the genesis of the diabetic, insulin-resistant dyslipidemia. In addition, accumulation of apo-C-III rich apoB-containing lipoproteins might have direct atherogenic consequences. (Reproduced with permission from Ginsberg HN, Brown WV. Arterioscler Thromb Vasc Biol 2011;31:471–3)

[153, 154]. New findings demonstrate that apoC-III can play an additional "feedback" role in PPAR-α-mediated metabolic and inflammatory functions by controlling lipolytic generation of PPAR-α ligands. Because apoC-III expression is suppressed and LPL activity is stimulated by PPAR-α, a positive feedback system may exist. Individuals with high apoC-III levels may have impaired generation of endogenous PPAR-α ligands. Such a scenario is likely in patients with IR [155].

Accelerated conversion of buoyant LDL with apoC-III to dense LDL raises the possibility that apoC-III positively modulates the action of hepatic lipase, contributing to an increase in the concentration of plasma dense LDL [156]. ApoC-III also interacts with SR-B1 and ABCA1, which will affect lipidation and delipidation of HDL. HDL particle size shifted toward smaller sizes with increases of plasma apoC-III levels; especially when the elevations of apoC-III and apoC-II was simultaneous. The higher apoA-I concentrations also modified the effect of apoC-III on HDL subclass distribution profile. Dynamic remodeling of HDL is impaired when large-sized HDL_{2b} particles decreased greatly in hypertriglyceridemic subjects characterized by elevated apoC-III and C-II and lower apoA-I [157].

Like apoC-I the majority of apoC-III is found in the HDL fraction in normolipidemic individuals and on TG-rich lipoproteins in patients with elevated levels of plasma triglyceride. In plasma, different lipoproteins (whether apoB- or apoA-I-containing) have different numbers of apoC-III molecules, which may be determined by both the structure and the composition of the lipoproteins. Whether all of the apoC-III is exchangeable or not, it significantly affects the fate of the particle on which it resides, affecting potentially atherogenic VLDL, IDL, and small LDL [158]. C-III also interacts with apoE and thus VLDL metabolism is influenced by both their content of apoE, and by the availability of apoC-III. VLDL E+ and IDL E+ had lower fractional catabolic rates and much higher apolipoprotein C-III (apoC-III) content than did the corresponding E-particles [159]. Reanalysis of data suggests that some VLDLs, IDLs and LDLs contain several molecules of apoC-III, whereas others contain none [160]. Less than half of HDLs contain apoC-III [161]. There are several mechanisms at play with respect to how apoC-III influences lipoproteins. Overproduction of apoC-III and apoB lipoproteins that contain apoC-III is a common feature of patients with hypertriglyceridemia. ApoC-III inhibits receptor-mediated uptake of these lipoproteins by the liver and thus VLDLs containing apoC-III are channeled down the lipolytic cascade to LDL, particularly to smaller LDLs that have a slower clearance rate from plasma leading to elevations of both small and total LDL-P. Indeed, increases of LDL particles containing apoC-III (LpB:C-III) are significantly associated with increases in small, dense LDL levels in healthy men independent of TG levels [162].

Many reports indicate that increased apoC-III content may contribute to inflammatory factors related to atherogenesis (Figs. 4.17 and 4.18) [163]. ApoC-III stimulates monocytes and endothelial cells to produce cytokines such as tumor necrosis factor-α and adhesion molecules via activation of the nuclear transcription factor NF-κB, and it potentiates insulin-resistance pathways in endothelial cells causing endothelial dysfunction [164]. ApoC-III also stimulates adipocytes to produce cytokines and suppresses their production of adiponectin [165, 166]. ApoC-III activates the NLRP3 (NLR family pyrin domain containing 3) inflammasome in human

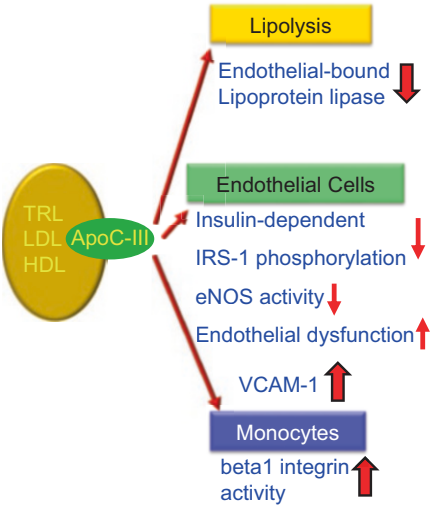

Fig. 4.17 Atherogenic mechanisms of apoC-III. ApoC-III on the surface of triglyceride-rich, low-density, or high-density lipoproteins can interact with endothelial-bound lipoprotein lipase to attenuate its activity. It may also more directly interact with endothelial cells to inhibit insulin dependent IRS-1 phosphorylation and eNOS activity and thereby induce endothelial cell dysfunction. Impairment of endothelial function augments proinflammatory responses to cytokines. This interaction of apoC-III with the endothelium also elevates vascular cell adhesion molecule-1, which can augment recruitment of leukocytes to developing atheromas. ApoC-III can also increase the activity of 1-integrins on monocytes, further augmenting their adhesion to endothelium. The presence of apoC-III on high-density lipoproteins may limit its anti-inflammatory properties. (Reproduced with permission from Bobik A. Circulation 2008;118:702–4)

monocytes by activating an alternative NLRP3 inflammasome via caspase-8 and dimerization of toll-like receptors 2 and 4 and may participate in tissue injury systemically [145]. ApoC-III induces arterial smooth muscle cell proliferation via the Akt signaling pathway [167]. In a cohort if 660 patients with established CAD, an apoC-III level ≥10.5 mg/dL predicted both total and cardiovascular mortality (HR for total mortality 2.22 with 95% CI 1.16–4.24; HR for cardiovascular mortality 2.35 with 95% CI 1.19–4.62) even after adjusting for mortality-associated covariates [168].

Although not commonly appreciated, apoA-II is not solely an HDL apoprotein, but also traffics with TG-rich lipoproteins and induces postprandial hypertriglyceridemia. In mice, several features of the metabolic syndrome were associated with moderate to high expression of human apolipoprotein A-II. Overexpression of human apoA-II in mice led to postprandial accumulation of intestinal TRL for several hours, in a manner that one expects in IR patients [169]. Brewer suggests increased levels of apoC-III, apoC-I, or apoA-II on the apoB-containing lipoproteins may alter lipoprotein metabolism causing increased levels of atherogenic remnant lipoproteins. In some patients with hypertriglyceridemia, apoA-II is associated with the apoB-containing lipoproteins suggesting that the lipoproteins containing

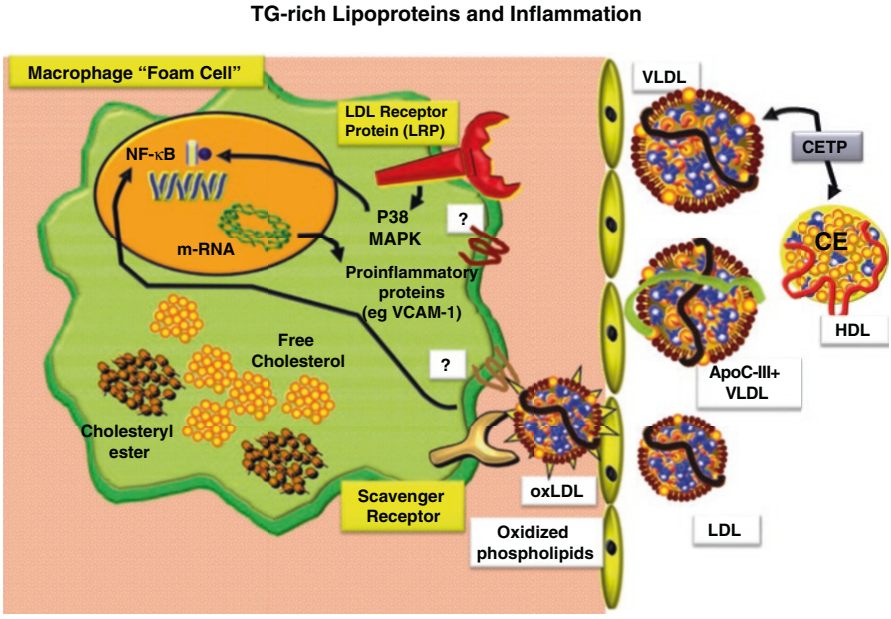

Fig. 4.18 Oxidation of LDL releases bioactive lipids that incite inflammation in vascular tissues through scavenger receptors and other putative receptors. Binding and internalization of TG-rich lipoproteins activate P38 MAP kinase and NFκB. ApoC-III+ VLDL and LDL also activate proinflammatory functions of endothelial cells via a pertussis-sensitive, proteins kinase C (PKC) mediated pathway that can stimulate via NFκB recruitment of leukocytes. (Adapted from Libby P. Circ Res 2007;100:299–301with permission)

apoA-II were not effectively metabolized by LPL, and the increased plasma levels of these triglyceride-rich remnants were due to defective lipolysis (Fig. 4.19) [170]. ApoA-II transfers from HDL to VLDL in vitro, resulting in VLDL that is a poorer substrate for LPL, suggesting one function of apoA-II is to regulate the metabolism of TG-rich lipoproteins, with HDL serving as a plasma reservoir of apoA-II. Mice which overexpress apoA-II exhibit a marked hypertriglyceridemia, hypercholesterolemia, and increased plasma FFA, as well as insulin resistance, increased adiposity, and increased atherosclerosis [171]. ApoA-II prematurely released from poorly maturing HDL particles in persons with certain hypoalphalipoproteimias may contribute to the elevated TG levels seen in such patients. Low serum levels of apoA-II correlate with increased risk for CAD as shown in multiple epidemiological cohorts [172–174].

ApoE has multiple effects on lipogenesis, lipid absorption, lipoprotein formation and catabolism, and receptor-mediated clearance. TG-rich lipoproteins typically carry several copies of apoE, which exist in several genotypes (*E3/*E3, *E3/*E4, *E2/*E3, *E4/*E4, *E2/*E4, and *E2/*E2), some of which are associated with lipid/lipoprotein disorders. ApoE isoforms associate with lipoproteins and mediate their capacity to bind to members of the LDL receptor family (LDL receptor, LDL

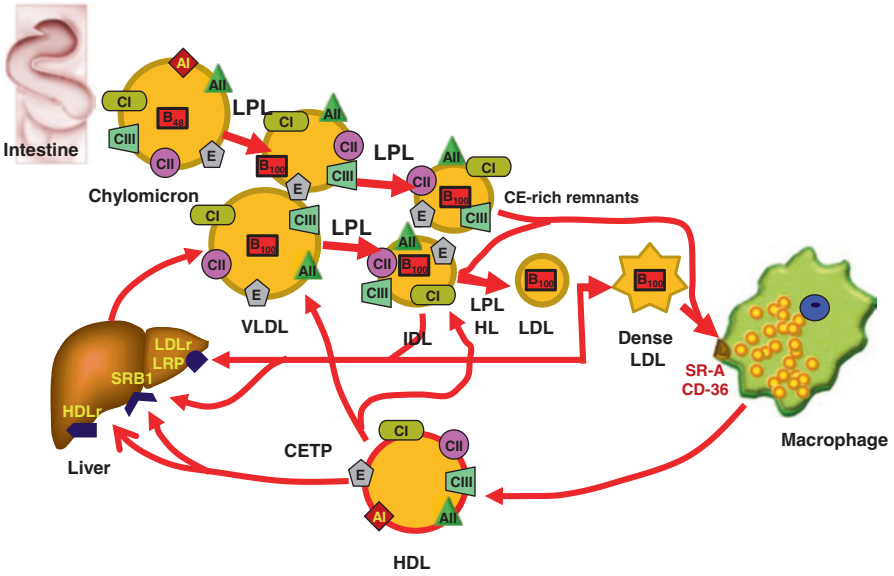

Fig. 4.19 Schematic overview of lipoprotein metabolism in patients with hypertriglyceridemia, dense LDL, and reduced HDL. (Adapted from Brewer HB, Jr. Am J Cardiol 1999;83:3f–12f with permission)

receptor related protein, and the VLDL receptor) on the surface of hepatocytes [175]. With respect to mice with STZ-induced diabetes, investigation reveals apoE4 causes severe dyslipidemia and atherosclerosis independent of its interaction with LDLr. ApoE4-expressing livers have reduced fatty acid oxidation, which contributes to the accumulation of tissue and plasma lipids [176]. Southern European ethnicity does not confer an independent survival advantage in community-based Australian type 2 diabetic patients, but the APOE4 carriers are at higher risk of cardiac death [177].

ApoE polymorphisms have been implicated in predisposition to diabetes, but the results of the individual studies have been inconclusive. A meta-analysis of population-based case-control genetic-association studies relating apoE polymorphisms and T2DM which included 30 studies, reported data of apoE genotypes in 5423 T2DM patients and 8197 healthy unrelated controls and revealed a significant role played by the E2 allele carriers, who were at elevated risk for T2DM (Odds Ratio = 1.18, 95% CI: 1.02, 1.35). Meta-regression analysis provided some weak evidence that the risk conferred by E2 allele is mediated through altering serum lipid levels [178]. The relationship between *APOE* and fatal and nonfatal CHD was examined among 10,035 men and 12,134 women, aged 440 to 79 years, from the Norfolk, England, arm of the European Prospective Into Nutrition and Cancer Study (1993–2007). During an average of 11 years of follow-up, 2712 CHD events were documented. In the largest prospective cohort study to date, CHD risk was not associated with *APOE* genotype after controlling for a variety of cardiovascular risk

factors, particularly the ratio of low- to high-density lipoprotein cholesterol [179]. A Turkish group assessed the apoE polymorphism in 295 patients with atherosclerosis, 124 of them had diabetes. Findings suggested that apoE polymorphism was not related to the development of atherosclerosis and was not associated with lipid levels in patients with T2DM [180]. In the CARE trial apoE concentrations in plasma or in VLDL + LDL were associated with CHD but were linked to apoC-III retarding their clearance. Also, apoE in HDL was an independent predictor of recurrent coronary events and explains the weaker relation between plasma triglycerides and coronary events [181]. In a more recent study, diabetic patients with the E3/E4 genotype were at 2.4-fold increased risk to develop CVD (95% CI 1.14–5.19, $p = 0.02$) and the ε4 allele associated with 2.23-fold higher CVD risk (95% CI 1.09–4.59, $p = 0.02$). After comprehensive adjustment for other risk covariates, the E3/E4 genotype was an independent risk factor for CVD (OR = 2.3, $p = 0.009$) but not for T2DM (OR = 1.7, $p = 0.28$). Of great interest is the observation that the ε4 allele is an independent risk factor for both T2DM (OR = 2.2, $p = 0.04$) and CVD (OR = 3.0, $p = 0.018$), with a 5.9-fold increased risk to develop CVD in T2DM patients ($p = 0.019$) [182].

Another apolipoprotein involved with TG-rich lipoproteins is apoL-I which was discovered in 1997 and has also been found in human atherosclerotic vascular tissue. ApoL-I is trypanolytic, plays a role in apoptosis and autophagy, and may participate in a variety of pro-inflammatory phenomena [183, 184]. Typically, apoL-I associates with HDL particles [185] but, in the HDL Atherosclerosis Treatment Study (HATS), there were significant associations between apoL-I and VLDL-TG and elevated glucose. VLDL-TG was the specific TG component associated with apoL-I and ~50% of patients with high apoL-I levels had an elevated glucose phenotype compared with <15% of those in the low apoL-I cluster. This supports the possibility that high apoL-I levels may be a novel marker of an atherogenic phenotype [186]. Proprotein convertase: subtilisin kexin type 9 (PCSK9) has emerged as a major regulator of hepatic LDLr expression, and it limits visceral adipogenesis likely via adipose VLDLr regulation. In vivo, endogenous hepatic PCSK9 has been shown to regulate VLDLr protein levels in adipose tissue. This regulation is achieved by circulating PCSK9 and thus helps regulate fat metabolism [187].

The TG/HDL Axis: The HDL, ApoA-I Containing Lipoproteins

The very large HDL lipidome is an area ripe for research. Ceramide, an HDL component, has been implicated in the pathogenesis of insulin resistance and has many proinflammatory properties such as impaired Huh7 cell (a well differentiated hepatocyte) viability, mitochondrial function, and insulin signaling [188]. A kinetic study revealed that increased HDL-apoA-I catabolism, a significant effector of low apoA-I in the metabolic syndrome, may be largely associated with dysregulation of VLDL-apoB metabolism (i.e. elevated plasma triglyceride and VLDL-apoB concentration and overproduction of VLDL-apoB), insulin resistance, and, to a lesser

extent, low adiponectin concentration [189]. Compared with lean individuals, overweight–obese individuals had significantly higher HDL apoA-I fractional catabolic rate (0.21 ± 0.01 vs. 0.33 ± 0.01 pools/day; $p < 0.001$) and production rate (PR; 11.3 ± 4.4 vs. 15.8 ± 2.77 mg/kg/day; $p = 0.001$). In the lean group, HDL apoA-I PR was significantly associated with apoA-I concentration ($r = 0.455$, $p = 0.004$), whereas in the overweight–obese group, both HDL apoA-I fractional catabolic rate ($r = -0.396$, $p = 0.050$) and HDL apoA-I PR ($r = 0.399$, $p = 0.048$) were significantly associated with apoA-I concentration. After adjustment for fasting insulin or Homeostasis Model Assessment (HOMA) score, HDL apoA-I PR was an independent predictor of apoA-I concentration [190]. In part, the catabolic rate is related to heterotypic exchange of TG for CE between apoB and apoA-I particles, resulting in TG-rich HDLs which are subject to lipolytic catabolism and release of apoA-I making it available for renal excretion.

The SR-B1 receptor is involved with lipidation and delipidation of mature HDL particles [191]. SR-BI is widely believed to be beneficial and anti-atherogenic because it has been shown to regulate hepatic uptake and hepatic secretion of HDL, participates in macrophage dependent reverse cholesterol transport, and stimulates endothelial cell nitric oxide synthase activity [192]. In a study of 16 men, postprandial lipemia caused structural changes to HDL so there was enhanced SR-BI and ABCG1-dependent efflux to large HDL_2 particles. Although that is seemingly beneficial, postprandial lipemia was equally associated with enhancing formation of CE-enriched, TG-rich lipoprotein particles through the action of CETP and by inducing structural changes in HDL particles that reduce the direct return of HDL-CE to the liver [193]. Also affecting SR-B1 and ABCA1 efflux in vivo in transgenic mice in a reciprocal manner was modulation of HDL PL content. The type of lipase acting on HDL in vivo may also determine which FC efflux pathway the HDL serves. Efflux was examined by overexpressing either endothelial lipase (EL) or phosphatidylserine phospholipase (PS-PLA1) in human apoA-I transgenic mice. Overexpression of EL led to large reductions in the serum PL/apoA-I ratio (−60%), total cholesterol (TC; −9%), and HDL cholesterol (−91%). Relative to the serum before overexpression of EL, the efflux potential of the serum via SR-BI decreased by 90% and ABCA1-mediated efflux increased by 63%. In contrast to overexpression of EL, overexpression of PS-PLA1 led to increases in the PL/apoA-I ratio (88%), TC (78%), HDL cholesterol (57%), and HDL size. The efflux potential of the serum increased by 60% via SR-BI and decreased by 57% via ABCA1 [194].

The TG/HDL Axis: Relating ApoB and ApoA-I Containing Lipoproteins

It has been known for decades the dyslipidemia or more aptly named dyslipoproteinemia associated with IR and T2DM is characterized by normal or abnormal levels of TC and LDL-C. but also elevated TG and reduced HDL-C. Szapary and Rader coined the term TG/HDL axis and noted its high association with CV risk

[108]. The National Cholesterol Education Program stated that low HDL-C is a major and independent risk factor for CV risk [55]. It continues to be debated whether TG levels have such independent predictive powers on CV risk although a large meta-analysis showed TG had moderate and highly significant associations and even though TG lost some predictive power when adjusted for HDL-C, it remained an independent predictor [195]. Major new insight as to the risk associated with TG comes from the Metabolic, Lifestyle, and Nutrition Assessment in Young Adults (MELANY) that followed 13,953 apparently healthy, untreated, young men (age 26–45 years) with TG levels less than <300 mg/dL over 5.5 years. The risk for CHD in men with high-tertile TG levels at time 1 changed depending on the tertile at time 2 (hazard ratios, 8.23 [95% CI, 2.50–27.13] for high (>131 mg/dL)/high (≥148 mg/dL), 6.84 [CI, 1.95–23.98] for high (131 mg/dL)/intermediate (94–147 mg/dL), and 4.90 [CI, 1.01–24.55] for high (>131 mg/dL)/low (≤93 mg/dL), compared with the stable low/low group). The risk for CHD in men with low-tertile levels at time 1 also changed depending on the tertile at time 2 (hazard ratios, 3.81 [CI, 0.96–15.31] for low/intermediate and 6.76 [CI, 1.34–33.92] for low (≤81 mg/dL)/high (≥148 mg/dL), compared with the stable low/low group). The conclusion was that TG measurements over time can help CV risk assessment in young men. A decrease in initially elevated TG levels was associated with a decrease in CHD risk compared with stable high TG levels. However, this risk remains higher than in those with persistently low TG levels [196]. Additional analysis showed two TG levels 5 years apart also identified young men at increased risk for diabetes, independent of traditional risk factors and of associated changes in BMI and lifestyle parameters. Two measurements of fasting triglyceride levels obtained 5 years apart can assist in identifying apparently healthy young men at increased risk for diabetes, independent of traditional risk factors and of associated changes in BMI and lifestyle parameters. Men in the lowest tertile of time 1 triglyceride levels (≤81 mg/dL) who progressed to the highest tertile (≥148 mg/dL) over follow-up (low-high) exhibited a hazard ratio (HR) of 12.62 (95% CI 3.52–31.34) compared with those remaining in the lowest tertile at both time points (reference group: low-low). Whereas men who were at the top triglyceride level tertile throughout follow-up [high (≥131 mg/dL)-high (≥148 mg/dL)] had a HR for diabetes of 7.08 (2.52–14.45), those whose triglyceride level decreased to the lowest tertile [high (≥131 mg/dL) – low (≤81 mg/dL)] exhibited a HR of 1.97 (0.67–6.13). Alterations in triglyceride levels during follow-up were associated with changes in BMI, physical activity, and eating breakfast habit ($p < 0.05$), but remained an independent modifier of diabetes risk even after adjustment for such changes [197].

More recently, a number of studies point to elevations in triglycerides as being etiologic in atherosclerotic disease. The Bezafibrate Infarction Prevention trial included a cohort 15,355 persons. Twenty two-year mortality data were obtained from a national registry. Patients were divided into 5 groups according to levels of fasting serum triglycerides: (1) low-normal triglycerides (<100 mg/dL); (2) high-normal triglycerides (100–149 mg/dL); (3) borderline hypertriglyceridemia triglycerides (150–199 mg/dL); (4) moderate hypertriglyceridemia triglycerides (200–499 mg/dL); (5) severe hypertriglyceridemia triglycerides (≥500 mg/dL).

Survival was 41% in the low-normal triglycerides group and 37%, 36%, 35%, and 25% in groups with progressively higher triglycerides ($p < 0.001$). After adjustment for risk factor covariates, each 1 unit of natural logarithm (Ln) triglycerides elevation correlated with a 6% ($p = 0.016$) increased risk of 22-year all-cause mortality. The 22-year mortality risk for persons with triglycerides >500 mg/dL increased by 68% compared to persons with triglycerides <100 mg/dL ($p < 0.001$) [198]. In the Progression of Early Subclinical Atherosclerosis study, associations between serum TG and subclinical atherosclerosis in individuals without an indication for lipid-lowering was evaluated in 4184 participants. After multivariate adjustment, TG levels ≥150 mg/dL correlated with subclinical noncoronary atherosclerosis (odds ratio [OR]: 1.35; 95% confidence interval [CI]: 1.08–1.68; $p = 0.008$). The association was significant irrespective of whether or not participants had high LDL-C (OR: 1.42; 95% CI: 1.11–1.80; $p = 0.005$) or normal LDL-C (OR: 1.85; 95% CI: 1.08–3.18; $p = 0.008$). No correlation was discerned between TG level and CAC score. TG levels ≥150 mg/dL correlated significantly with arterial inflammation (OR: 2.09; 95% CI: 1.29–3.40; $p = 0.003$) [199]. In retrospective study**,** persons aged 45 years or older with diabetes and/or atherosclerotic CV disease were included and analyzed in an elevated TG cohort (≥150 mg/dL) vs a comparator cohort with TG levels less than 150 mg/dL [200]. In the elevated TG vs propensity-matched comparator cohorts (both $N = 23{,}181$ patients), the mean age was 62.2 vs 62.6 years, mean follow-up was 41.4 vs 42.5 months, 49.7% (11,518) vs 49.5% (11,467) were female, 83.7% (19,392) vs 84.0% (19,478) had diabetes, and 29.8% (6915) vs 29.3% (6800) had atherosclerotic CV disease. In the high TG ($N = 27{,}471$ patients) vs comparator ($N = 32{,}506$ patients) cohorts, multivariate analysis demonstrated significantly higher risk of a composite of major CV events (hazard ratio [HR], 1.26; 95% CI, 1.19–1.34; $p < 0.001$), nonfatal myocardial infarction (HR, 1.32; 95% CI, 1.20–1.45; $p < 0.001$), nonfatal stroke (HR, 1.14; 95% CI, 1.04–1.24; $p = 0.004$), and need for coronary revascularization (HR, 1.46; 95% CI, 1.33–1.61; $p < 0.001$) but not unstable angina ($p = 0.53$) or CV death ($p = 0.23$). These associations remained significant even with the addition of non-HDL-C to the multivariate model and in high and low HDL-C subgroup analysis.

The key to understanding TG and its relationship to CV risk is to study its relationship to atherogenic lipoproteins, especially a change in the core TG of lipoproteins has a significant influence on how those particles are trafficked and catabolized. The lipoprotein hallmark of IR is the synthesis and secretion of large VLDL1 particles. As noted, normolipemic patients do not create significant amounts of VLDL1. A normal VLDL particle has a core TG/CE ratio of 5 to 1 [29]. Normally the TG-rich VLDL particles and chylomicrons undergo rapid lipolysis and vanish within 2–6 h but such is not the case when IR is at play where delayed catabolism and increased plasma residence time are the rule, leading to increased fasting and postprandial TG levels (Fig. 4.20) [201]. The elevated TG by itself leads to endothelial dysfunction, elevation of inflammatory markers, hypercoagulability, and increased blood viscosity. The delayed catabolism is due to several factors already discussed, including imbalance of apoA-II, apoC-I, apoC-III, CETP activity, and impaired LPL function. The longer the residence times of TG-rich lipoproteins, the greater the chance that

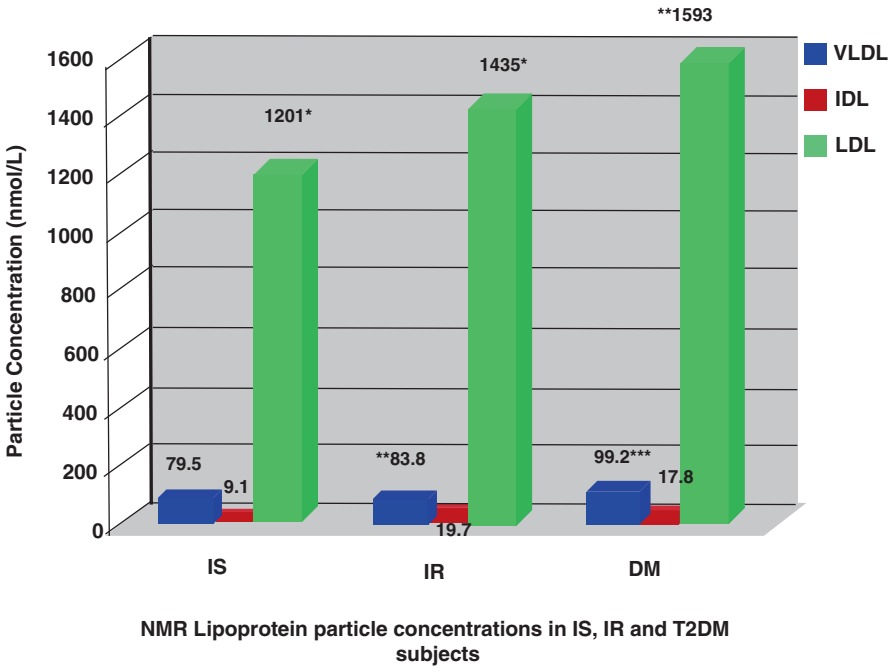

Fig. 4.20 Effects of insulin resistance on lipoprotein concentrations. Dyslipidemia was evaluated using both NMR lipoprotein subclass analysis and conventional lipid panel, and insulin sensitivity as the maximal glucose disposal rate (GDR) during hyperinsulinemic clamps in 56 insulin sensitive, 46 insulin resistant, and 46 untreated subjects with type 2 diabetes. The more insulin resistant the individual, the higher the concentration of VLDL-P and the higher the concentration of LDL-P. The majority of atherogenic lipoproteins in individuals with insulin resistance or T2DM are not triglyceride enriched VLDL-P, but cholesterol-depleted LDL-P. These compositional changes in LDL particles explain the lack of association between LDL-C and insulin resistance. (Reproduced with permission from Rosenson RS, et al. Atherosclerosis 2010;213:1–7)

both homotypic and heterotypic exchange of neutral lipids occur between lipoproteins utilizing CETP. The TG-rich VLDLs and chylomicrons send their core TG to IDLs and LDLs or to HDLs in exchange for CE. In the process, the VLDLs and chylomicrons become TG-poorer and CE-rich. Once LPL-mediated hydrolysis of core TG occurs, the particles reduce in size and shed surface phospholipids, creating atherogenic remnant lipoproteins [1H]. In essence, the remnants are very large, formerly TG-rich, but converted to CE-enriched particles. One must keep in perspective that despite the risk associated with remnants, that risk is not solely due to VLDL-P per se but also rather marked elevations of LDL-P (Fig. 4.21) [124, 202]. In a Japanese study, it was $apoB_{100}$ carrying lipoproteins (VLDL remnants), not $apoB_{48}$ lipoproteins, which were the major subset of remnants associated with sudden cardiac death in the postprandial state, regardless of the severity of coronary atherosclerosis [203]. Using newer analytical methods data suggest that the major

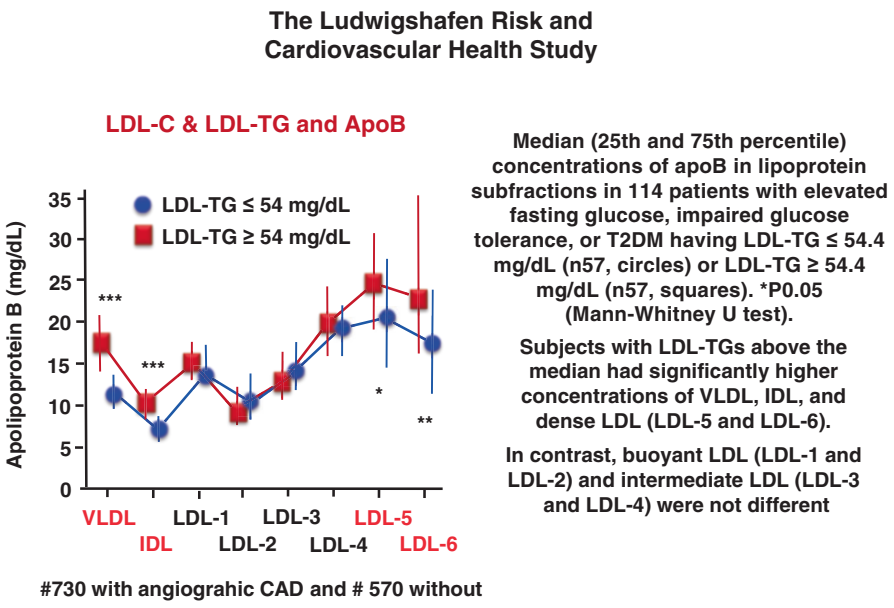

Fig. 4.21 ApoB levels in lipoprotein subfractions among patients with dysglycemia in the Ludwigshafen Risk and Cardiovascular Health study. (Reproduced with permission from März W, et al. Circulation 2004;110:3068–74)

part (approximately 80% or more) of remnants are VLDL remnants not chylomicron remnants. It was also found that plasma TG vs. remnant-TG concentrations in the postprandial state correlated significantly higher with risk than in the fasting state [204]. The increased TG in the postprandial state mainly consisted of TG in remnant lipoproteins. In normal volunteers, postprandial TG vs. remnant lipoprotein concentrations were significantly more correlated when compared with fasting TG vs. RLP concentrations and the authors concluded increased sensitivity of nonfasting TG in predicting the CV risk (events) may be directly explained by the increase of remnant lipoproteins in the postprandial state [205]. However, in the Copenhagen General Population Study, lipid and apolipoprotein concentrations, as a function of time since the last meal were evaluated in 58,434 individuals (participation rate 45%) from the general population, 2270 of whom had diabetes. TG increased up to 17.7 mg/dL after normal food intake in individuals with and without diabetes. No statistically significant differences in postprandial apoB were seen although apoB fluctuates to higher levels more in diabetics [206]. Nakajima has also suggested that remnant-like lipoprotein particles, not LDL particles are the major oxidized lipoproteins in plasma [207]. Lipolysis of TG-rich LP will be delayed with an excess of C-III, and the apoC-II/apoC-III ratio has been used as a predictor of lipolytic rate with high ratios associated with increased plasma residence time.

The increased CETP-mediated exchange of core lipids in T2DM results in TG-rich and CE-poor LDLs and HDLs. The size of the LDL or HDL does not affect the lipid transfer, and small or large LDLs and HDLs can be TG acceptors or CE

donors as can large TG-rich and CE poor LDLs and HDLs. TG-rich LDLs are an underappreciated part of dyslipoproteinemia. Patients with elevations of LDL-TG (defined as >54 mg/dL) may have low, normal, or elevated LDL-C levels, but because these LDLs are CE depleted, they are almost always associated with elevated LDL-P or apoB. This was studied in 1309 patients not taking lipid-lowering drugs in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Among these, 739 individuals had angiographic CAD (>20% stenosis), and 570 subjects served as control subjects. The association of LDL-TG (odds ratio [OR], 1.30; 95% CI, 1.19–1.43; $p < 0.001$) with CAD was stronger than that of LDL-C (OR, 1.10; 95% CI, 1.00–1.21; $p = 0.047$). The predictive value of LDL-TG for CAD was independent of LDL-C. High sensitivity C-reactive protein (hs-CRP), serum amyloid A, fibrinogen, interleukin 6, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) increased in parallel to LDL-TG. CRP, ICAM-1, and VCAM-1 were inversely related to LDL-C. The authors speculate that since HL is subject to modulation by inflammatory cytokines, low-grade systemic inflammation might be the cause rather than the consequence of high LDL-TG. In 114 individuals with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes mellitus subjects with high LDL-TG, LDLs were depleted of CE, and VLDLs, IDLs, and dense LDLs were significantly elevated, i.e., apoB was elevated. The authors concluded that LDL-TG is a better indicator for atherogenic alterations of LDL metabolism than is LDL-C at any given concentration of LDL particles, LDL-C would be low once LDL-TGs were high (Figs. 4.21 and 4.22) [208].

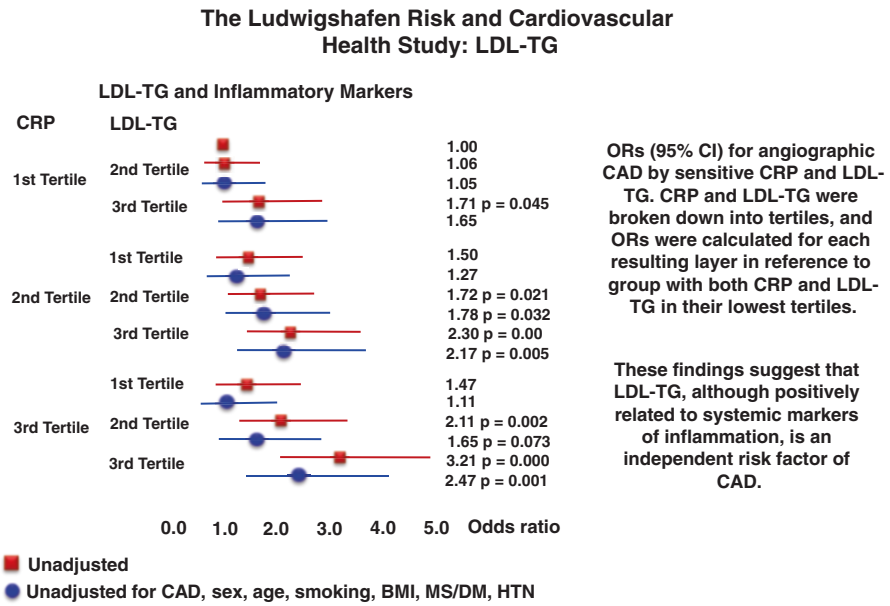

Fig. 4.22 Relationship between tertile of LDL-triglyceride and C-reactive protein levels. (Reproduced with permission from März W, et al. Circulation 2004;110:3068–74)

Of additional interest is the actual molecular structure and shape of TG-rich LDLs. LDL particles vary in their receptor-binding affinity and susceptibility to oxidative modifications. LDLs must be thought of as dynamically remodeling particles with changes in particle composition, shape, and size as well as apoB conformation; all of these features can affect LDL function and receptor-binding. Small LDLs show lower affinity for the LDL receptor but increased unspecific binding to cell surfaces [209]. LDLs also may undergo a structural transition at body temperature which may affect LDLr recognition. Below the transition temperature, the core-located lipids are arranged in an ordered liquid-crystalline phase, whereas above the temperature, the neutral lipids are organized in a fluid, oil-like, randomly distributed state. If the LDLs are TG-rich, the core lipids remain in their fluid phase, independent of temperature and such LDLs have less affinity for LDLr compared to normolipidemic LDL. When LDL core TG is normal, the core CE is immobilized causing a higher core viscosity. Under these conditions, the activity of CETP is lower [210]. It has also been speculated that when core lipids are in the liquid-crystalline state, surface phospholipids can be altered which could change the LDL shape from spherical to elliptical [211]. If the TG-rich LDs and HDL particles undergo additional lipolysis with HL, they can transform into small LDL or HDL with the latter being subject to break up with renal excretion of surface apoA-I. Atherogenesis is related to the accumulation and retention (binding to proteoglycans) of LDL in the arterial subendothelial space [212]. Several studies have suggested that the small LDL is highly prone to oxidation and binding to HSPG [213, 214]. Subintimal oxidation of LDL is an initial phase in atherogenesis and is a key step in activating macrophage lipoprotein scavenging and foam cell formation via increased expression of the LOX-1 (oxidized LDL receptor-1) and SRA (scavenger receptor A) [215]. The oxidation of LDL particles can be driven by such enzymes as NAD:NADH oxidase, xanthine oxidase, a variety of lipoxygenases, and myeloperoxidase [216]. These enzymes are activated as part of the inflammatory cascade and can incur significant molecular damage with the oxidation of phospholipids, fatty acid, amino acids, and sterols within the core of the particle [217, 218]. Lp-PLA_2 is known to have high affinity for and traffic with the small LDL species [219, 220].

Despite the discussion of LDL core composition and size, the major factor driving the particle into the arterial wall is particle number (particles flow down along a concentration gradient as dictated by thermodynamics). Older studies have related atherogenesis to the smaller, higher density LDL but several newer studies which adjusted for LDL-P reveal the LDL size does not maintain a statistically significant independence as a CV risk factor [220]. A major area of lipid/lipoprotein clinical importance in IR and T2DM is the significant discordance between two measurements that typically have excellent correlation, specifically LDL-C and apoB and LDL-P. As discussed in the Garvey study, although VLDLs contribute to apoB, the vast majority of apoB particles are LDLs [124] and apoB measurement should be regarded as an assay of LDL-P. The American Association of Clinical Chemistry (AACC) in a position statement [13] reiterated that apoB is a measure of LDL-P and not a measure of VLDL or VLDL remnants. Cromwell in an evaluation of CV death over 16 years in the Framingham Offspring trial (fourth examination cycle

1987–1991) showed that CV risk was related not *per se* to high or low LDL-C but rather elevated or not elevated LDL-P. Adding VLDL-P to the equation added little to risk prediction. There were far fewer events in those in the lowest quartile of LDL-P than the equivalent quartile of LDL-C (Fig. 4.23) [221]. Recent data from MESA also highlighted the fact that when LDL-C and LDL-P are discordant, abnormal changes in CIMT follow LDL-P better than LDL-C [222]. In another study of T2DM patients, 84% of patients who had an LDL-C < 100 mg/dL (the 20th percentile population cut point in FOS) had an LDL-P > than the equivalent 20th percentile LDL-P cut point. Of more concern was that in those with an LDL-C < 70 mg/dL (5th percentile cut point), 41% had an LDL-P above the 20th percentile cut points (Fig. 4.24) [223]. Sniderman has demonstrated this discordance in multiple clinical trials comparing CV risk to LDL-C vs. ApoB [224]. The level of TG in metabolic syndrome patients that is associated with at-risk levels of LDL-P is far lower than previously imagined. In the FOS as triglyceride levels increased from 80 to 250 mg/dL, the number of total LDL particles rose dramatically beginning with TG > 130 mg/dL while the levels of LDL-C remained low (Fig. 4.25) [225].

Low HDL-C is of course the other component of the TG/HDL axis. Often underappreciated is that TG also have a profound influence on HDLs affecting HDL-P, HDL-TG, HDL-C, and HDL functionality. As discussed, due to heterotypic CETP

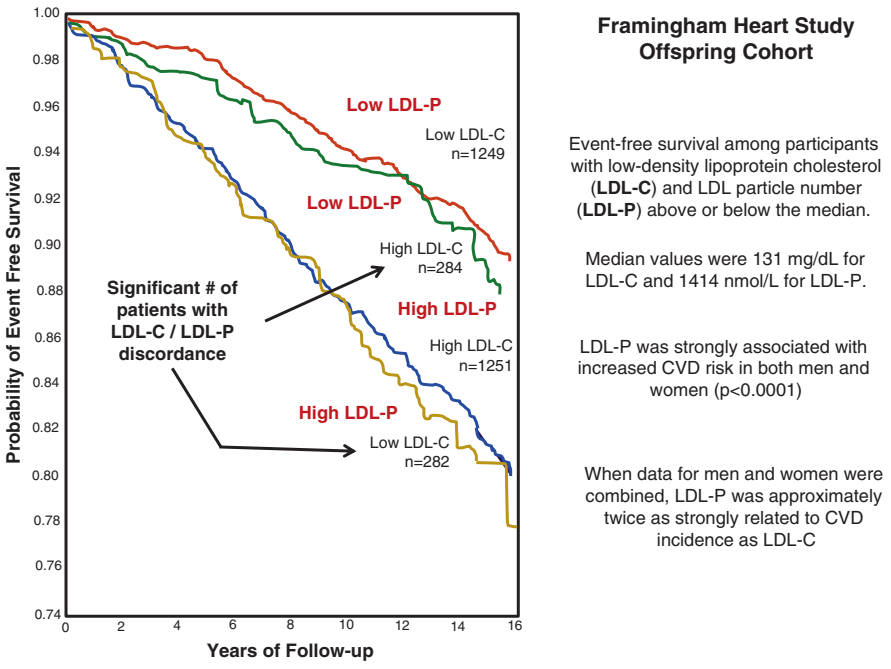

Fig. 4.23 Discordance between LDL-C/LDL-P and its impact on event free survival in the Framingham Offspring Study. (Reproduced with permission from Cromwell WC, et al. J Clin Lipidol 2007;1:583–92)

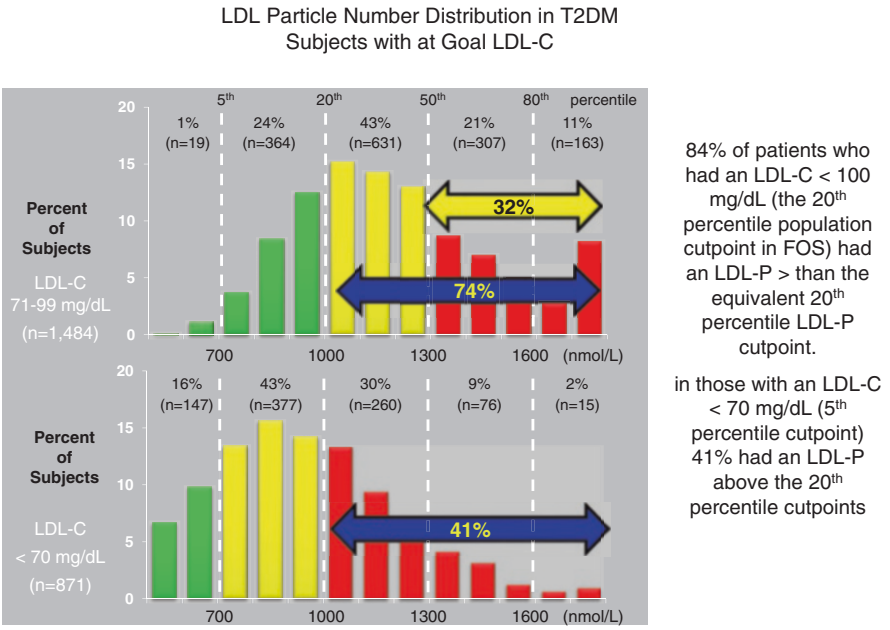

Fig. 4.24 LDL particle number distribution in T2DM subjects with at goal LDL-C. (Reproduced with permission from Cromwell WC, Otvos JD. Am J Cardiol 2006;98:1599–602)

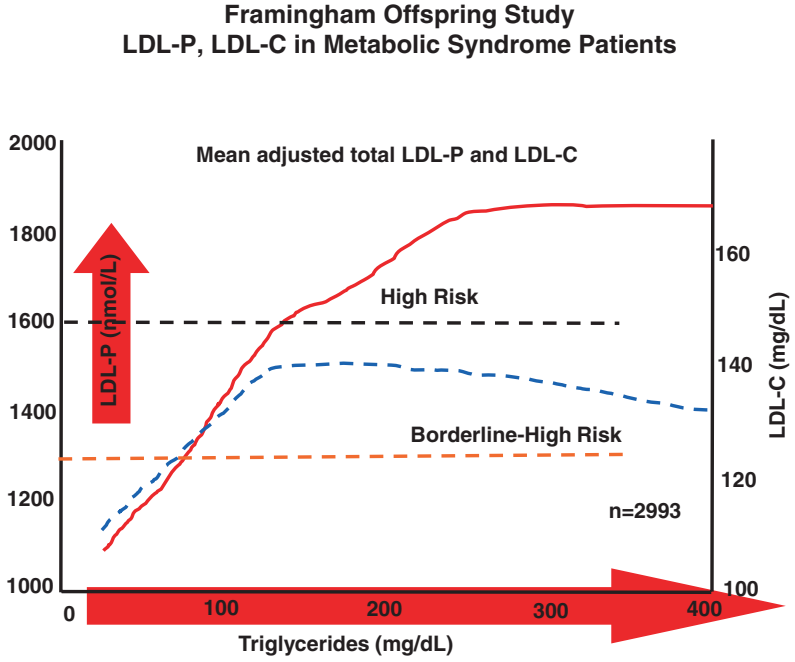

Fig. 4.25 Discordance between LDL-C and LDL-P in patients with metabolic syndrome in the Framingham Offspring Study. (Reproduced with permission from Kathiresan S, et al. Circulation 2006;113:20–9)

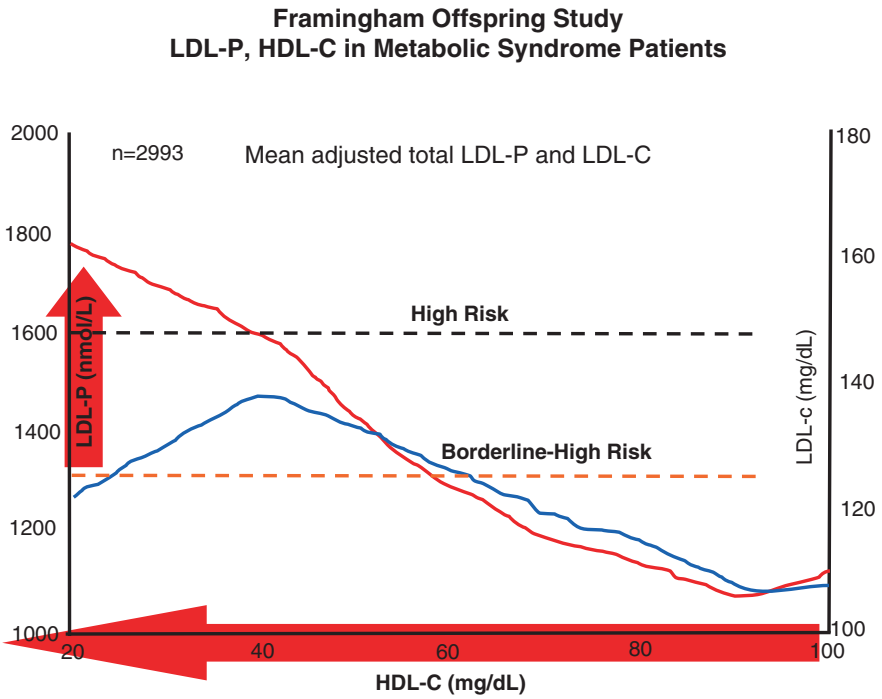

Fig. 4.26 Relationship between LDL-C, HDL-C, and LDL-P in patients with metabolic syndrome at either borderline or high risk for ASCVD events. (Reproduced with permission from Kathiresan S, et al. Circulation 2006;113:20–9)

exchange of neutral lipids, HDL particles become TG-rich and CE poor. It is one reason that low HDL-C (<40 mg/dL) is associated with abnormal, at-risk levels of LDL-P (Fig. 4.26). Rader suggests TG-enrichment of HDL, and its subsequent hydrolysis by HL adversely impacts HDL function [85, 226]. Such HDLs are subject to further lipolysis by HL and endothelial lipase resulting in smaller, denser HDL species, and apoA-I dissociates from the smallest of those particles [227]. EL and HL are upregulated in IR and may act in tandem with HL being more of a triglyceridase and EL, a phospholipase. Most lipoproteins cannot pass into the glomerulus, but lipids bound to filtered apolipoproteins can be excreted after binding to the renal tubule proteins, megalin and cubilin. Dissociation of apoA-I from HDL or failure of apoA-I to incorporate into HDL enhances renal apoA-I catabolism via cubilin-mediated tubule secretion (Fig. 4.27) [92, 228].

The preβ-1 HDL particles are poorly lipidated HDL particles composed of one or two molecules of apolipoprotein A-I and small amounts of PL and UC. Hypertriglyceridemic patients including those with metabolic syndrome exhibit significantly higher plasma preβ-1 HDL concentrations compared to healthy individuals. CETP and HL/EL induced remodeling of HDLs results in increased production and levels of preβ-1 HDL particles. The increased HL activity that has been observed in patients with high TG generates preβ-1 HDL. The net result of these changes is the elevation of preβ-1 HDL levels and the reduction in the

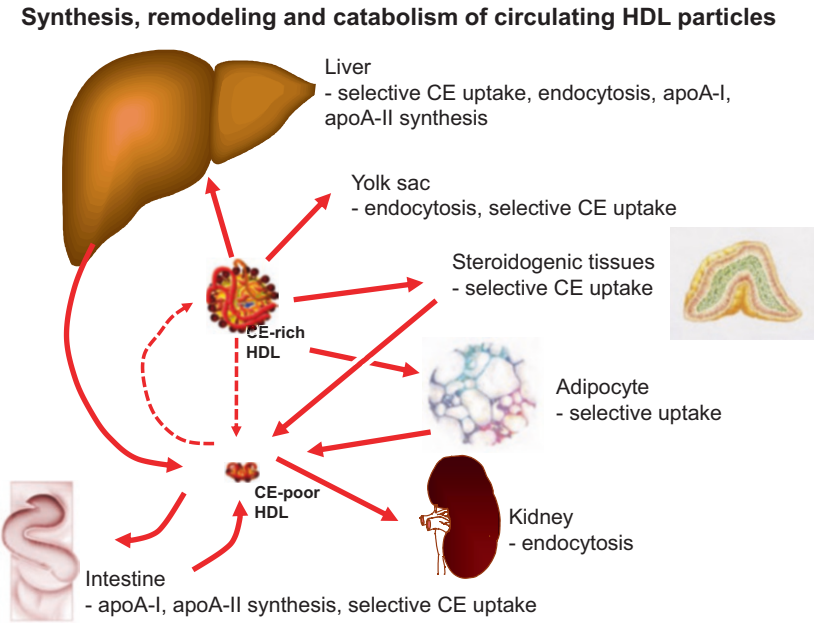

Fig. 4.27 (1) Formation of small (discoidal) HDL particles from apolipoprotein A-I and A-II synthesized in the intestine and complexed with phospholipid and free cholesterol transferred from other lipoprotein particles and tissues. (2) Formation of spherical HDL of increased size promoted by further lipid loading and lecithin cholesterol acyl transferase-catalyzed esterification of cholesterol. (3) Selective CE uptake (mediated by scavenger receptor BI; SR-BI) and endocytosis (unknown receptor) in the liver. (4) Selective CE uptake (mediated by SR-BI) in steroidogenic tissue, e.g., adrenals, testis, and ovaries. (5) Formation of small cholesterol-poor HDL particles originating from HDL particles subjected to selective uptake in the steroidogenic tissues. (6) Formation of small cholesterol-poor HDL particles originating from (a) HDL particles subjected to selective uptake in the liver or (b) apolipoprotein AI and AII synthesized in the liver. (7) Reduction of HDL size as a result of lipolysis (effect of hepatic lipase, lipoprotein lipase, and endothelial lipase), and transfer of CE and phospholipid to other lipoproteins as promoted by CE transfer protein and the phospholipid transfer protein. (8) Renal filtration and subsequent endocytosis (mediated by cubilin) of lipid-poor apolipoprotein AI and probably also some small filterable HDL particles (smaller than 8 nm). (9) Endocytosis (mediated by cubilin) of HDL and selective CE uptake in the yolk sac/early placenta of the pregnant organism. (Adapted and modified from Moestrup SK, Kozyraki R. Curr Opin Lipidol 2000;11:133–40)

concentrations of large α-migrating HDL [229]. It has been shown that increases in preβ1HDL concentrations reflect an impairment in HDL maturation and in dynamic remodeling of HDL and is a sign of impaired RCT [230–232]. PLTP activity is also increased in patients with high triglyceride values [233] and LCAT activity, required for maturation of HDL is also decreased [234]. In a study evaluating the functional effects of HDL in healthy persons, this lipoprotein decreased superoxide production, increased endothelial nitric oxide secretion, and improved both endothelium-derived vasodilatation and early endothelial progenitor cell-mediated endothelial repair. These endothelial effects of HDL were impaired in HDL from T2DM patients [235].

Conclusions

In summary, examination of lipoprotein changes present in drug naive IR and T2DM patients with or without TG/HDL axis abnormalities reveals elevations of apoB particles specifically increased remnants, significantly increased numbers of LDL particles, and decreased apoA-I and total HDL-P characterized by decrease in the larger alpha HDL species and increase in the prebeta-1 species. Many of these abnormalities are related to abnormal cholesterol absorption, synthesis, cellular efflux, and its trafficking in lipoproteins whose function is modulated by numerous apolipoproteins and cell surface receptors. Such patients therefore have high apoB/apoA-I ratios and high LDL-P/HDL-P ratios which were identified as the best predictors of CV risk in INTERHEART and VA-HIT and Women's Health Study, respectively [236–238]. More readily available to practicing clinicians is the TG/HDL-C ratio of which there are several studies linking high ratios >3.0 with insulin resistance [239], small LDL size [240], CV outcomes and all-cause mortality in women [241], as a predictor of residual risk in those treated to LDL-C goal [242], as a predictor of first coronary event in men [243] and with microvascular complications of diabetes [244]. In children with a TG-to-HDL-C ratio ≥2.0, there was a two- to three-fold higher risk of elevated ALT levels and concentric LV hypertrophy than those with a TG-to-HDL-C ratio <2.0, independent of confounding factors [245]. There are other factors influencing HDL-C concentrations in diabetes. ABCA1 expression and protein concentrations in leukocytes, as well as function in cultured skin fibroblasts were evaluated in drug naive T2DM men with variable degrees of hyperglycemia. All were abnormal and associated with low HDL-C. There are other conflicting studies with some showing ABCA1 directly influences glycemia via its action on β-cell insulin secretion, but other data suggest that it is glucose which modifies ABCA1 [246, 247].

A review of the NMR-measured lipoprotein changes typical of patients with TG/HDL axis abnormalities reveals increased total LDL-P and reduced total HDL-P and high LDL-P/HDL-P ratios. Subparticle examination identifies increased large VLDL-P, increased VLDL size, increased small LDL-P, decreased LDL size, decreased large HDL-P, and decreased HDL size. These parameters have been examined in the large (n = 28,345) Women's Health Study of whom over 13.3 years 1687 cases of T2DM occurred. Lipoproteins subfractions differed substantially by size in T2DM patients compared to normal patients. Small LDL and small HDL were positively associated with diabetes (quintile 5 vs. 1 [adjusted hazard ratios and 95% CIs], 4.04 [3.21–5.09] and 1.84 [1.54–2.19], respectively). By contrast, large LDL and large HDL were inversely associated with diabetes (quintile 1 vs. 5, 2.50 [2.12–2.95] and 4.51 [3.68–5.52], respectively). For VLDL, large particles imparted higher risk than small particles (quintile 5 vs. 1, 3.11 [2.35–4.11] and 1.31 [1.10–1.55], respectively). Lipoprotein particle size remained significant after adjusting for standard lipids (HDL-C and TG) and non-lipid factors [248].

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Chapter 5
Lipoprotein Metabolism and Alterations Induced by Insulin Resistance and Diabetes

Gerald H. Tomkin and Daphne Owens

Global warming continues to excite the imagination, even though in Ireland we have had the coldest summer on record and it is now fashionable to talk, not about global warming, but climate change. Similarly there is tremendous enthusiasm and interest in the explosion of obesity and diabetes that has occurred in the past 30 years, yet at the same time, people are living longer and we worry how to fund pension plans that will cover this increase in longevity. Obesity without diabetes, hypertension or dyslipidaemia may be less of a risk factor for cardiovascular disease than was originally thought, but with accompanying risk factors it is certainly a dangerous condition, with an increase in risk for diabetes mellitus, cardiovascular disease, cancer, stroke and osteoarthritis [1]. The link between obesity, insulin resistance and diabetes is complex and poorly understood, and the coining of the term "metabolic syndrome" has not helped to foster understanding of this complex disease process. Diabetes is primarily a disease of pancreatic beta cells leading to partial or complete loss of insulin production. It is an ongoing process with little evidence of reversibility, and we have yet to find a robust method of reversing the destruction of the beta cell whether it is due to apoptosis or necrosis. Obesity plays a major part in insulin resistance, and it is rare to find insulin resistance without obesity. Insulin resistance without a defect in pancreatic function (recognised by hyperinsulinaemia in the absence of hyperglycaemia) is also associated with increased risk for atherosclerotic disease. The purpose of this chapter is to explore the relationship between insulin or lack of insulin in the presence or absence of insulin resistance on lipid metabolism, and secondly to explore the effect of insulin resistance in the absence of a defect in the beta cell on lipid metabolism.

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Lipoprotein Metabolism Overview

The lipoprotein level in the blood depends on the balance of synthesis and degradation or turnover. Synthesis of the lipoprotein particle depends on cholesterol and fat absorption, de novo cholesterol synthesis and de novo fatty acid synthesis. Cholesterol absorption depends on the availability of cholesterol in the diet and the availability of re-circulated cholesterol via the enterohepatic circulation. De novo cholesterol synthesised in the intestine is also included in the cholesterol pool since it also enters the lipoprotein pool through the intestinal villi [2]. De novo synthesis of cholesterol occurs mainly in the liver, but virtually every cell in the body has the ability to synthesise cholesterol and the intestine is an important site of cholesterol synthesis. The larger lipoprotein particles consist of a triglyceride-rich core, and fatty acids which have been esterified to form phospholipid and cholesterol esters [2]. Phospholipids play an important part in the outer coat of the chylomicron particle, and the particle is solubilised by the addition of intestinally derived apolipoprotein (apo) B48, which is a better carrier protein for large amounts of triglyceride than apo B100 [3]. Apo B100 is the structural protein for very low density lipoprotein (VLDL), the major hepatically derived triglyceride-containing lipoprotein. VLDL is converted to low density lipoprotein (LDL) by delipidation in the circulation. The VLDL particle acquires its cholesterol from the cholesterol which is taken up into the liver through receptors such as the LDL B/E receptor, VLDL receptor, LDL receptor-related protein (LRP), and perhaps other receptors such as the apo E receptor 2 (apo ER2) [4]. Cholesterol that has been newly synthesised in the liver is the other source of cholesterol for the VLDL particle. Some cholesterol is also derived from high density lipoprotein (HDL), which is a cellular scavenger of cholesterol, and transports it back to the liver from peripheral tissue. The HDL cholesterol may be directly taken up by the liver through the scavenger receptor class B type1 (SR-B1) [5] (Fig. 5.1).

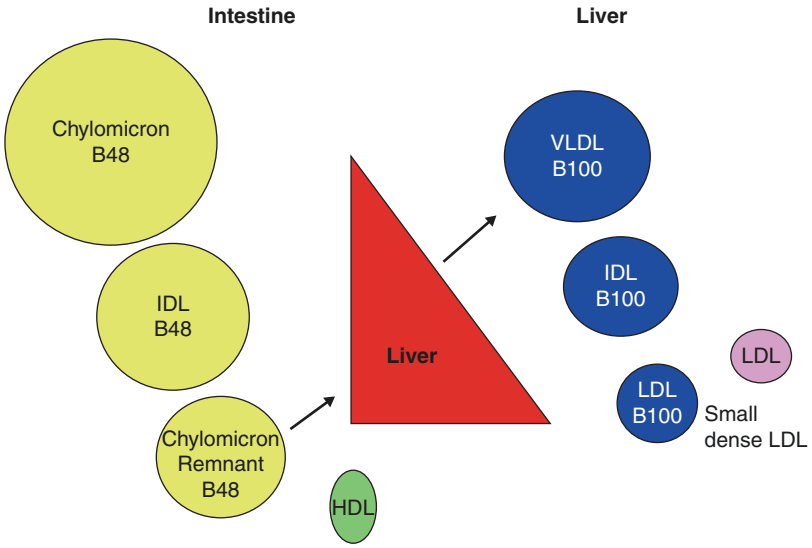

Fig. 5.1 The lipoprotein cascade

Apolipoproteins and Triglyceride-Rich Lipoprotein Metabolism

So far, we have given a very simplified version of lipoprotein metabolism. Apolipoproteins other than apo B48 and apo B100 are important for the chylomicron and VLDL story. Apo E for example, which is synthesised in the liver and other extrahepatic tissues, including macrophages, is transferred to the chylomicron and VLDL particles in the circulation. Apo E is necessary for clearance of the triglyceride-rich particles by the B/E receptor in the liver. Chylomicrons from diabetic patients have less apo E per particle than those from non-diabetic control subjects [6]. Once released from the chylomicron particle, or indeed from the VLDL particle since apo E is also attached to VLDL and is involved in its uptake by the liver, apo E is transferred to HDL.

Apo E is a very interesting protein in that it appears also to mediate cellular cholesterol efflux when attached to apo B. Apo E increases cholesterol uptake by the liver [7]. Apo E recycling in the hepatocyte is associated with an increase in ABCA1, a mechanism by which apo E increases cholesterol uptake in the liver. Many extrahepatic cells including the macrophage secrete apo E [8]. Apo E genotype is very interesting and has gathered a lot of research interest due to the increase in Alzheimer's disease and its association with apo E genotype, in particular E4. The mechanism whereby apo E4 confers Alzheimer risk is being investigated extensively, but remains elusive. Diabetes alas confers considerable risk of early dementia, but due to vascular damage rather than Alzheimer's disease, and the apo E4 genotype does not seem to confer extra risk [9]. A study by Shinohara et al. [10] examined whether diabetes affects cognitive decline depending on apo E genotype and potential relationships with neuropathology. They found that diabetes affected cognitive decline in apo E3 carriers and apo E2 carriers but not apo E4 carriers [11]. An earlier study found that apo E4 genotype in Type 2 diabetic patients did confer an extra risk. Perhaps the risk in diabetes of the apo E4 genotype is a much weaker one than in non-diabetic patients [12].

Apo CI is another apoprotein attached to the chylomicron and VLDL; however, 70% of apo CI is associated with HDL. During the postprandial rise of triglyceride-rich lipoproteins in serum, apo CI is transferred from HDL to VLDL [13]. Apo CI, at least experimentally, modulates lipoprotein production by increasing the production rate of hepatic VLDL, inhibiting lipoprotein lipase activity, interfering with apo E-mediated uptake of VLDL, and inhibiting cholesterol ester transfer protein (CETP). CETP transfers cholesterol from HDL to VLDL in exchange for triglyceride [14, 15]. Apo CII, on the other hand, is a cofactor for lipoprotein lipase which hydrolyses the triglyceride in chylomicron and VLDL and promotes their uptake by liver receptors, and thus is associated with a decrease in triglyceride-rich lipoproteins. Apo CIII is yet another constituent of triglyceride-rich lipoproteins which impairs lipoprotein uptake and is involved in hypertriglyceridaemia and fatty liver disease [16, 17].

It has also been shown to enhance hepatic triglyceride-rich VLDL assembly and secretion under lipid-rich conditions [18]. The risk of hypertriglyceridaemia and low HDL has been shown to predict outcomes in patients with stable angina. Of 355 patients with stable angina studied over a 4.5 year time-frame, as expected, patients with high triglycerides and low HDL more frequently had the metabolic syndrome, insulin resistance or diabetes. The authors concluded that the ratio of triglycerides to HDL could be used to identify patients with considerable residual risk [19].

Low Density Lipoprotein

The LDL particle is a cholesterol-rich, triglyceride-poor particle (Fig. 5.2). LDL is composed of a hydrophilic surface layer of phospholipid, free cholesterol, and hepatically derived apo B100 which packages the particle and adds stability. The core of the LDL particle includes esterified cholesterol and triglyceride together with the fatty acid tails of the phospholipid. LDL may act as a carrier for other insoluble particles such as free fatty acids and proteins which may be loosely attached [20]. Perhaps more importantly, lipoprotein lipase attaches to the particle and facilitates attachment of the particle onto the endothelial cell surface.

The atherogenicity of triglyceride-rich lipoproteins is increasingly recognised. This is not surprising as these particles, whether they be apo B100 particles synthesised in the liver or apo B48 particles synthesised in the intestine, are transporters of a considerable amount of cholesterol. It is true that there is very much less cholesterol per particle than in LDL, but LDL takes 3 or more days to transport cholesterol, whereas the chylomicron particle takes minutes and the VLDL particle only a little more. The analogy is the small bus carrying a few people but running very frequently as compared to a large bus carrying many people, but which only operates infrequently [21].

Triglyceride-rich lipoproteins are usually measured in the fasting state and therefore ignore chylomicrons. Even so, these triglyceride-rich particles, called remnant particles, have been shown to be predictive of cardiovascular events

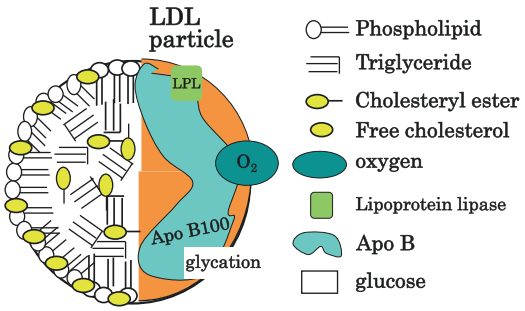

Fig. 5.2 The LDL particle

even when LDL is taken out of the equation [22, 23]. An excellent review of modern concepts of the aetiology of atherosclerosis has recently been published [24].

LDL can be sub-divided into sizes by gradient gel electrophoresis and separated into a pattern A and a pattern B, pattern B being termed small dense LDL [20]. This pattern B has been associated with an increase in atherosclerosis but it has been difficult to define changes in composition of the LDL that create the increased atherogenicity. The usual way to separate the different apo B containing lipoproteins is by density-based ultracentrifugation but the correlation between the denser particles on ultracentrifugation and size-based electrophoresis is uncertain. Amore recent addition to the methods to investigate lipoproteins is magnetic resonance (MR) spectroscopy which can sort particle size in large numbers of samples over very short time, but this technique still does not define small dense LDL [20]. Some years ago, a subfraction of LDL with oxidised characteristics was described and was named electronegative LDL (LDL^-) based on its properties of electrical mobility [24, 25]. It was later re-named minimally oxidised LDL. More heavily oxidised LDL is more electronegative than LDL^- and is identified as LDLOx. It now appears that electronegative LDL may also be produced by phospholipase (PL) A2. Rosenson et al. [26] in the PLASMA11 Trial showed that an inhibitor of PLA2 reduced LDL by 7% and small dense LDL by 11%.

Enrichment of LDL with apo CIII contributes to the electronegativity [27]. AntiLDL-monoclonal antibody had a protective effect against atherosclerosis in LDL receptor knockout mice [28]. It has been suggested that LDL^- is a potential stress biomarker present in health and disease [29]. Small dense LDL isolation by various methods has been compared by Chung [30].

The suggestion is that LDL's atherogenicity resides in the large amount of cholesterol being packaged in a relatively small volume; hence the surface area of the particle is relatively large, making it more easily amenable to modification and therefore more avidly taken up by scavenger receptors. Small dense LDL is also thought to be more susceptible to non-enzymatic glycation, even in non-diabetic people [31]. The association between small dense LDL and VLDL has been investigated, not least because of the difficulty of demonstrating hypertriglyceridaemia as an independent risk factor for atherosclerosis.

VLDL, like LDL, comes in many sizes depending on its triglyceride load. The Scottish and Finnish groups [32–34] many years ago demonstrated the relationship between large triglyceride-rich VLDL and small dense LDL: the larger the VLDL, the smaller and denser the LDL. Oxidation of the LDL particle depends on oxidation of its constituent protein and/or fatty acids. Polyunsaturated but not monounsaturated fatty acids are amenable to oxidation, hence a particle rich in linoleic acid is more susceptible to oxidation than one rich in oleic acid [35] (Fig. 5.3).

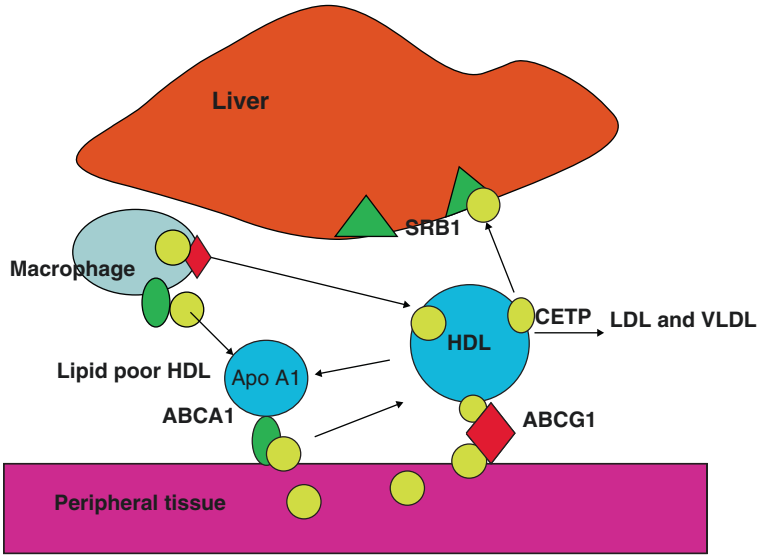

Fig. 5.3 HDL cholesterol uptake by the liver. Cholesterol uptake by HDL from macrophages and peripheral tissue is facilitated by ABCA1 and ABCG1 receptors. The HDL particle then docks with the liver giving up free cholesterol and lipid through the scavenger receptor (SR) B1 pathway and becomes free to circulate as nascent HDL

High Density Lipoprotein

Apoprotein A1 is the major apoprotein in HDL and functions as a cholesterol acceptor in the periphery through a complex set of interactions. ATP binding cassette transporter A1 (ABCA1) facilitates the efflux of cellular phospholipid and free cholesterol to assemble with apolipoprotein A1 (apo A1), forming nascent HDL particles. ABCG1 is another protein involved in cholesterol efflux from peripheral tissue to apo A1 for reverse transport and binds larger, more spherical HDL species. Lipid poor apo A1 accepts cholesterol released from macrophages forming nascent HDL [36]. The esterification of cholesterol to cholesteryl esters by lecithin: cholesterol acyltransferase (LCAT) is important for the process of mobilising cholesterol from the periphery. Once HDL becomes mature, it may transfer cholesterol and phospholipid through the action of cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) to apo B containing lipoproteins, in exchange for triglyceride, which is then hydrolysed by the action of hepatic lipase [37]. The HDL particle docks with the liver and gives up its remaining cholesterol and lipid through the scavenger receptor (SR) B1 pathway and becomes free to circulate as nascent HDL. There is an inverse relationship between HDL cholesterol and hepatic expression of SR-B1 [38]. In passing, it should be noted that the kidney plays an important part in apo A1 metabolism both by synthesis and clearance [39].

Apo AII is another apoprotein constituent of HDL and facilitates cholesterol efflux, HDL remodelling and cholesterol ester uptake. Apo AII has been found to be a strong risk factor for cardiovascular disease, and it has been suggested that variation in Apo AII production may exert an influence on apo B production [40]. The composition of HDL reflects on its various functions. For example, its ability to act as an antioxidant to apo B containing lipoproteins through PON-1 [41], and reconstituted HDL has been shown to have an antithrombotic effect [42]. HDL may also play a role in inflammation, and it has been shown that serum amyloid A1, which is elevated in inflammation, may be deposited in atheroma plaque and may promote endothelial dysfunction. HDL may reverse this process at least partially [43]. Further studies have suggested that HDL may modulate glucose metabolism in muscle and effect insulin secretion [44], and this is discussed in another chapter in this book by Dr. Jenkins. Although HDL-C levels are usually low and triglycerides usually high in obesity, the metabolic syndrome and type 2 diabetes, HDL functionality has not been well defined. A study which examined this in participants in the Dallas Heart study found that functionality of HDL was linked to the metabolic syndrome, but only to waist circumference and low HDL [45].

The Chylomicron

To explore the effect of diabetes and insulin resistance on lipoproteins, we will start at the beginning. In this chapter, the beginning must be the chylomicron and its synthesis, since without food, there would be little interest in diabetes or insulin resistance and in the rural areas of the world where starvation occurs, there is little talk of type 2 diabetes or insulin resistance.

The apo B48-containing chylomicron transports both cholesterol and triglyceride from the intestine to the circulation and has a dominant role in distributing fatty acids/triglyceride to the tissues prior to being taken up by the liver [46]. The second function of the chylomicron is to transport cholesterol to the liver, although on the way the cholesterol may be taken up by tissues, including the atheromatous plaque where the macrophage sits in waiting with a specific apo B48 receptor, as well as VLDL and scavenger receptors [47]. Apo B48 has been demonstrated in plaque by a number of workers [48–51]. Apo B48 is the solubilising protein necessary for the transport of cholesterol and lipid in aqueous solution in humans. The amount of triglyceride available for the chylomicron particle is limitless, the normal gut managing to limit the amount of triglyceride/fatty acids in the stool to under 5g/day. On the other hand, serum cholesterol is very tightly regulated and varies very little throughout one's lifetime due to a hugely efficient regulatory process. Absorbed cholesterol varies considerably from person to person, and high absorbers of cholesterol have been shown to have low synthesis rates and to be less sensitive to cholesterol lowering with statin therapy [52]. The mechanism that regulates cholesterol absorption in the intestine is complex, and both diabetes and insulin resistance have been shown to affect the regulation, [53, 54] causing the initiation of the dyslipidaemia of insulin resistance and diabetes (Fig. 5.4).

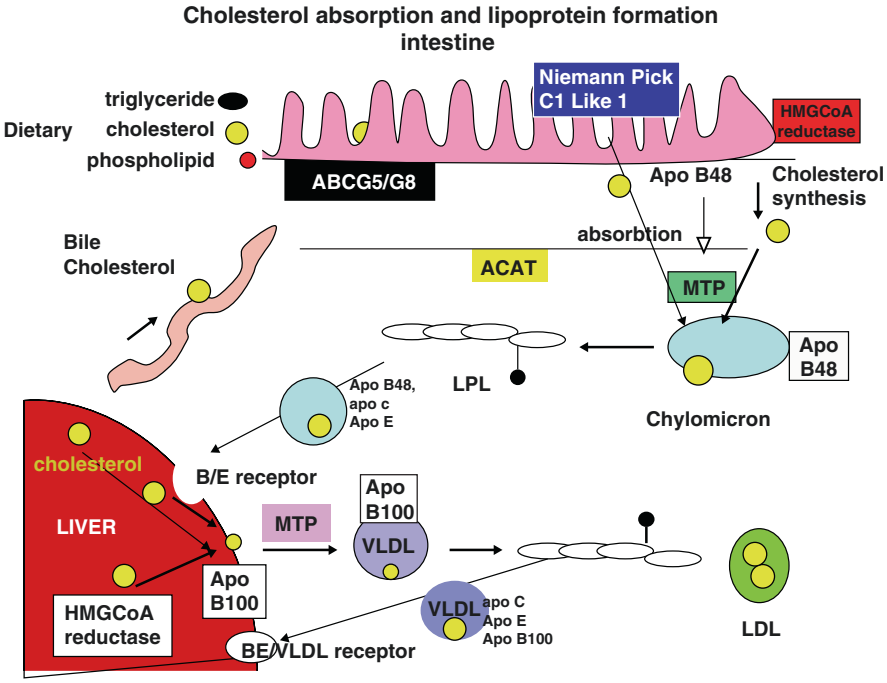

Fig. 5.4 Cholesterol absorption and lipoprotein formation. Dietary cholesterol, biliary cholesterol, and cholesterol synthesised in the intestine for which HMGCoA is the rate limiting enzyme, is transported across the cell membrane by NPC1L1 and, together with triglyceride, phospholipid, and the intestinally derived apo B48 protein, is assembled, under the influence of MTP into the triglyceride-rich chylomicron. Some of the absorbed cholesterol is excreted back into the lumen of the intestine under the influence of ABCG5/G8. The chylomicron is partially hydrolysed in the circulation by lipoprotein lipase and acquires apo CIII and apo E. The resulting chylomicron remnant is taken up by the B/E receptor in the liver. The cholesterol and triglyceride released are reassembled with hepatically synthesised cholesterol and apo B100 to form VLDL. Lipoprotein lipase in the artery wall releases the triglyceride from VLDL and it acquires apo CIII and apo E. Some of the VLDL is taken up again by the liver, and the rest is further hydrolysed and loses apo CIII and E to become IDL and then LDL

Intestinal Niemann-Pick C1-Like 1 Protein

The first step in cholesterol absorption in the intestine appears to be through the transmembrane protein Niemann-pick C1-like1 (NPC1L1) which is highly expressed in the jejunum. In humans, it is localised to the brush borders of the enterocytes and acts as a unidirectional transporter of cholesterol and non-cholesterol sterols [55, 56]. The mechanism of action of NPC1L1 has been elucidated. It has been shown that cholesterol promotes the formation and endocytosis of NPC1L1 flotillin-cholesterol membrane microdomains which is an early step in cholesterol uptake. Zhang et al. [57] discovered that it is the N-terminal domain of NPC1L1 that binds cholesterol. It is interesting that this domain does not bind to plant sterols;

thus, it now seems that plasma membrane bound NPC1L1 binds exogenous cholesterol and this binding facilitates the formation of the NPC1L1-flotiln-cholesterol micro domains that are then internalised into cells through the clathrin adaptor protein 2 pathway. Twenty rare NCP1L1 alleles have been found in the low cholesterol absorbers and appear to impair NPC1L1 cholesterol uptake through various mechanisms [58, 59] (for review see Calandra [60]). It has been shown that the effectiveness of ezetimibe, which blocks NPC1L1 and inhibits cholesterol absorption, depends on the NPC1L1 genotype [59].

There are other transporters of cholesterol; for example, SR-B1 is located both in the apical and basolateral membranes of the enterocyte [61]. Scavenger receptors (SR) are cell surface proteins that can bind and internalise modified lipoproteins. SRB1 which is involved in cholesterol uptake in the intestine and may play an important part in intestinal chylomicron production, and the fatty acid transporter CD36, which is also involved in the uptake of oxidised LDL, are members of the class B scavenger receptor family [61, 62]. Hayashi et al. [63] investigated gene expression of key proteins involved in the active absorption of dietary fat and cholesterol in response to the development of insulin resistance. They used two models of diet-induced insulin resistance, the fructose-fed hamster and the high fat fed mouse. Expression of SR-B1 was increased in both these animal models of insulin resistance. In the CaCo2 adenocarcinoma cell line, SRB1 over-expression increased apo B100 and apo B48 secretion. The authors concluded that apical or basolateral SR-B1 may have an important role in cholesterol absorption and may play a part in cholesterol over-absorption in insulin resistant states. SR-B1 in the intestine may play an important role in chylomicron production. CdC42, a member of the Rho family of small guanidine triphosphatases with numerous functions, has been shown by Xie et al. [64] to interact with NPC1L1 and to control its movement from the endocytic recycling compartment to plasma membranes in a cholesterol dependent manner. Glucose stimulated CDc42 signalling appears to be essential for second stage insulin secretion [65]. It is probable that in insulin resistance, the signalling of NPC1L1 is disturbed through this pathway but we have been unable to find any studies in the intestine that have explored the pathway in diabetes/insulin resistance. In animal studies, we have demonstrated an increase in cholesterol absorption in diabetes [66]. We then asked the question as to whether diabetes might be associated with an increase in cholesterol absorption through stimulation of NPC1L1. We demonstrated in animal models of diabetes that NPC1L1 was upregulated [67] and in diabetic patients we demonstrated an increase in NPC1L1 mRNA [68], suggesting a mechanism for an increase in cholesterol absorption. In the Sammomas Obesus, a model of type 2 diabetes, the animals exhibiting weight gain, hyperinsulinaemia and hypercholesterolaemia, NPC1L1 protein and gene expression were both significantly reduced in the intestine, and the authors found a lower capacity to absorb cholesterol compared to controls [69]. This may suggest interspecies variation, but it is a surprising finding considering that this animal model of diabetes has been shown to have increased production of intestinal apo B48-containing lipoproteins [70]. Ezetimibe has been shown to bind to the brush border and to NPCILI expressing cells [71]. There is a sterol regulatory element in the promoter and a

sterol sensing domain of NPCILI which appears to regulate cholesterol absorption in response to cholesterol intake. Huff et al. [72] have shown that NPC1L1 is suppressed in mice given a cholesterol-rich diet and increased in the cholesterol depleted porcine intestine. The nuclear receptor, peroxisome proliferator-activated receptor (PPAR) δ/β, appears to control the expression of NPC1L1. Activation by a synthetic agonist of PPARδ has been shown to reduce cholesterol absorption and reduce expression of NPC1L1 without altering expression of the adenosine triphosphate (ATP) binding membrane cassette transport proteins (ABC) G5/8 [73]. ABCG5/8 is a transmembrane heterodimer that transports plant sterols and excess cholesterol out of jejunal enterocytes (discussed in greater detail below).

Fenofibrate, a PPARα agonist, has been shown to inhibit cholesterol absorption, and the mechanism has been shown to be through reduced NCP1L1 transcription by binding to a PPARα response element upstream of the human NPC1L1 gene. In a human construct, Iwayanagi et al. [74] showed that PPARα positively regulated human NPC1L1 transcription, and Valasek et al. [75] showed that fenofibrate reduced intestinal cholesterol absorption by PPARα modulation of NPC1L1. Tremblay et al. [76] have shown that atorvastatin increases NPC1L1 in the intestine and decreased ABCG 5/8 which leads to an increase in cholesterol absorption. These findings were accompanied by an increase in the transcription factors, sterol regulatory binding protein (SREBP) 2, and hepatic nuclear factor (HNF)-4.

Intestinal ATP Binding Cassette Proteins G5/G8

Once cholesterol has been transported across the brush border membrane, it faces another regulatory process and may be excreted back into the intestinal lumen rather than being further processed for absorption into the perimesenteric lymphatic circulation. ABCG5/G8 expression is mostly confined to the human small intestine and liver [77]. These two proteins act in tandem to re-excrete both cholesterol and, in particular, non-cholesterol sterols such as plant sterols from the body. Much of the understanding of ABCG5/G8 comes from the rare mutations that cause a defect in ABC G5 and G8 and result in high levels of sitosterol in the blood. Beta-sitosterolaemia is a condition which manifests itself in children as tendon xanthomas or in young adults as severe coronary heart disease (CHD) with massive accumulation of sterols and stanols in monocyte derived macrophages [78].

Ma et al. [79] found in an animal model that dietary calcium had a beneficial effect on the lipoprotein profile by up-regulating the mRNA levels of intestinal ABCG5/8 and cholesterol-7α-hydroxylase (CYP7A1), whereas it downregulated the intestinal NPC1L1 and microsomal triacylglycerol transport protein (MTP) due to enhanced biliary cholesterol excretion. Méndez-González et al. [80] investigated the effect of ABC G5 and G8 deficiency on lipoproteins in mice.

They found that postprandial triglycerides were five-fold higher in the ABCG5/G8$^{-/-}$ mice due to a lower fractional catabolic rate with lower post-heparin lipoprotein lipase activities. They also showed that liver triglyceride secretion and intestinal

triglyceride secretion were higher, and there was a relationship between this and the HOMA index as a measure of insulin resistance. Since diabetes is so frequently associated with dyslipidaemia and atherosclerosis, the ABC translocases became a target for research. Blocks et al. [81] examined mRNA and protein expression of ABCG5 and G8 in the intestine of streptozotocin diabetic rats and found significant reduction in expression of both ABCG5 and G8. They found that levels were partially normalised on insulin supplementation. We have shown that ABCG5 and G8 were reduced by more than 50% in the intestine of Zucker diabetic fa/fa rats compared with lean rats although these changes did not reach statistical significance [68]. Insulin treatment caused a non-significant increase in ABCG5 and G8 mRNA. In another study of streptozotocin, diabetic rats ABCG5 and G8 were both very significantly reduced in the intestine [67]. There was a negative correlation between ABCG5 and G8 and chylomicron cholesterol. In the Psamonas Obesus, another model of diabetes, Levy et al. [69, 70] showed a reduction in ABC G5/G8 in the intestine. In the intestine of human subjects with type 2 diabetes, ABCG5 and G8 mRNA were both significantly lower compared to controls [68]. There was a negative correlation between ABCG5 and G8 and NCP1-L1 in the combined diabetic and control subjects, and there was a significant negative correlation between chylomicron cholesterol and both ABCG5 and G8 [69]. These two genes appear to play an important role in the dysregulation of cholesterol metabolism in diabetes.

Microsomal Triglyceride Transport Protein

The cholesterol that has evaded ABCG5 and G8 in the intestine is now ready to be solubilised for transport through the lymphatic system. The assembly of the chylomicron occurs under the direction of microsomal triglyceride transfer protein (MTP). MTP has the ability to combine cholesterol, triglyceride, and phospholipid into the triglyceride-rich chylomicron particle. The cholesterol that becomes available however is not only cholesterol that has been absorbed from the diet, but is also cholesterol that has been excreted through the bile duct under the influence of hepatic ABCG5/G8. Finally, there is the cholesterol that has been synthesised in the intestine through the 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase pathway. This pathway in the intestine accounts for up to 25% of body synthesised cholesterol, the amount varying depending on whether the people are high or low cholesterol absorbers. Intestinal MTP plays a major role in the assembly of the chylomicron particle and therefore of cholesterol and triglyceride metabolism. MTP has become a hot topic since inhibitors of intestinal MTP have been shown to lower triglyceride without causing hepatic steatosis, at least in animal studies [82, 83]. In short-term human studies, a specific intestinal MTP inhibitor did not appear to effect liver function tests [84]. Although many polymorphisms of MTP have been described, some of which have considerable impact on LDL cholesterol in both non-diabetic and diabetic subjects, it is difficult to know whether the results mainly stemmed from the effect in the liver rather than the intestine [85, 86]. The intestinal

inhibitors of MTP which have no effect on the liver should answer this question in the future. In animal studies, diabetes is associated with an increase in MTP mRNA with close correlation between MTP mRNA and chylomicron cholesterol. In the diabetic rabbit, increased intestinal MTP mRNA is associated with an increase in chylomicron particle numbers [87], but in the rat it is associated with larger particles [88]. The fructose-fed insulin resistant hamster model had an increase in MTP protein mass, and this was associated with an increase in the triglyceride-rich intestinally derived lipoproteins [89]. Zolotowska et al. [90] in 2003 examined the B48 containing lipoprotein assembly in the small intestine of *Psammomys obesus*, a model of nutritionally induced diabetes and insulin resistance. De novo triglyceride synthesis, apo B48 biogenesis, and triglyceride-rich lipoprotein assembly were all increased. MTP activity and protein expression, however, were not altered. In the enterocyte of fructose-fed golden hamster, MTP mRNA and protein mass were increased by TNFα but apo B levels in the enterocyte were not affected suggesting that there is considerable interspecies variation [90]. In humans with type 2 diabetes, we demonstrated an increase in MTP mRNA in intestinal biopsies [66, 91]. Diabetic patients who were on statin therapy had lower MTP mRNA compared to those not on statins [91]. We found positive correlations between MTP mRNA and chylomicron fraction cholesterol and apo B48 [91]. A novel intestinal specific inhibitor of MTP has been shown to ameliorate impaired glucose and lipid metabolism in Zucker diabetic fatty rats, but whether this effect was due to impairment of food intake or to inhibition of fat absorption is not clear [92].

The signals that upregulate chylomicron formation to cope with excess fat in the diet are slowly being elucidated. Another non-specific inhibitor of MTP, which reduced serum levels of triglycerides by more than 70%, was also associated with significant improvements in glucose tolerance and insulin sensitivity in Zucker fatty rats [93]. Hepatic MTP mRNA expression is negatively regulated by insulin, and it is suggested that insulin might also directly inhibit apo B48 secretion independently of MTP even though it is probable that upregulation of MTP stimulates apo B secretion. The membrane glycoprotein CD36 binds long chain fatty acids. CD 36 deficiency reduces chylomicron production [94]. It has been shown that binding of lipid by CD36 upregulates apo B48 and MTP through CD 36 signalling via the ERK 1/2 pathway [95]. Interestingly, polymorphisms of MTP which have been associated with differences in serum lipids appear to alter cholesterol absorption but not synthesis in women [96].

Apolipoprotein B48 and B100

Apo B48, the structural protein for the chylomicron, is produced in the intestine by editing of the hepatic version, apo B100 [97]. The enzyme apobec cuts the apo B100 form into the shorter version, apo B48. It has been suggested that apo B is in excess of body needs. In the liver, it has been shown that insulin silences apo B translation by introducing intracellular traffic into mRNA granules [98]. The authors showed

that the availability of apo B mRNA for translation was regulated by the rate of release from translationally silenced mRNPs processing bodies (p bodies). Insulin specifically silences apo B mRNA translation by reprogramming its mRNA into p bodies and reducing the size of translationally competent mRNA pools. Translational control via traffic into cytoplasmic RNA granules may be an important mechanism for controlling the rate of apo B synthesis and hepatic lipoprotein production, the authors suggest. It is however not clear that this silencing plays a part in reducing chylomicron production or influences nascent chylomicron size. In diabetes, it may be that there is an increase in apo B48 production, but then if meaningful, one would expect smaller chylomicron particles containing less triglyceride per particle to be produced. Our studies in an animal model demonstrated that the particles in the cannulated lymphatic duct of the rabbit was associated with an increase in chylomicron particle numbers [99], but in the rat it was associated with larger particles [66]. In patients with type 2 diabetes, apo B48 is increased but it is difficult to ascertain whether the increase is due to delayed delipidation, increased synthesis, or both [100]. We injected labelled chylomicrons, collected by canulation of the lymph duct into diabetic and non-diabetic rabbits, into another group of diabetic and non-diabetic rabbits and found evidence of both increased synthesis and delayed clearance [101]. Our animal research therefore suggests that the increase in apo B48 particles in diabetes may be due to both and increase in synthesis and a decrease in turnover.

Cholesterol Synthesis and HMGCoA Reductase

Cholesterol synthesis is regulated by HMGCoA reductase, the rate limiting enzyme in the synthetic pathway. Sterol regulatory element binding protein 2 (SREBP2) is a regulatory protein of cholesterol homeostasis and regulates HMGCoA gene expression. In isolated rat hepatocytes, we demonstrated significant reduction in HMGCoA reductase activity in the presence of insulin [101]. In animal studies, we reported the different effects of pioglitazone, an insulin sensitiser which acts through peroxisome proliferator-activated receptor (PPAR) gamma, as compared to insulin on expression of hepatic HMGCoA reductase mRNA [53].

We found a highly significant increase in expression of HMGCoA reductase in the liver of diabetic animals (Zucker diabetic fatty fafa rats). There was a small, but insignificant reduction in HMGCoA reductase mRNA in the intestine when the animals were treated with insulin. There was a larger reduction in HMGCoA reductase in the liver of the insulin-treated animals, but this reduction did not reach statistical significance [53]. In type 1 diabetes Sittiwet et al. [102] presented some evidence to suggest that improved glycaemic control increases cholesterol synthesis. However, the study was perhaps a little unsatisfactory in that, although there was a significant increase in cholesterol synthesis, there was no change in serum or lipoprotein cholesterol, nor was there any change in markers of cholesterol absorption. Inhibition of HMG CoA reductase with a statin has been shown to decrease ABCG5/8, as well as increasing NCP1L1, thus increasing cholesterol absorption [88].

Hepatic steatosis is common in diabetes, insulin resistance and obesity. Inflammatory stress is present in these conditions and is also associated with obesity, insulin resistance and diabetes. It is therefore of interest that Zhao et al. [103] demonstrated that interleukin 1b and interleukin 6 stimulation of HepG2 cells increased SREBP2 and HMGCoA mRNA. Further high fat loading in mice or LDL loading in HepG2 cells suppressed the above genes but this suppression could be overridden by the above inflammatory proteins. Severe calorie restriction in patients with steatosis results in rapid reduction of liver fat, insulin resistance and improvement in diabetes control. In contrast, insulin resistance and the accompanying hyperinsulinaemia are associated with an upregulation of SREPB-2 through extracellular signal regulated pathways involving the kinases ERK-1 and 2, another example of the interaction between fat and carbohydrate metabolism [104] (for review see Van Rooyen and Farrell [105]).

Very Low Density Lipoprotein

Before discussing clearance of the chylomicron particle with reference to insulin resistance and diabetes, it is necessary to discuss VLDL, the other major triglyceride transport particle which is produced by the liver and has as its structural protein apo B100. The synthesis of the VLDL particle in the liver is somewhat similar to that of the chylomicron in the intestine. Through a series of steps, the lipid and cholesterol are assembled under the influence of MTP with apoB100 yielding VLDL. The VLDL particle will contain some de novo synthesised cholesterol. As with the chylomicron, apo E attaches itself to the particle and is necessary for clearance by the liver through the LDL B/E receptor. The VLDL particle is distinguished from the LDL particle, not only by its triglyceride content, which is much higher than LDL, but also by the attachment of apo E onto the particle. There are three common polymorphisms of apo E. Apo E2/2 although rare is associated with hypercholesterolaemia but E4/4 with hypertriglyceridaemia [106]. Compared with individuals with the E3/3 genotype, E2 carriers have a 20% lower risk of coronary heart disease and E4 carriers have a slightly higher risk. It has been suggested that the apo E4 allele is a risk factor for the metabolic syndrome [107]. Apo CIII can be present on apo B-containing lipoproteins but is not integral to the basic lipoprotein particle structure, thus lipoproteins exist both with and without apo CIII. Apo B-containing lipoproteins with apo CIII are enriched in triglyceride and cholesterol and have slow clearance from plasma. The concentration of apo CIII in VLDL and LDL is highly and independently predictive of coronary heart disease, more so than triglyceride alone [108]. LDL particles with apo CIII, a remnant particle produced by partial lipolysis in plasma of VLDL, are the lipoprotein particle type most predictive of CVD in type 2 diabetes [109]. Apo CIII inhibits lipoprotein lipase and triglyceride hydrolysis as well as direct clearance of VLDL particles from plasma, resulting in the formation of less LDL. In passing one might mention apo A5, a key gene regulating triglyceride levels and thought to be exclusively in the liver [110]. Lee et al.

[111] have described the expression of the gene in the mouse and human small intestine. The function here has yet to be explained.

Dallinga-Thie Guardiola et al. [112] examined apo A5 in diabetes in relation to triglycerides and found the same positive relationship between apo A5 as in non-diabetic subjects. They found in a group of 215 subjects with type 2 diabetes taken from the Diabetes Atorvastatin Intervention Study that 6% of the variation in plasma triglycerides was due to apo A5, whereas 52% was explained by apo CIII. Diabetes sometimes results from pancreatitis which may be caused by severe hypertriglyceridaemia. Apo A5 has not been shown to play a part in diabetes secondary to pancreatitis [113].

Cholesterol Synthesis and Transport in the Liver

Cholesterol may be either synthesised in the liver through the HMGCoA reductase pathway and packaged for transport by association with apo B100 or the cholesterol may have been delivered to the liver by the chylomicron particle. Insulin plays a major part in regulating many of the steps in the production of cholesterol [114, 115]. HMGCoA reductase is increased in animal models of diabetes in the liver [99]. In isolated rat, hepatocytes we have demonstrated significant reduction in HMGCoA reductase activity in the presence of insulin [116]. In animal studies, we have reported the different effects of pioglitazone, an insulin sensitiser through peroxisome proliferator-activated receptor (PPAR) gamma, as compared to insulin on expression of intestinal and hepatic HMGCoA reductase mRNA [53]. In that study, we also found a highly significant increase in expression of HMGCoA reductase in the liver of diabetic animals (Zucker diabetic fatty fafa rats). There was a large reduction in HMGCoA reductase in the liver of insulin-treated animals but this reduction did not reach statistical significance. The liver, like the intestine, can regulate, at least to some extent, the amount of cholesterol in the VLDL particle by regulating the excretion of cholesterol in bile.

Hepatic NPC1L1

NPC1L1 is localised to the canalicular membrane in hepatocytes where it plays a part in the regulation of cholesterol transport. Hepatic nuclear factor-1 (HNF-1) alpha and sterol regulatory element binding protein-2 (SREBP-2) appear to be important regulators of NPC1L1 in the liver [117]. It has also been shown that they have important binding sites within the human NPC1L1 promoter. The role of NPC1L1 in the liver is probably to divert cholesterol away from excretion in the bile [118]. A recent study in female Chinese women with gall stones has shown reduced NPC1L1 mRNA and protein in the liver and super saturation of cholesterol in the bile [119].

Ezetimibe has not been shown to increase the risk of gall stones, perhaps because the drug has its primary effect in reducing cholesterol absorption. Indeed in the golden Syrian hamster, ezetimibe reduced the diet-induced increase in biliary cholesterol [120] and, in gallstone susceptible mice fed lithogenic diets, ezetimibe prevented gall stone formation [121]. Inhibition of NPC1L1 by ezetimibe is associated with an improvement in hepatic steatosis. Jia et al. [122] investigated the mechanism by deleting NPC1L1 in mice and inducing hepatic steatosis with a high fat diet. The knockout mice did not develop steatosis. Hepatic fatty acid synthesis and mRNA for genes regulating lipogenesis were reduced, and the knockout animals did not develop hyperinsulinaemia. Nomura et al. [123] demonstrated in Zucker rats that ezetimibe improved hepatic insulin signalling as well as hepatic steatosis in both the liver and in cultured steatotic hepatocytes. The drug recovered insulin induced Akt activation and reduced gluconeogenic genes. The relevance of this study to humans is not clear as patients with diabetes who are treated with ezetimibe do not improve blood glucose control [124]. Over-expression of NPC1L1 in the liver inhibits biliary cholesterol secretion and raises serum cholesterol suggesting that inhibitors of NPC1L1 may have a role in the liver [125]. NPC1-L1 mRNA has been shown to be increased in the liver of diabetic rats with a positive correlation between NPC1L1 mRNA and VLDL cholesterol [67]. Interestingly, we found that although pioglitazone significantly reduced NPC1L1 in the liver of diabetic fafa rats, insulin had no effect [53]. ABCG5 and G8 play a role in the regulation of cholesterol excretion by promoting neutral steroids into the bile. Diet-induced lipid loading into liver causes a significant increase in the expression of ABCG5 and ABCG8 in bile canaliculi [126].

Hepatic ABCG5 and G8

The liver X receptor (LXR) helps to regulate expression of ABCG5/G8 [127], and ABCG5/G8 expression is also stimulated by LXR agonists, but not to the same extent as by feeding. It has recently been demonstrated that NCP2, a cholesterol binding protein secreted by the biliary system, positively regulates biliary secretion of cholesterol through stimulation of ABCG5 and G8. There is a well-documented relationship between gallstones, diabetes and the metabolic syndrome.

Under conditions of obesity and insulin resistance, the serine/threonine protein kinase Akt/PKB is required for lipid accumulation in liver. Two forkhead transcription factors, FoxA2 and FoxO1, have been suggested to function downstream of and to be negatively regulated by Akt and are proposed as key determinants of hepatic triglyceride content [128].

In mice with isolated insulin resistance, there was increased expression of biliary transporter ABCG5/G8 through disinhibition of the forkhead transcription factor FOX01 [129]. However, these findings do not fit well with the increased VLDL synthesis that has been described in insulin resistance and diabetes [129, 130] even if they explain the increased gallstones found in diabetes [131, 132]. In diabetic fafa

rats, insulin, but not pioglitazone, significantly increased hepatic expression of ABCG5 and G8 [53].

MTP in the Liver

The final stage for the production of the VLDL particle is the assembly of the cholesterol, triglyceride, and phospholipid with apo B100 under the regulation of MTP. In animal studies, MTP mRNA is increased in streptozotocin diabetic rats in the liver [66]. We have shown modest suppression with insulin treatment in the liver of Zucker diabetic rats [53]. In the alloxan-diabetic rabbit model, we found no difference in MTP mRNA or activity in the liver, whereas there was a significant increase in the intestine [85]. The disturbances in chylomicron and VLDL production reflect the increase in chylomicron and VLDL particles found in diabetes. Although chylomicron production has only been measured in animal models of diabetes and insulin resistance, VLDL over production has been shown in diabetes in humans as well [129–135]. The driving force is the non-compressibility of FFA postprandially due to the malfunction of adipose tissue by adipose triglyceride lipase (ATGL) which leads to an increase in postprandial triglycerides which are taken up by the liver and re-secreted in VLDL [134]. VLDL overproduction is also found in insulin resistance [135].

Triglyceride-Rich Lipoproteins in Diabetes

One of the causes of the disturbance in the metabolism of the triglyceride-rich lipoproteins in diabetes is the inhibition of lipoprotein lipase (LPL) activity. Lipoprotein lipase is an insulin sensitive enzyme. In type 2 diabetes, insulin treatment significantly increases LPL activity in adipose tissue [136, 137]. Among the causes of lipoprotein lipase, dysfunction is the effect of glucose on enzyme dimerization and has been related to the severity of diabetes [138]. Clearance of both VLDL and chylomicrons is severely impeded due to the inability of lipoprotein lipase to function in a relative or absolute insulin deficient environment. The VLDL remnant is cleared by the LDL B/E receptor or the VLDL lipoprotein receptor (VLDLr) which is found mainly in adipocytes and muscle cells [139]. The LDL receptor-related protein which clears postprandial lipoproteins is also insulin dependent [140]. Perhaps one of the most important results of this delay in clearance is the influence that the triglyceride-rich lipoproteins have on LDL size (decreasing it) and atherogenicity (increasing it) [141].

Large triglyceride-rich VLDL is associated with both increased small dense LDL and reductions in HDL. The atherogenicity of triglyceride-rich lipoproteins, particularly VLDL remnants, is recognised. Particularly with their shorter half-life than LDL, they transport considerable amounts of lipids, including cholesterol [142].

Truly fasting triglyceride levels do not include chylomicrons, but would include VLDL and VLDL remnants. Levels of these remnants predict cardiovascular events, even when LDL is taken out of the equation [143, 144].

LDL in Diabetes

The concentration of LDL is often normal in people with diabetes, when compared to non-diabetic people of similar BMI. However, since turnover of the LDL particle is increased in diabetes [145] and production is increased through the increase in hepatic VLDL secretion, a similar serum level of LDL in diabetes should not be taken as meaning that the atherogenic risk of LDL is similar to that in non-diabetic people, and is one of the reasons why emphasis is placed on maximum reduction of LDL in diabetes.

HMGCoA reductase and the LDL receptor are insulin sensitive, and the receptor is down-regulated and HMGCoA reductase is upregulated in the setting of insulin resistance [145].

As stated previously, small dense LDL is more common in diabetes, particularly Type 2 diabetes, and small dense LDL has been shown to promote macrophage foam cell formation [146]. LDL composition in diabetes is also abnormal in other ways, i.e. it contains more esterified cholesterol [142], an increase in linoleic acid and more FFA [12]. However, another study suggests that patients with diabetes and the metabolic syndrome have lower cholesterol ester and lower linoleic acid in the cholesteryl esters [36]. This may of course be due to differences in diets between the two studies. Both studies agree that markers of lipid peroxidation are increased in diabetes. Colas et al. [147] have also shown that LDL from metabolic syndrome and type 2 diabetic patients were potent in activating the platelet arachidonic acid signalling cascade potentiating platelet aggregation as compared to control LDL.

An increase of free radical production occurs in the hyperglycaemia of diabetes [148] causing non-enzymatic LDL glycation. Some studies show that glycated LDL is more susceptible to oxidation, one reason for the increased LDL oxidation that occurs in poorly controlled diabetes [149]. The foam cell is perhaps the hallmark of the atherosclerotic lesion. The development of the foam cell depends on macrophage uptake of cholesterol. A novel mechanism has been described which facilitated uptake of oxidised LDL through uptake by the toll-like receptor 4 (TR4) which is found on the macrophage surface [150, 151]. It has been demonstrated that cholesterol ester hydroperoxides are an indigenous ligand for TR4. The increase in free fatty acids in poorly controlled diabetes is associated with an increase in fatty acids attached to the LDL particle, a further potential cause for increased oxidation of the particle [36]. Oxidised LDL can be taken up by the macrophage through scavenger receptors in an unregulated manner. We have shown that lipoprotein lipase mass is increased on diabetic LDL [36], a factor which also increases LDL uptake into plaque (Fig. 5.5). Like all good theories, the oxidation theory has recently been challenged and Libby [24] writes "we should seek explanations beyond the oxidation hypothesis".

Normally LDL is cleared through the LDL receptor which is downregulated by receptor-mediated cholesterol uptake into the cell. The LDL receptor is upregulated

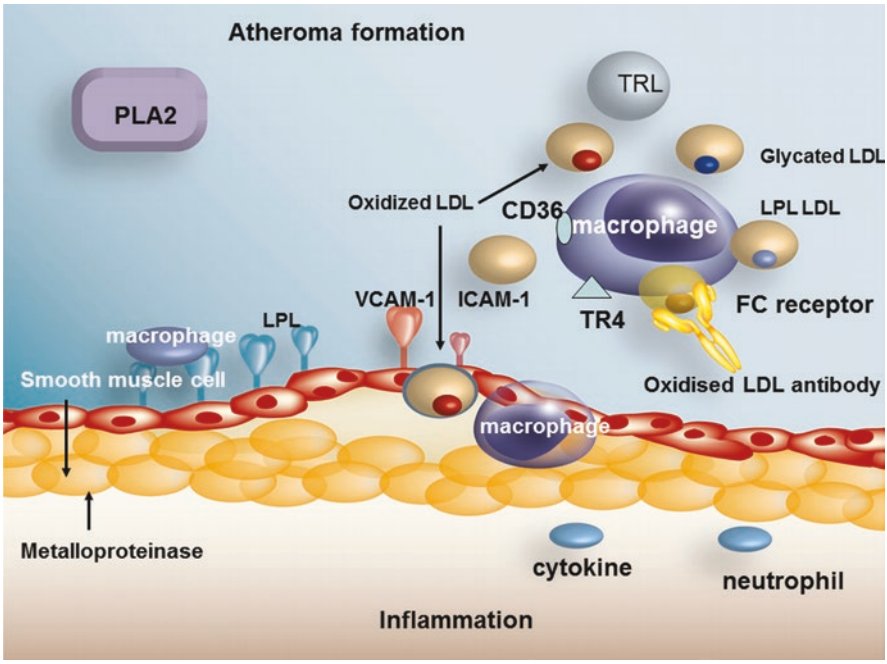

Fig. 5.5 Atherosis formation. The atherosclerotic plaque is composed of a lipid-rich core containing cholesterol, fatty acids and necrotic tissue and is covered by a fibrous smooth muscle cell cap. Low density lipoprotein (LDL) is the major contributor to plaque cholesterol. LDL may attach to the endothelium through lipoprotein lipase and heparin sulphate proteoglycans (HPSG) which facilitate their uptake into the subendothelial space. Macrophages, which have accumulated large amounts of cholesterol, attach to chemotactic factors such as VCAM and ICAM on the artery wall, and slip between the endothelial cells into the intima where they are trapped, mature into foam cells and eventually disintegrate providing the cholesterol for the lipid-rich atherosclerotic core

by insulin. Insulin signalling via AKT2 and mTORC1 has emerged as having a key role in the regulation of hepatic lipogenesis in obese mice. The underlying mechanism involves induction of proprotein convertase subtilisin kexin type 9 (PCSK9) via mTORC1 leading to post-transcriptional down regulation of hepatic LDL receptors. The glucose insulin pathway is perhaps another story, but very exciting, since it involves both an understanding of how glucose stimulates insulin secretion in the beta cell and how insulin stimulates glucose disposal in all other cells. A few years ago, PKCs were all the rage and an editorial in Diabetologia on The Rising Star of PKC, started with the sentence " In the pubs of Ireland there is talk of little else" [152]. PKCs are involved in insulin signalling and glucose transport but another pathway, and in particular a pathway involved in glucose disposal in exercise is signalled by AKT2. Thus the understanding that AKT2 is also involved in cholesterol lowering through its effect on the LDL receptor is another link in the chain tightening the link between dyslipidaemia and hyperglycaemia in diabetes (and of course supports the idea diabetes mellitus being re-named diabetes lipidus) (Fig. 5.6).

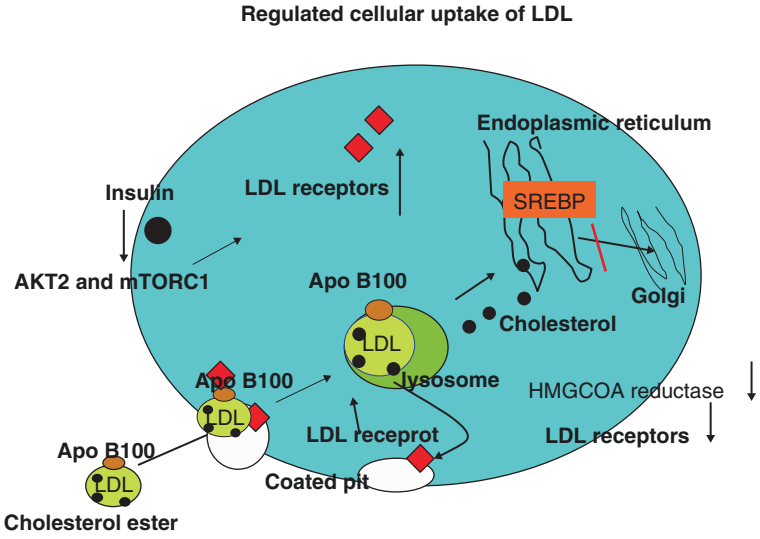

Fig. 5.6 Receptor-mediated LDL clearance. LDL is normally taken into the cell through the LDL receptor pathway. In the lysosome, the apo B is degraded and the cholesterol released is transferred to the endoplasmic reticulum. This blocks the transport of SREBP to the Golgi complex preventing transcription of HMGCoA reductase, thus reducing de novo cholesterol synthesis and also blocking LDL receptor synthesis, preventing further LDL uptake

The excitement of this new information, apart from a better understanding of the control mechanism for the receptor is that it opens up new avenues for pharmacological intervention. An example of translational science at its best might become the discovery of antibodies that inhibit PCSK9. PCSK9 is the gene which regulates recycling of the LDL receptor, diverting it to the lysosomal compartment for degradation. PCSK9 binds tightly to the LDL receptor and channels it towards the lysosomal compartment for degradation which results in decreased LDL receptor numbers and increased plasma LDL levels. An interesting loss of function polymorphism of PCSK9 increases the number of LDL receptors and increases LDL removal from the plasma, reducing LDL levels. There is strong evidence that PCSK9 and LDLR transcription are both activated by cellular cholesterol depletion via sterol regulatory element binding protein-2 (SREBP-2). This notion is supported by human studies that plasma PCSK9 concentration is increased with statin therapy. It has been shown that fibrates also significantly increase circulating PCSK9 levels. Thus an inhibitor of PCSK9 would be a useful addition to statin and fibrate therapy. Dramatic lowering of LDL-cholesterol levels has been described in non-human primates using monoclonal antibodies (mAB) against PCSK9, and the New England Journal of Medicine has reported on a clinical trial of mAB against PCSK9. The authors showed in healthy volunteers, up to 65% reduction in LDL cholesterol. These experiments were repeated in patients with familial hypercholesterolaemia who were already on atorvastatin and the results were similar to healthy volunteers. The authors found a good correlation between reduction in free PCSK9 and reduction in LDL cholesterol, and the authors demonstrated that the drug had an

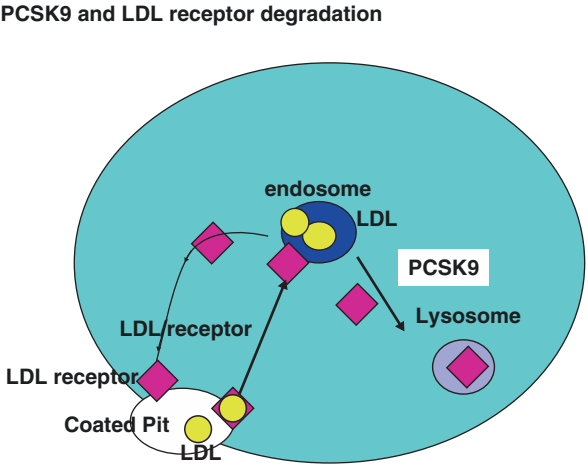

Fig. 5.7 PCSK9. PCSK9 is a regulator of liver LDL receptor expression. Normally the LDL receptor, once it has delivered LDL to the lysosome, re-circulates to the coated pit on the cell surface. Insulin signalling via AKT2 and mTORC1 induces PCSK9 which binds tightly to the LDL receptor and instead channels it toward the lysosomal compartment for degradation resulting in decreased LDL receptor numbers and increased plasma LDL cholesterol

additive rather than synergistic effect since the mean reductions were similar between normal and FH patients when administered alone or in subjects receiving atorvastatin and this is to be expected since atorvastatin increases hepatic LDL receptor activity by enhancing production of LDL receptors whereas the antibody decreases the degradation of receptors. There was a significant increase in HDL cholesterol. There was no clear evidence of drug related events which perhaps was the most important finding in the study.

PCSK9 treatment is now main stream treatment for hypercholesterolaemia unresponsive to statin treatment and in particular, familial hyperlipidaemia but ineffective in those with LDL polymorphisms that affect recognition of LDL by the LDL receptor. PCSK9 agonists also lower Lp(a) which may be an extra benefit in those with high levels. Lowering Lp(a) may of course have unknown adverse effects, but a recent study from the Copenhagen population study suggested that low Lp(a) carries no risk and in fact may be protective with regards to certain cancers [153, 154] (Fig. 5.7). PCSK9 inhibitors are discussed further in the chapter by Dr. P. Toth.

High Density Lipoprotein in Diabetes

The lipoprotein cascade which starts with the chylomicron, ends with HDL, the smallest of the lipoprotein particles and the only non-apo B containing particle. The solubilising proteins for HDL are apo AI and AII, although no HDL species contain only apo AII. Diabetes is associated with low HDL and in many studies low HDL

has been associated with premature/accelerated atherosclerosis. The reasons for the low HDL in diabetes include an increase in apo AI catabolism. Chan et al. [155] examined the relationship between the fractional catabolic rate between apo A1 and VLDL kinetics in a group of obese men compared to non-obese men. They found that variations in VLDL apo B production and therefore triglyceride concentrations exerted a major effect on the catabolism of apo AI. They further found that insulin resistance and adiponectin, an insulin sensitising hormone produced by adipocytes, and reduced in obesity, were contributing factors. They found that in a study of 87 non-diabetic men, plasma adiponectin was one of the best predictors of HDL apo AI fractional catabolic rate (FCR). The significant relationship between plasma adiponectin and HDL apo AI FCR was independent of HOMA IR score, an index of insulin sensitivity [156]. The authors suggest that adiponectin may have a direct role in HDL catabolism. It has been shown that low plasma adiponectin levels are associated with increased hepatic lipase activity in vivo. So low plasma adiponectin levels may enhance the catabolism of HDL apo AI by an increase in the lipolysis of HDL triglyceride and the dissociation of apo AI from HDL particles.

In keeping with atherogenic effects of low HDL and apo AI, in the Swedish Amoris cohort study, major adverse cardiovascular events were predicted best by an increased apo B/apo AI ratio [157].

The causes and consequence of low levels of HDL in patients with diabetes have been reviewed by Barter [158]. The low HDL is associated with smaller and denser particles, again thought to be secondary to the elevated level of plasma triglycerides. Patel et al. [159] studied the influence of plasma glucose on expression and function of a key mediator in reverse cholesterol transport, the ATP binding cassette transporter-A1 (ABCA1) and expression of its regulators liver X receptor-α (LXRα) and peroxisome proliferator-activated receptor-γ (PPARγ). They found that ABCA1 expression and protein concentrations in leukocytes, as well as function in cultured skin fibroblasts, were reduced in type 2 diabetes. ABCA1 protein concentration and function were associated with HDL-C levels. These findings indicate a glycaemia-related, persistent disruption of a key component of reverse cholesterol transport. Cholesterol ester transfer protein (CETP) is increased in diabetes and may account, at least in part, for lower cholesteryl ester and higher levels of triglyceride in diabetic HDL [160]. Hepatic lipase is increased in diabetes and insulin resistance [161] and accounts for the increased catabolism of triglyceride giving a smaller, less active HDL particle.

There are about 100 HDL associated proteins which makes the function, or rather functions, of HDL extremely complex. In diabetes, glycation and oxidation of HDL are increased and may affect HDL function [162]. The formation of advanced glycation end products impairs HDL function and its ability to activate LCAT. Hyperglycaemia increased LCAT activity and lowered PON 1 activity, which has been suggested to contribute to the impaired antioxidant capacity of HDL in diabetes. Interestingly, Loued et al. [163] have recently shown that the anti-inflammatory effect of PON 1 appears to depend on its association with HDL. A study in type 2 diabetic subjects showed that the antioxidant function of HDL was impaired because of lower HDL cholesterol [164]. Phospholipid transfer protein (PLTP) is elevated in type 2 diabetes. Dullaart et al. [165] found that it was raised in

diabetic patients, particularly in those with enlarged waist circumference compared to control subjects. HDL with low clusterin (apo J) may be associated with insulin resistance. Apo J is low in patients with reduced insulin sensitivity, perhaps related to alteration in the anti-inflammatory function of HDL [166].

There has recently been interest in the relationship between HDL and retinopathy [167]. Sasongko et al. [168] have demonstrated that apo A1 and B are stronger biomarkers of diabetic retinopathy than traditional lipids. This has recently been confirmed by Deguchi [169] who also showed a relationship between apo B/A1 and retinopathy.

The beta cell in the pancreas contains LDL receptors and it has been suggested that increased oxidation of LDL as occurs in diabetes due to increased free radical production may lead to further destruction of the already damaged beta cell. HDL in diabetes is functionally abnormal and often low and thus can be considered to be an accomplice in the death of the beta cell due to its inability to prevent LDL oxidation. Fryirs et al. [170] have demonstrated that lipid free and lipid associated apo AI and apo AII increase beta cell insulin secretion and it has also been shown that HDL can decrease beta cell apoptosis [171].

It is interesting that insulin sensitivity is affected by the hormone adiponectin secreted by the adipocyte. HDL has been shown to mediate release of adiponectin [172], hence dysfunctional HDL may play a part in insulin resistance and type 2 diabetes.

Conclusions

The complexity of lipoproteins and their metabolism has meant that it has been possible to study many aspects of the pathways in diabetes and insulin resistance [173]. There are two excellent reviews of lipoproteins which describe the complexity in an exciting and intimidating way [24, 174]. It is clear that diabetes and insulin resistance play a major part in disturbing lipoprotein metabolism and many of the disturbances have been shown to be atherogenic, either directly or indirectly through the influence on the lipoprotein cascade. Evidence continues to accumulate that improvement in glycaemic and lipid control influences atherosclerosis disease progression. Further research and the translation of proven therapies into clinical practice so as to reduce vascular disease in people with diabetes are merited.

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Chapter 6
The PPAR System in Diabetes

Jean Claude Ansquer

Introduction

The chapter is divided into five sections:

1. PPAR gene and gene variants, proteins and natural ligands
2. Synthetic ligands: from PPAR activators to PPAR agonists
3. The PPAR machinery with subsections on
   (a) Coactivators and corepressors
   (b) Metabolic modifications (phosphorylation, ubiquitination and sumoylation, acetylation and methylation)
   (c) Partial agonists or selective PPAR modulators (SPPARMs)
4. Effect of PPAR agonists in diabetes
   (a) Pharmacology, in particular in the pancreas
   (b) Effects in type 1 diabetes
   (c) Effects in type 2 diabetes and/or dyslipidemia with products reaching clinical development
5. Conclusions and perspectives

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PPAR Gene and Gene Variants, Proteins and Natural Ligands

Peroxisome proliferative activated receptors (PPARs) belong to a subfamily of the nuclear receptors which includes the retinoic acid receptors, the thyroid hormone receptors, and the revErbA-related orphan receptors [1]. The PPAR subfamily contains three isoforms, namely PPAR α (PPARA, NR1C1), PPAR β/δ (NR1C2 identified here as PPAR δ) and PPAR γ (PPARG, NR1C3, PPAR γ1 and PPAR γ2 sub-isoforms) that are encoded by different genes on different chromosomes.

In humans, PPAR α is mapped on chromosome 22 on the regions 22q12-q13.1; 22q13.31 with a linkage group of six genes and genetic markers [2]. The human PPAR γ gene is located on chromosome 3 at position 3p25, close to the retinoic acid receptor beta (RAR β) and the thyroid hormone receptor beta genes [3–5]. Two different human PPAR γ transcripts are expressed in hematopoietic cells: a 1.85-kb transcript, which corresponds to the full-length mRNA (PPAR γ1), and a shorter 0.65-kb transcript (PPAR γ2) [5]. PPAR γ2 is mostly expressed in adipose tissue where the PPAR γ2/PPAR γ1 ratio of messenger RNA is directly correlated with body mass index and where a low-calorie diet downregulates PPAR γ2 messenger RNA in subcutaneous fat [6]. Several variants in the PPAR γ gene have been identified, with the Pro12Ala variant having been the most extensively examined in epidemiologic studies. A strong association between PPAR γ 12Ala polymorphism and a reduction in type 2 diabetes risk (odds ratio: 0.86, 95% CI: 0.81–0.90) was recently described in an updated meta-analysis of 60 studies involving 32,849 subjects with type 2 diabetes mellitus (T2DM) and 47,456 controls evaluated by the Human Genome Epidemiology Network [7].

The human PPAR δ, which was cloned from a human osteosarcoma cell library, is located on chromosome 6, at position 6p21.1-p21.2 [8]. In the mouse, where the first PPAR, PPAR α was identified in 1990 by Issemann and Green [9], PPAR α is found on chromosome 15, PPAR γ is located on chromosome 6 at position E3-F1, while PPAR δ is found on chromosome 17 [10]. In both human and mouse, PPAR transcript is encoded by six exons (one in the A/B domain, two in the C domain, one for the hinge region and two for the ligand binding domain).

PPAR isoforms share a common domain structure as shown in the schematic view in Fig. 6.1. Five domains designated A/B, C, D, E and F are distinguishable, and each has a different function. The N-terminal A/B domain contains at least one constitutionally active transactivation region (AF-1) and several autonomous transactivation domains (AD) [1]. The specificity of gene transcription is granted by the isoform-specific sequence of the A/B domain of the receptor [11]. Chimeric proteins generated by fusion with the A/B domains of other receptor proteins attenuate the specificity of target gene activation [11]. The DNA-binding domain (DBD, C domain) is the most conserved region, which contains a short motif responsible for DNA-binding specificity (P-box) on sequences called peroxisome proliferator response elements (PPREs), typically containing the AGGTCA motif.

The D domain, called a hinge, permits the change in shape of PPARs. The C terminal E/F domain contains the ligand binding domain (LBD), a large pocket in

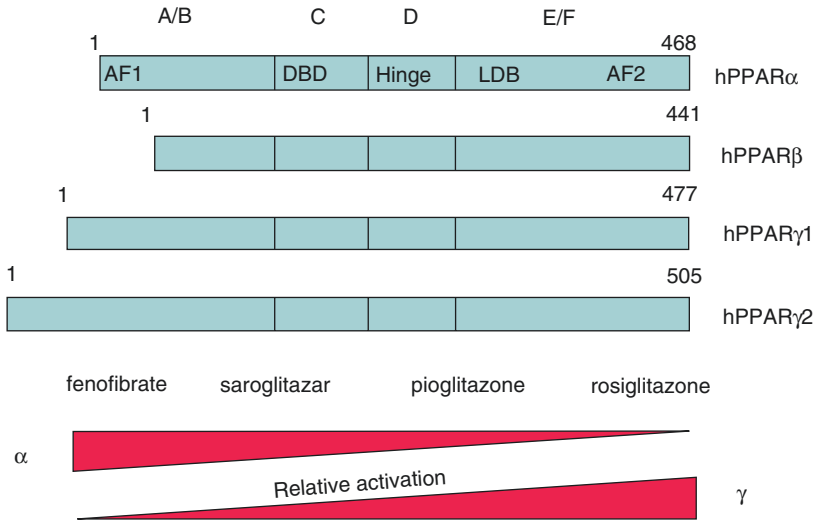

Fig. 6.1 Structure of PPARs. In the upper panel, the structure of PPARs with their four domains: 1 is the NH2 terminal and 468 the COOH terminal for PPAR α. The bottom panel illustrates the relative activation for PPAR α and PPAR γ for major agonists with fenofibrate and rosiglitazone as behaving as specific activators and saroglitazar or pioglitazone with mixed effects

the shape of the letter Y of polar character and the AF-2 region for binding co-activators and co-repressors. When activated by ligands, PPARs heterodimerize with another nuclear receptor, the retinoid X receptor, and alter the transcription of target genes after binding to specific PPREs on target genes.

Natural ligands for PPARs are long chain fatty acids, saturated or not, such as EPA: eicosapentaenoic acid, DHA docosahexaenoic acid, and eicosanoids: 8-HETE (hydroxyeicosatetraenoic acid), and to some extent leukotriene B4 (LTB4) for PPAR α, 9- and 13-HODE (hydroxyoctadecadienoic acid), two 15 lipoxygenase metabolites of linoleic acid and 15-deoxy PGJ2, for PPAR γ and prostacyclin (PGI2) for PPAR δ [12–14]. However, tissue concentrations are probably too low for them being the active ligands [15]. A new candidate endogenous ligand for PPAR α in the liver is a glycerophosphocholine esterified with palmitic and oleic acids 16:0/18:1-GPC or POPC (1-palmitoyl, 2-oleoyl-sn-glycero-3-phosphocholinehydroxyeicosatetraenoic acid) which was identified in the liver of mice by tandem mass spectrometry [16]. This phosphatidylcholine is displaced from PPAR α by the synthetic agonist Wy14643. Its portal infusion induces dependent gene expression of carnitine palmitoyltransferase 1 (CPT1) in wild-type mice, but not in PPAR α deficient mice. Recently, two other phosphatidylcholines, DLPC and DUPC (1,2-dilauroyl-sn-glycero-3-phosphocholine and 1,2-(cis-cis-9,12-octadecadienoyl)-sn-glycero-3-phosphatidylcholine respectively), have been shown to improve glucose control in two mouse models of insulin resistance [17]; however, they did not affect rosiglitazone binding to PPAR γ, and their effects are linked to stimulation of another nuclear receptor liver receptor homologue (LRH)-1.

Synthetic Ligands: From PPAR Activators to PPAR Agonists

PPAR α was first cloned from a mouse liver cDNA library at ICI, the pharmaceutical company which developed clofibrate, the first fibrate [9], and subsequently in humans [2, 18]. Fibrates, which were in clinical use as lipid-lowering agents for 20 years before this discovery, are weak PPAR α agonists, effective on human PPAR in the micromolar range, explaining the observation that they are given in the range of 100–1200 mg/day. Fibrates, such as fenofibrate, mainly act via activation of PPAR α in the liver to regulate genes involved in fatty acid oxidation [19]. They were then called PPAR α activators and their main laboratory effects are to reduce triglycerides and increase high density lipoprotein (HDL) cholesterol levels. The first potent and selective PPAR α agonist acting in the nanomolar range with clinical data was LY518674, the development of which was stopped in 2007 when phase 2 studies showed no advantage over existing fenofibrate [20].

The link between PPAR γ activation and the thiazolidinedione insulin-sensitizing agents pioglitazone and rosiglitazone was established by researchers at Upjohn and Glaxo in 1994 and 1995, respectively [21, 22]. PPAR γ increases adipocyte differentiation and storage of fat. The short-term marker of PPAR γ activation in plasma is an increase in levels of the adipocytokine named adiponectin, which increases insulin sensitivity in liver and muscle [23, 24]. First animal results with PPAR δ agonists L165041 and GW501516 were reported in 1999 by researchers at Merck and in 2001 at Glaxo [25, 26].

The PPAR Machinery

The PPAR machinery is similar to other nuclear receptors with sequential complexes of coactivators and corepressors with enzymatic activities (for review see Rosenfeld 2006) [27] and a series of metabolic transformations that turn PPARs towards activation or direct them to degradation (Fig. 6.2). The role of these different proteins, their metabolic transformations and the concept of selective PPAR modulator are summarized in the next sections. Without ligand, the transcription of DNA into messenger RNA is usually repressed by the binding of corepressors on the heterodimer PPAR-RXR and chromatin is compacted (Fig. 6.3). With the presence of ligand in the ligand binding domain, the structural changes in the AF-2 region permit to replace corepressors by coactivators, to associate remodelling of chromatin by acetylation of histones, in order for RNA polymerase to access the DNA and initiate transcription (Fig. 6.4). One important aspect common to PPAR activation is transrepression of inflammatory genes under the control of nuclear factor kappa B (NFκB) or activated protein (AP) 1. This transrepression is an indirect effect since there is no PPRE in the promoter. This was shown for PPAR γ on induction of tumour necrosis factor (TNF) α by phorbol myristate acetate in human monocytes/macrophages [28], for PPAR α on human aortic smooth muscle cells and interleukin (IL) 1-induced IL6 expression [29, 30] and for PPAR δ with expression

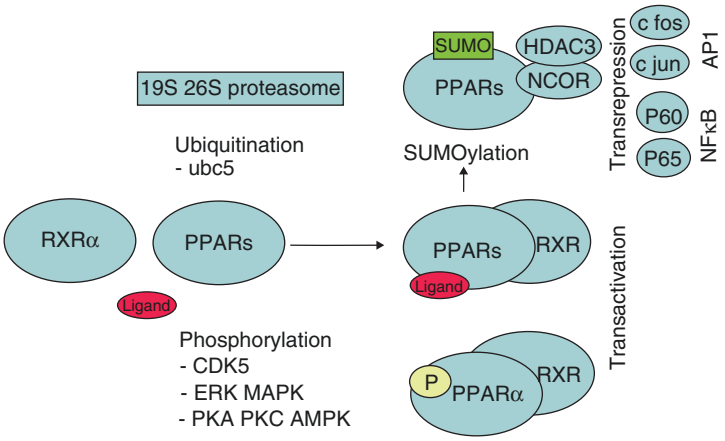

Fig. 6.2 PPAR network. Upon activation with ligand, PPAR heterodimerizes with RXR α and activate target genes (transactivation). Phosphorylation has opposite effect transactivation for PPAR α or its inhibition for PPAR γ. Sumoylation of PPAR is associated with transrepression which prevents transcription of NFκB or AP-1 dependent inflammatory genes and with a reduction of degradation in the proteasome. *CDK5* cyclin dependent kinase 5, *ERK MAPK* mitogen activated kinase, *PKA PKC AMPK* protein kinase A or C and AMP activated kinase, *NCoR* nuclear corepressor, *HDAC3* histone deacetylase 3

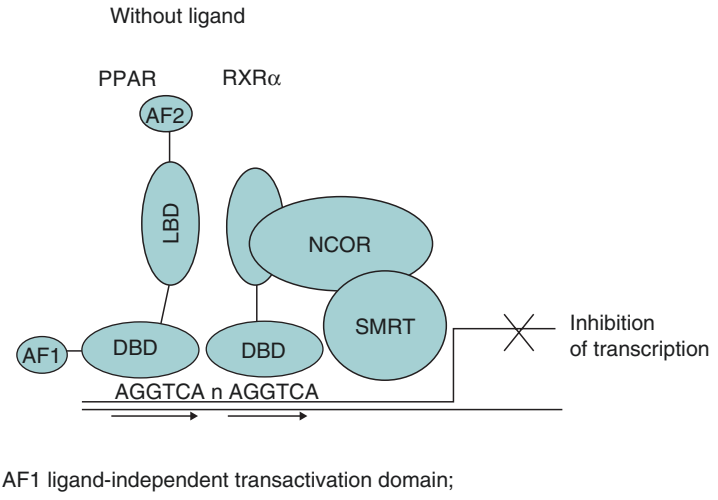

Fig. 6.3 Corepressor complex: without ligand, PPAR and RXR α are linked to their PPRE direct repeat (AGGTCA) n AGGTT by the DNA binding domain; the corepressors NCoR and SMRT prevent DNA transcription. *AF1 AF2* ligand-independent transactivation domains 1 and 2, *DBD DNA* binding domain, *LBD* ligand binding domain, *Nco R* nuclear corepressor, *SMRT* silencing mediator for retinoid and thyroid hormone

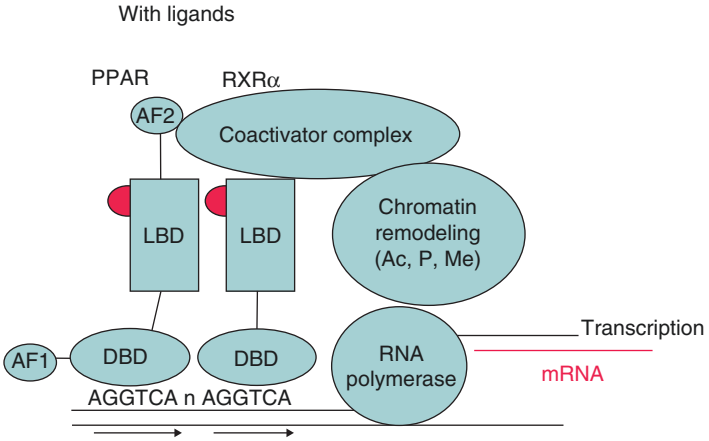

Fig. 6.4 Coactivator complex: with fixation of ligands, conformational changes in ligand binding domain permit replacement of corepressors by coactivators, of which the enzymatic activities, acetylate, phosphorylate or methylate the chromatin allowing access to DNA of RNA polymerase and initiation of transcription into copies of messenger RNA

of monocyte chemoattractant protein (MCP)-1 [31]. In human endothelial cells, fenofibrate and L165041, but not rosiglitazone, inhibited TNF α-induced monocyte adhesion, Vascular Cell Adhesion Molecule-1 (VCAM-1) expression, and Monocyte Chemotactic Protein-1 (MCP-1) secretion through inhibition of nuclear P65 translocation, necessary for NFκB activation [32].

PPAR Coactivators and Corepressors

The main PPAR coactivator, or at least the best studied one, is peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) [33]. Through a number of transcription factors, including PPARs, PGC-1α modulates numerous metabolic pathways in liver, skeletal and cardiac muscle, and adipose tissue, including gluconeogenesis and glycolysis, fatty acid synthesis and oxidation. Indeed, PGC-1α itself is subject to the same modulations as PPAR (see below through phosphorylation, ubiquitination or sumoylation). Other PPAR coactivators are steroid receptor coactivator1 (SRC1) and cyclic adenosine 5′-monophosphate (cAMP) response element binding protein (CBP/P300) which possess histone acetyl transferase activity, leading to the decondensation of chromatin necessary for gene transcription.

The main PPAR corepressors are named as nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone (SMRT) which are associated with histone deacetylase activity which maintain chromatin in a compact state. The role of NCoR was studied by specifically knocking out its gene in mouse adipocytes (AKO) or muscle (MKO). MKO mice were able to run longer than normal mice [34]. AKO mice had higher insulin sensitivity in liver, muscle and adipose

tissue than normal mice, with limited additional effect of rosiglitazone since PPAR γ target genes were already derepressed by NCoR deletion [35]. The effects of rosiglitazone to cause hemodilution were the same in AKO and normal mice. In MKO mice, exercise capacity and mitochondrial oxidation are enhanced by the loss of a transcriptional cofactor in muscle cells through modulation of transcription factors that includes PPAR δ. SMRT is a protein structurally similar to NCoR, which possesses different receptor interaction domains (RID) for different nuclear receptors, called RID2 for PPAR or RXR or RID1 for retinoid acid receptor [36].

Phosphorylation

Phosphorylation of PPAR γ by mitogen activated kinase (MAPK)-extracellular signal related kinase (ERK) 1 at serine 112 inhibits adipogenesis [37]. Phosphorylation of PPAR α on serine residues in the ligand-independent transactivation domain AF1 in response to insulin increases transcription activity through dissociation of corepressors [38]. HMG CoA reductase inhibitors ('statins') have been shown to stimulate PPAR α transcription by reducing its phosphorylation in HepG2 cells, a synergistic effect with fenofibric acid [39]. Transcriptional activation of PPAR α by bezafibrate was dose dependently increased by statins in human kidney 293T cells. In addition, concomitant administration of fenofibric acid and pitavastatin decreased the transactivation of NFκB induced by phorbol myristate acetate (PMA) [40]. Data on PPAR δ phosphorylation are limited to the location of predicted consensus phosphorylation sites and inhibition of PPAR δ activation by kinase inhibitors [41].

It was shown that phosphorylation of PPAR γ at Serine-273 by activated CDK5 leads to a loss of transcription of PPAR γ in adipocytes [42]. The cyclin dependent kinase (CDK) 5, which is present in the cytoplasm and the nucleus, is activated by phosphorylation at tyrosine 15 within a high glucose milieu and IL1β, by TNF α or by high fat diet. This finding permitted the same authors to discover new small molecules binding to PPAR γ blocking CDK5 serine 273 phosphorylation, like thiazolidinediones (TZDs), with potent antidiabetic activity in insulin-resistant mice fed a high fat, high sugar diet, without causing fluid retention and weight gain [43]. However, to date no clinical development has been reported blocking CDK5 pathway.

Ubiquitination

Proteins are degraded in the proteasome after fixation on lysine residues of repeated sequences of a small 76AA polypeptide called ubiquitin. In the absence of their ligands, PPARs are rapidly degraded by this process. The degradation of PPAR γ is increased by different TZD ligands [44]; conversely, ubiquitination of PPAR α is reduced transiently with different fibrate ligands [45] and ubiquitination of PPAR δ is markedly reduced by PPAR δ agonists [46].

Sumoylation

Sumoylation is the attachment of another polypeptide of 101 amino acids called SUMO, for small ubiquitin like modifier. Sumoylation at a lysine in the ligand-binding domain of PPAR γ is the mechanism which converts activation of transcription by rosiglitazone into repression of NFκB or activator protein (AP) 1 in murine macrophages. This prevents ubiquitination of NCoR to maintain repression of inflammatory genes such as inducible NO synthase [47]. In adipose tissue, sumoylation of PPAR γ, which reduces the effect of rosiglitazone, is increased in the absence of the hepatokine fibroblast growth factor (FGF) 21 [48].

Similarly, sumoylation at lysine 185 has been identified in the hinge region of PPAR α [49]. To date, a potential sumoylation site for PPAR δ has also been suggested on lysine 185.

Post-translational regulation of PPARs by different patterns of mono- or polyubiquitination, as well as by mono- or polysumoylation, has been reviewed by Wadosky and Willis [50]. This review also reports that the coreceptor RXR α and the coactivators PGC-1α can be ubiquitinated or sumoylated, adding to the complexity of these regulatory processes.

Acetylation

Acetylation and deacetylation of genes are major procesess affecting gene expression through decondensation and recondensation of chromatin. It also affects proteins. The first nuclear receptors shown to be acetylated were the androgen oestrogen receptors; this has not been shown clearly for PPAR [51]. However, their key coactivator PGC-1α is inactivated by acetylation in high energy states or deacetylated by sirtuin 1 in low energy states [52]. The nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases or sirtuins by interacting with PPARs and their coactivators thus provide a new level of complexity to the regulation of nuclear receptors [53].

Methylation

Methylation of histones is another prominent histone posttranslational modification in response to environmental and pharmacological factors. The methylation of histone lysine residues is a reversible process with interplay between lysine methylation by methyltransferases (KMTs) and demethylation by lysine demethylases (KDMs).

Methylation of PPAR γ promotor decreases PPAR γ in murine 3T3L1 adipocytes [54].

Partial Agonists or SPPARMs

A partial agonist is a ligand that induces a submaximal response even at full receptor occupancy. It can also reduce the full PPAR γ agonist response. For instance, in comparison with rosiglitazone, troglitazone is a full agonist on murine 3T3L1 adipocytes, but a partial agonist in muscle C2C12 myotubes and HEK293T kidney cells [55]. Olefsky proposed to name selective PPAR modulators (SPPARMs); such products differ from full agonists by differential regulation of target genes [56]. SPPARMs are designed to separate efficacy and adverse effect dose–response curves. This concept was already developed in nuclear receptor pharmacology, with selective oestrogen receptor modulators (SERMs), such as tamoxifen or raloxifene, which recruit corepressors such as NCoR to the AF2 region, whereas oestradiol recruits coactivators such as the glucocorticoid receptor interacting protein 1 (GRIP1) [57] or with selective vitamin D modulators such as paricalcitol with differential recruitment of coactivators than calcitriol, the active form of vitamin D [58].

Pemafibrate has been described as a SPPARM α due to different binding to PPAR α ligand binding domain and recruitment of coactivators/corepressors than fenofibrate [59]. Pemafibrate was first approved in Japan with the same indications than fenofibrate in hyperlipidemia. A large-scale intervention study PROMINENT has recruited 10391 participants with T2DM and dyslipidemia [60] to assess the reduction in cardiovascular events. Results were expected at the end of 2022 but the study was discontinued for futility in April 2022.

Increasing concentrations or doses with full PPAR γ agonists lead to greater efficacy, but greater adverse events, such as weight gain and volume expansion.

PPAR γ partial agonists such as balaglitazone or INT131 displace a full agonist such as rosiglitazone. Metaglidasen, the (−) stereoisomer of halofenate, tested as racemate in the 90s as a lipid lowering agent, is another selective partial PPAR γ modulator and was in clinical development for its uricosuric activity. Partial agonists bind the same pocket as TZDs, which is required to block PPAR γ phosphorylation, but induce different conformational changes in PPAR γ, leading to different recruitment of coactivator/corepressor. As an example, INT131 induces less recruitment of DRIP205 (vitamin D-interacting protein 205), a coactivator involved in lipid accumulation than rosiglitazone or pioglitazone in HEK cells [61]. The same finding was reported with fibrates: gemfibrozil induced less recruitment of DRIP205 than fenofibrate and behaves as a partial agonist to increase apoA-I activation. This translated in a comparative trial in dyslipidemic patients to a larger increase in ApoA-I, a protective apoprotein in HDL, with fenofibrate than with gemfibrozil [62].

Effects of PPAR Agonists in Diabetes

This review is limited to PPAR activators or agonists which are marketed or remain in clinical development in diabetes and/or dyslipidemia (Table 6.1). Several PPAR antagonists were synthesized but they were not developed for the treatment of

Table 6.1 Phase of clinical development reached by PPAR agonists

	PPAR α	PPAR γ	PPAR α/γ	PPAR α/δ	PPAR δ	Pan PPAR
Marketed	Bezafibrate Ciprofibrate Fenofibrate Gemfibrozil Clinofibrate Pemafibrate (K-877)	Pioglitazone Rosiglitazone	Lobeglitazone (CKD501)			
No more marketed	Clofibrate etofibrate	Troglitazone				
Phase 3		Deuterated pioglitazone (PXL065) Azemiglitazone (MSDC0602)		Elafibranor (GFT505)	Seladelpar (MBX8025) Fonadelpar[a]	Chiglitazar (CS038) Lanifibranor (IVA337)
Phase 2		INT131 Leriglitazone[b]				
Discontinued	AVE8134 GW590735 KRP105 LY518674 CP778875 KDT501	Balaglitazone Metaglidasen Rivoglitazone Ciglitazone Farglitazar[c] MBX2044 FK614 Efatutazone	Aleglitazar Muraglitazar Ragaglitazar Tesaglitazar Imiglitazar MK767 Cevoglitazar Naveglitazar Saroglitazar		GW501516 GW0742 L165041	Chiglitazar Indeglitazar Sodelglitazar Netoglitazone

[a] In dry eye disease
[b] Hydroxypioglitazone in X-linked adrenoleukodystrophy
[c] Discontinued in hepatic fibrosis

diabetes [63]. GW6471, a potent PPAR α antagonist, is mostly used as a pharmacological agent to test whether an effect is PPAR dependent or PPAR independent. GW9662 is a PPAR γ antagonist which promotes the recruitment of NCoR. Finally, GSK0660 and GSK3787 are PPAR δ antagonists for pharmacological use which compete with the binding of full agonists. However, GSK0660 when used alone behaves as an inverse agonist activity to inhibit the TNF α-induced expression of multiple chemokines in human retinal microvascular endothelial cells [64, 65].

The organs implicated in glucose control are listed in Table 6.2. With their direct effects on gene expression and their indirect effects on inflammation, and according to their tissue distribution, PPARs affect most of these organs, beyond the liver for PPAR α, the adipose tissue for PPAR γ and the skeletal muscle for PPAR δ. In the kidney, they have different locations: PPAR α is located mainly in the proximal tubule, the medullary thick ascending limb and in the mesangium; PPAR γ in the distal medullary collecting duct and glomeruli; and PPAR δ in a diffuse fashion as in other organs [66]. In the brain, the interplay of PPAR subtypes has been shown in cultures of astrocytes, where the three subtypes are present. PPAR α (fenofibrate), PPAR δ (GW501516) and PPAR γ (rosiglitazone) agonists and their respective antagonists (GW6471, GSK0660 and GW9662) decreased the release of the proinflammatory cytokine, TNF α in rat astrocytes stimulated by lipopolysaccharide

Table 6.2 Organs implicated in glucose control

	PPAR α	PPAR γ	PPAR δ
Liver	Increase in fat oxidation and apoA-1 Increase in insulin sensitivity	Decrease in steatosis Increase in insulin sensitivity	
Skeletal muscle		Increase in insulin sensitivity	Increase in fat oxidation and energy expenditure
Adipose tissue	Reduction in inflammatory adipocytokines	Increase in adipocyte differentiation and adiponectin release	
Pancreas			Amplification of glucose induced insulin secretion
Gut		Anti-inflammatory	Increase in GLP1 production
Vascular wall	Increase in NO synthesis		

(LPS) [67]. Combined application of PPAR γ and PPAR δ activators increased cyclooxygenase 2 expression induced by LPS, whereas the additional application of a PPAR α agonist abolished this effect [68].

In the pancreas, the three PPARs are expressed in pancreatic β cells. PPAR α modulates fatty acid oxidation, and PPAR γ directs them toward esterification. Although PPAR δ is the most abundant PPAR in the pancreas at the mRNA and the protein level, until recently its effects on fatty acid oxidation have been less well-studied [69]. PPAR δ activation increases fatty acid oxidation and to a larger extent than PPAR α activation. In the pancreas, fatty acids acutely potentiate glucose-stimulated insulin secretion (GSIS) but their chronic exposure elevates basal insulin secretion and alters GSIS, a phenomenon called lipotoxicity.

Discordant results are reported in the literature with PPAR α or PPAR γ agonists. PPAR α was described to potentiate and PPAR γ to attenuate GSIS in INS-1E cells, an immortalized insulinoma rat cell line [70]. On the contrary, in vivo, the PPAR α agonist fenofibrate impaired GSIS in neonatal rats receiving monosodium glutamate to induce obesity, while pioglitazone, a PPAR γ agonist, increased it in db/db mice [71, 72]. This discordance might be explained by the low expression level of PPAR γ in INS-1E cells.

Reduced amounts of sulfatide, 23% of the levels in control participants, in pancreatic islets of individuals with newly diagnosed type 1 diabetes, have been associated with reduced expression of enzymes involved in sphingolipid metabolism. Fenofibrate, which activates sulfatide biosynthesis, completely prevented diabetes in NOD mice [73]. Fenofibrate treatment initiated 7 days after diagnosis eliminated the need for insulin therapy in a 19-year-old girl newly diagnosed type 1 diabetes [74].

Activation of PPAR δ by unsaturated FAs or a synthetic ligand enhanced GSIS in primary rat islets or INS-1E cells without affecting basal insulin secretion [69]. In order to maintain β cell function, PPAR δ would play a role of lipid sensor to adjust

the mitochondrial fatty acid oxidation. It was recently suggested that 4-hydroxynonenal (4-HNE) was one endogenous activating ligand of PPAR δ [75]. The level of reactive oxygen species (ROS), such as 4-HNE, is essential to β cell function, as low-0level ROS production increases glucose-induced insulin secretion, whereas high levels of ROS can induce β cell apoptosis.

GSIS is also linked to influx of calcium ions to the cytosol induced by depolarization from the voltage-dependent Ca^{2+} channel. In INS-1 cells, the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA2) pump maintains intracellular Ca^{2+} homeostasis, in particular a high Ca^{2+} level in the endoplasmic reticulum. The expression of this pump is decreased in animal models of diabetes and in diabetic human islets. Pioglitazone directly increases expression of SERCA2 through transcription of the gene and indirectly through prevention of CDK5-induced phosphorylation of PPAR γ [76]. This experiment suggests that blocking CDK5 could permit to dissociate positive effects on glucose homeostasis from other effects from PPAR γ agonists.

Effects of PPAR Agonists in Type 1 Diabetes

Clinical studies with PPAR agonists in type 1 diabetes (T1DM) are limited to their effects on lipid or glucose markers. One placebo-controlled randomized study was conducted with fenofibrate in 44 patients with T1DM to assess its effect alone or in combination with vitamin E for 8 weeks on in vitro copper-induced oxidation of LDL and VLDL particles [77]. The lag time of oxidation was significantly prolonged by fenofibrate 200 mg + vitamin E 400 IU. A placebo-controlled randomized study is evaluating the effects of fenofibrate on progression of diabetic retinopathy in 450 adults with T1DM (http://clinicaltrials.gov/ct2/show/NCT01320345) [78].

The lipid-modifying effects of bezafibrate in T1DM were evaluated in earlier placebo-controlled studies [79, 80]. Of note, this fibrate, now considered as an archetype pan-PPAR agonist in transactivation assays, did not improve HbA1c after 3 months of treatment [40, 81].

Three placebo-controlled randomized studies have been reported with TZDs in T1DM patients on insulin therapy, with modest insulin-sparing effects as compared to those observed in type 2 diabetes mellitus (T2DM). In 50 overweight adults with T1DM, an 8-month intervention to achieve glycated haemoglobin level of 7.0% required an 11% increase in the daily dose of insulin in the placebo group, but no change in the rosiglitazone group [82]. In 36 T1DM adolescents aged 10–18 years, the dose of insulin was increased 9% with placebo and reduced by 6% with rosiglitazone after 6 months of treatment, with HbA1c remaining stable around 8.5% [83]. In 60 lean T1DM patients aged 14 years or more, 6 months treatment with pioglitazone was associated with a significant decrease in HbA1c (0.2%) and in postprandial glucose levels (0.7 mmol/L) in the intervention group only, with no changes in insulin doses [84]. In patients with slowly progressive T1DM, diagnosed by the

presence of glutamic acid decarboxylase (GAD) antibodies, an insulin-requiring state defined by HbA1c and post glucose C-peptide levels was reached at 4 years in 4/4 subjects randomized to pioglitazone as compared to 1/5 subjects randomized to metformin [85]. Thus, the effects of TZDs in T1DM sharply differ from those reported for T2DM prevention with troglitazone in TRIPOD [86], rosiglitazone in DREAM [87], and pioglitazone in ACT-NOW where development of T2DM in patients with impaired glucose tolerance over 2.4 years decreased from 19.7% with placebo to 7.0% with pioglitazone [88].

Effects of PPAR Agonists in Type 2 Diabetes and Dyslipidemia

For the treatment of T2DM, the first TZD PPAR γ agonist troglitazone was introduced in the US in October 1997 and was withdrawn in March 2000 for hepatic toxicity. Rosiglitazone and pioglitazone were introduced in the US in 1999 and in Europe in 2000. In Japan, pioglitazone was introduced in 1999 and rosiglitazone in 2003. The effects of pioglitazone on macrovascular events in 5238 T2DM patients were reported in 2005 [89]. Although the study primary endpoint was not reached, there was a significant 16% reduction in the main secondary endpoint, which included death from any cause, acute non-fatal myocardial infarction or stroke. The effect of TZDs on diabetes control and the controversy about their hazard on cardiovascular events have been the subjects of numerous reviews in the early 2010s [90–92].

The first PPAR α/γ dual agonist muraglitazar was submitted for treatment of diabetes to the Food and Drug Administration (FDA) for registration but the file was withdrawn in May 2006 after a combined analysis of clinical studies indicated an increased cardiovascular risk [93]. Such an increase in cardiovascular risk led to the suspension of registration of rosiglitazone in Europe in September 2010 and severe limitations to its use in the US. Finally, in June 2011, pioglitazone was withdrawn from some European markets due to increased risk of bladder tumours, a decision not endorsed by the European Medicines Agency.

Discontinuation of the development of PPAR agonists occurred for multiple reasons: toxicity of the compound (vascular or bladder tumours in rodents with MK767 or ragaglitazar, respectively), long duration of development, clinical adverse events, expectation not to be better than existing drugs, and stopping development efforts in the cardiometabolic domain. In particular, the FDA requested in July 2004 that 2-year rodent carcinogenicity studies be completed and reviewed before proceeding to phase 3 studies of more than 6-months duration. This decision was made after the evaluation of carcinogenicity in rodents for 11 PPAR agonists, with the observation of haemangioma/haemangiocarcinoma with 8/11 compounds and urinary bladder/renal pelvic transitional cell carcinomas with 5/6 PPAR α/γ dual agonists and pioglitazone (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071624.pdf) [94]. In addition, the FDA requested in December 2008 that new antidiabetic agents had to demonstrate through randomized, prospective

clinical trials that they do not increase risk for cardiovascular events (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071627.pdf) [95]. The thiazolidinedione intervention with vitamin D evaluation (TIDE) study, a large intervention study to assess the effect of the existing TZDs pioglitazone and rosiglitazone on cardiovascular events, planned in 16000 T2DM patients at risk of CVD events was initiated in 2009 but stopped by the FDA 1 year later leaving uncertainty about the risks and benefits from TZDs (TIDE 2012) [96]. The authors stated that, had this study been initiated earlier, it would have provided clear evidence regarding the efficacy and safety of rosiglitazone and pioglitazone. Evaluation of pioglitazone was continued in T2DM patients on metformin in comparison with sulfonylureas (TOSCA-IT) [97] and in insulin resistant patients after a stroke or transient ischemic attack (IRIS) [98]. To date, pioglitazone remains a unique agent to improve insulin sensitivity.

Currently the number of PPAR agonists in phase 2 or phase 3 of clinical development in diabetes and/or dyslipidemia has been markedly reduced as compared to the mid-2010s (Table 6.3).

Two PPAR α agonists have reached the market for treatment of dyslipidemia: pemafibrate K877 from Kowa in Japan and saroglitazar ZYH1 from Zydus in India, the later having a PPAR γ component [103]. The PPAR γ SPPARMs balaglitazone, now discontinued in development, and INT131 appear to be as effective as pioglitazone on HbA1c levels but caused less weight gain in 6-month trials [99, 104]. Indeed, glitazones are chiral drugs marketed as racemates where the S stereoisomer possesses the PPAR γ activity and the R stereoisomer inhibits mitochondrial pyruvate transport while maintaining insulin sensitizing properties. Two PPAR γ derivatives from pioglitazone, R pioglitazone deuterated (PXL065) [105] and azemiglitazone (MSDC0602) [106], described as mitochondrial membrane transport protein modulators, reproduce part of the effect of pioglitazone without its adverse effects. Hydroxypioglitazone (leriglitazone MIN102) has increased brain entry which could be of benefit to improve mitochondrial function in neurodegenerative diseases [107].

Clinical studies with the first PPAR δ activators have been limited to short-term mechanistic studies. In moderately obese volunteer subjects with dyslipidemia, GW501516 10 mg once daily (od) for 2 weeks reduced fasting and postprandial TG levels by 30%, liver fat measured by magnetic resonance imaging by 20%, and urinary isoprostane levels, a marker of oxidative stress, by 30%. In a skeletal muscle biopsy of the thigh, the expression of carnitine palmitoyltransferase 1b, which permits fatty acid to enter the mitochondria, was increased suggesting increased fat oxidation [108]. In a randomized, placebo-controlled, cross-over trial 13 obese dyslipidemic subjects received GW501516 2.5 mg od for 6 weeks. The GW501516 reduced apo CIII production, increased VLDL-apoB catabolism, and increased apoA-II production and HDL-C levels [109]. MBX8025, another specific PPAR δ agonist, was recently reported to reduce TG and increase HDL-C levels alone or in combination with a statin in 181 dyslipidemic patients treated for 8 weeks [100].

Initially, the most studied PPAR dual agonist was aleglitazar, an α/γ agonist with a large intervention study ALECARDIO in 7226 T2DM patients after a recent acute

Table 6.3 Effects of recent PPAR agonists on lipids, glycated haemoglobin and weight

	Design/PPAR agonist	Study groups	HDL-C change	TG change	HbA1c change	Weight change kg
Nissen (2007) [20]	R,DB,6PG, 12 weeks *N* = 309 dyslipidemic LY518674 PPAR α	Placebo Feno 200 mg LY 10 μg LY 25 μg LY 50 μg LY 100 μg	−1% +14% +10% +16% +11% +2%	+1% −33% −36% −41% −42% −35%	N/A	N/A
DePaoli (2014) [99]	R,DB,6PG, 24 weeks *N* = 367 T2DM on metformin/ sulfonylurea INT-131 PPAR γ	Placebo Pio 45 mg 0.5 mg 1 mg 2 mg 3 mg	+1 mg/ dL +4 mg/ dL +2 mg/ dL +1 mg/ dL +4 mg/ dL +4 mg/ dL	+10 mg/ dL −49 mg/ dL −1 mg/ dL −12 mg/ dL −22 mg/ dL −8 mg/ dL	−0.1% −0.9% −0.3% −0.6% −0.9% −1.0%	−0.3 +3.6 +1.6 +1.2 +3.3 +3.9
Bays (2011) [100]	R,DP,6PG, 8 weeks *N* = 181 dyslipidemia MBX-8025 PPAR δ	Placebo Atorva 20 mg M50 mg M100 mg A20+M50 mg A20+M100 mg	+1% +2% +10% +13% +13% +2%	−5% −18% −32% −33% −35% −31%	N/A	Unchanged
Cariou (2011) [101]	R,DB,2PG, 5 weeks *N* = 47 prediabetes Elafibranor GFT505 PPAR α/δ	Placebo GFT505 80 mg	−3% +7%	−4% −32%	N/A	N/A
Lu (2020) [102]	R,DB,4PG, 24 weeks *N* = 1274 T2DM Chiglitazar PPAR α/γ/δ	Placebo Sitagliptin 100 Chigli 32 mg Chigli 48 mg	N/A	N/A	−0.45% −1.4% −1.4% −1.5%	
IVA 337 Inventiva [118]	R,DB,4PG, 4 weeks *N* = 61 T2DM Lanifibranor PPAR α/γ/δ	Placebo Lani 400 mg Lani 800 mg Lani 1400 mg	+3% +18% +28%	−3% −25% −28%	FBG −16 mg/ dL −24 mg/ dL	

R randomized, *DB* double-blind, *PG* parallel group, *Atorva* atorvastatin, *Feno* fenofibrate, *N/A* not available, *Pio* pioglitazone, *T2DM* type 2 diabetes. If not provided percent changes are estimated from figures or calculated from actual means before and after treatment

coronary syndrome randomized to aleglitazar 150μg or placebo [110]. The study was terminated after a median 2 years of follow-up for lack of efficacy on the primary endpoint combining cardiovascular death, non-fatal myocardial infarction and non-fatal stroke and increased risk of hospitalization for heart failure. However, this

risk was only present in those treated with the antiplatelet agent clopidogrel due to previously unknown pharmacokinetic interaction [111]. In addition, aleglitazar compared with placebo caused a larger reduction in HbA1c and haemoglobin and a larger increase in serum creatinine and adiponectin in patients who were concomitantly using clopidogrel versus patients who were not. Another PPAR α/γ dual agonist, lobeglitazone or CKD-501, has been marketed in Korea with a 6-month comparative trial with pioglitazone [112].

The first pan-PPAR agonist advanced to phase 2 was GW677954 or sodelglitazar which was discontinued from clinical development due to safety concerns. Chiglitazar is another pan-PPAR agonist with full gamma and partial alpha and delta agonist activities in preregistration in China [102]. Lanifibranor is described as a moderately potent and well-balanced modulator of the three PPARs isoforms with partial PPAR γ agonist activity [113, 114].

The development of these new agents, initially evaluated in T2DM or dyslipidemia, has moved recently after the results obtained in a pilot study with pioglitazone in patients with impaired glucose tolerance or T2DM and liver biopsy-confirmed nonalcoholic steatohepatitis (NASH) [115]. The presence of T2DM in patients with metabolic-associated fatty liver disease increases the risk of disease progression to NASH and advanced fibrosis.

Reduction in fibrosis score with pioglitazone 4 mg compared with placebo was shown in a 18-month study in 101 patients with prediabetes or T2DM and biopsy-proven NASH [116]. Phase II studies with pioglitazone derivatives are underway with pioglitazone deuterated PXL065 (NCT04321343) or completed with azemiglitazone in NASH patients with or without diabetes [105]. The expected endpoint in long-term phase III, reduction in NASH score without worsening of fibrosis, is felt more likely to occur in diabetic patients in the azemiglitazone study in the planning stage in 1800 patients (NCT03970031). Elafibranor (GFT505) is a PPAR α/δ agonist with an initial 3-month study in T2DM [101]. After positive results in phase II studies with elafibranor, the dual PPAR α/δ agonist [117], in the interim analysis of the phase III RESOLVE-IT, the response rate in the 717 patients enrolled on study drug was 19.2% for patients who received elafibranor 120 mg compared to 14.7% for patients in the placebo arm. With the pan PPAR agonist lanifibranor, the primary endpoint of the phase II trial NATIVE was reduced in the combined inflammation and ballooning score, with no worsening of fibrosis after 6 months in 247 participants with similar effects in those with and without T2DM [118].

Conclusion and Perspectives

The pharmacology of PPARs, one family of nuclear receptors, is extremely complex as it regulates energy stores in major organs through modulation of genes in lipid and carbohydrate metabolism as well as adaptation to stress, fasting and feeding. The natural ligands for PPARs are fatty acids and prostaglandins. Their first synthetic ligands are fibrates for PPAR α, thiazolidinediones for PPAR γ, few PPAR δ

agonists and then dual and pan-PPAR agonists. Most of these well-designed products have been discontinued from clinical development for various reasons from animal toxicity, clinical safety, to no advantage over existing drugs or hurdles to substantiate it. When compared with the initial version of this chapter in 2014 only three products have been marketed. Currently, the most advanced new PPAR agonist is pemafibrate, a PPAR α agonist, which is being evaluated for the prevention of cardiovascular events in people with type 2 diabetes and dyslipidemia. The prevention and treatment of microvascular events such as diabetic retinopathy, as shown with fenofibrate, now in clinical use for almost 50 years, should represent another area of research for new products. The anti-inflammatory effects of PPAR agonists have been well documented in animal experiments, although their potential in human disease is yet to be demonstrated. Dual PPAR α/γ and pan PPAR agonists may offer additional protection in diabetes and metabolic-associated fatty liver disease such as NASH. The search for natural PPAR ligands has been encouraged by the discovery that phosphatidylcholine derivatives can activate PPAR α and should continue for other.

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Chapter 7
Production and Metabolism of Triglyceride-Rich Lipoproteins: Impact of Diabetes

Angela Pirillo, Giuseppe D. Norata, and Alberico L. Catapano

Introduction

Apolipoprotein B (apoB)-containing lipoproteins include chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and their remnants. Plasma triglycerides (TG) are produced in the liver and the intestine; chylomicrons, which transport lipids derived from diet, are produced by the intestine, whereas VLDL, which transport endogenous lipids, are produced by the liver. Both are assembled at the surface of the endoplasmic reticulum (ER). After secretion, lipoprotein TG are hydrolyzed by lipoprotein lipase (LPL), and fatty acids are taken up by the cells to enter beta oxidation and provide energy (muscles) or to be stored (adipose tissue). The resulting remnant lipoproteins, which are enriched in cholesterol, are scavenged by cells through specific receptors.

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TG-Rich Lipoproteins Secretion by Liver and Intestine

TG-rich lipoproteins (TGRLs) comprise both hepatically derived apoB100-containing VLDL and intestinally derived apoB48-containing CM [1, 2]. These lipoproteins are spherical particles, consisting of a neutral lipid core (cholesteryl esters and TGs) surrounded by a monolayer of lipids (phospholipids and free cholesterol) interacting with apolipoproteins. Each particle of VLDL and CM contains a single apoB molecule.

ApoB is synthesized in two isoforms: apoB100 in the liver and apoB48 (generated from the same gene of apoB100, but differentially processed) produced exclusively in the intestine [3, 4]. TGRLs are synthesized following the interaction of apoB with microsomal triglyceride transfer protein (MTP), a protein mainly present in the ER of hepatocytes and enterocytes that is crucial for the first step of lipoprotein assembly [5]. When apoB translocates into the lumen of the ER, lipid droplets are added to apoB, a process facilitated by the activity of MTP, thus resulting in a nascent form of apoB-containing particle. Next, the addition of neutral lipids increases the size of the particle, which is transported through the Golgi and secreted into the hepatic vein (hepatic lipoproteins) or the lymphatic system (intestinal lipoproteins). In fact, in the presence of lipids, nascent apoB is quickly lipidated by MTP; when lipid availability is low or MTP activity is reduced, apoB is targeted for ubiquitinylation and degradation by the proteasome [6–8].

VLDL Assembly and Secretion

ApoB100, the major structural protein of VLDL, exhibits a highly lipophilic nature and contains two domains that interact irreversibly with the neutral lipids present in the lipoprotein core [9]. Due to its lipophilic nature, apoB folding and stability depend upon the simultaneous addition of lipids by MTP [10, 11]. Following heterodimerization with the small subunit protein disulfide isomerase (PDI), MTP mediates the transfer of phospholipids and TGs to nascent apoB during its translocation through a protein channel in the membrane of the rough ER [12, 13] (Fig. 7.1). This lipidation step results in the formation of small (max 25 nm) dense particles. Maturation of these precursors into VLDL particles with larger diameter (30–80 nm) involves a posttranslational acquisition of the bulk of TGs by fusion of apoB-containing precursor with large TG droplets produced in the smooth ER [14], generating TG-rich VLDL. The size of VLDL particles secreted by the liver is determined by the availability of TGs [15], which mainly derive from the lipolytic mobilization of the hepatic storage pool [16] rather than newly synthesized TGs [17].

TGs may derive from different sources, including uptake of albumin-bound fatty acids, uptake of circulating remnants of VLDL and chylomicrons, and de novo hepatic synthesis. A reduced lipid availability targets apoB for degradation and reduces VLDL secretion [18]. Fatty acids derived from the diet or released from

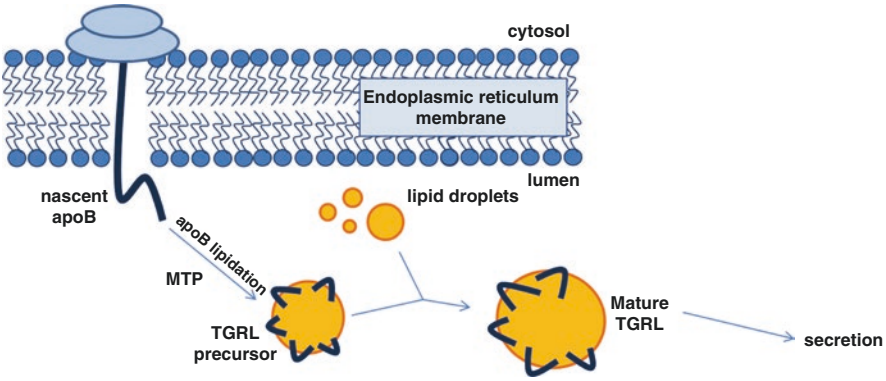

Fig. 7.1 Intrahepatic assembly of apoB into VLDL

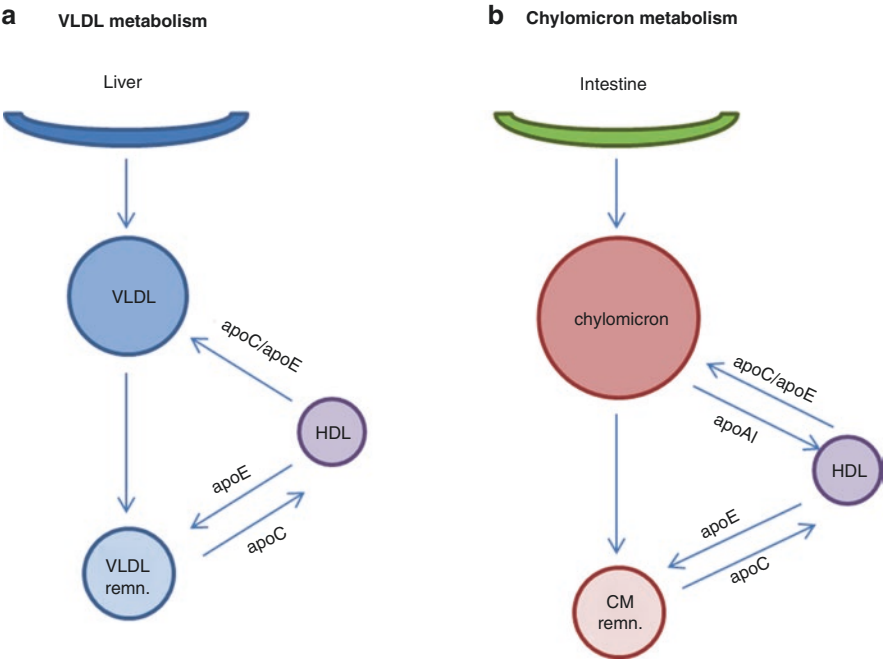

Fig. 7.2 Metabolism of VLDL (**a**) and chylomicrons (**b**)

adipose tissue enter the liver where they are re-esterified forming TG droplets [19]. Not all mobilized TGs enter the secretory pool to contribute to VLDL formation; a relatively large proportion (determined by MTP and insulin activity) is returned back to the cytosolic pool. Newly synthesized VLDL contain small amounts of apoC; after secretion in the circulation, they acquire apoE and additional apoC from HDL (Fig. 7.2).

Two major subfractions of VLDL exist, the larger VLDL1 fraction and the smaller VLDL2 fraction. VLDL1 secretion depends on fatty acid availability and is inhibited by insulin [20]. After secretion, VLDL1 are delipidated following hydrolysis of TG by LPL; the delipidation process of VLDL1 is not complete, and only a minor fraction is converted to LDL, most remnants being directly removed from plasma [21]. On the contrary, VLDL2 production is not modulated by insulin and these particles are poorly lipidated and rapidly converted to LDL [22].

Chylomicron Assembly and Secretion

Biosynthesis and assembly of CM is a multistep process highly regulated by several factors and pathways, and dysregulation of this process may have implications for health and disease [23]. Three proteins play a key role in CM assembly: apoB48, MTP, and apoA-IV. ApoB48 is produced from the same gene encoding apoB-100 and derives from a post-transcriptional mRNA editing process in enterocytes; it lacks the LDL receptor (LDLR)-binding domain, and thus apoB48 containing lipoproteins are primarily cleared by the heparan sulfate proteoglycan (HSPG) pathway [24]. ApoA-IV is a lipid binding protein expressed mainly in the small intestine and is immediately incorporated into nascent CM, thus playing an important role in determining CM size and metabolism in the circulation [25]; after CM secretion, apoA-IV dissociates from the particles and circulates mostly as lipid-free protein.

CM are highly enriched in triacylglycerols and are responsible for the transport of dietary medium- and long-chain fatty acids and cholesterol from the intestinal lumen to the liver. CM are assembled in the ER and then transported to the Golgi via specialized vesicles (prechylomicron transport vesicles, PCVs). During the first assembly step, apoB48 is translated into the ER lumen and immediately lipidated by intestinal MTP (Fig. 7.1), forming a precursor particle. During the second step, MTP mediates further addition of lipids to the precursor, and, in this phase, apoA-IV is added at the particle surface; apoA-IV increases MTP activity, thus further increasing CM lipidation [26].

Lipoprotein Lipase-Mediated Lipolysis

VLDL and CM leave the liver and intestine, respectively, and enter the circulation, where they acquire apoC-II and apoE from plasma HDL. In the capillaries of adipose tissue and muscles, triacylglycerols are hydrolyzed by LPL (activated by apoC-II) to produce free fatty acids (FFA), which are then absorbed by the tissues [27]. During the removal of fatty acids, a large part of phospholipids and apoproteins are transferred to HDL, resulting in the formation of VLDL and CM remnants (Fig. 7.3).

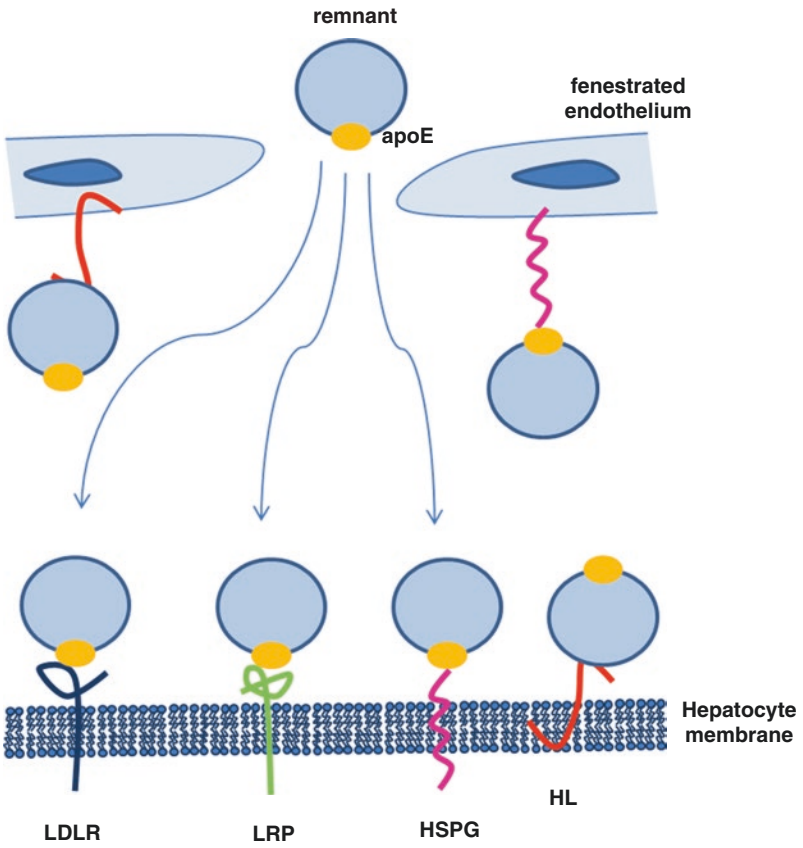

Fig. 7.3 Pathways of hepatic clearance of remnants

Hepatic Clearance of Remnants

The main organ involved in the clearance of remnant lipoproteins is the liver, where hepatocytes express LDLR, LDLR-related protein 1 (LRP1), and HSPG in high amount. In concert with LPL and hepatic lipase (HL), these surface proteins facilitate the rapid hepatic clearance of remnant lipoproteins [28–31], which are atherogenic [32] (Fig. 7.3). The most critical molecule in the remnant clearance is apoE, involved in the binding of lipoproteins to LDLR family and HSPGs [30]. Multiple steps are involved in the uptake of remnants by hepatocytes. HSPGs interact with apoE present on the remnant particle surface and sequester them in the space of Disse [31]; moreover, HSPGs can bind LPL and HL, that eventually may continue their lipolytic activity and prepare the particles for the successive internalization process [31, 33], which is mediated by LDLR, HSPGs, and the HSPGs/LRP complex.

CM remnants contain mainly cholesteryl esters, apoE and apoB48, and are rapidly removed from the circulation by the liver via interaction with LDLR which requires apoE [34]. Moreover, CM remnants can acquire additional apoE, allowing the remnants to be taken up via the CM remnant receptor, a member of the LRP family [34]. Alternatively, CM remnants can remain sequestered in the space of Disse by binding of apoE to HSPGs and/or binding of apoB48 to HL [34]. During this phase, CM remnants may be further metabolized, thus increasing apoE and lysophospholipid content and allowing the interaction with LDLR or LRP for hepatic uptake. VLDL remnant particles are immediately cleared by the liver or alternatively, further modified by HL and cholesteryl ester transfer protein (CETP) to generate LDL.

The Role of Insulin in TGRLs Metabolism

The VLDL assembly process in the liver is tightly regulated by insulin [35, 36]: under fasting conditions, VLDL production is induced; on the contrary, in response to postprandial insulin release, hepatic VLDL production is repressed [20, 37, 38]. This tight regulation allows the liver to rapidly adapt to the metabolic shift between fasting and feeding and to maintain plasma lipids within the physiological range [20, 38, 39] (Fig. 7.4).

Several observations suggest that insulin inhibits apoB secretion by inducing its degradation [40–43]; in addition, insulin reduces FFA availability by reducing their mobilization from adipose tissue, thus inhibiting apoB secretion [44]. The *APOB* gene is believed to be constitutively expressed, as hepatic mRNA levels in vivo tend to be stable in most animal systems. However, several studies suggest that apoB mRNA abundance can be influenced by insulin in vivo [45, 46]. Hepatic apoB production is regulated at the posttranslational level by lipid availability, a process that is inhibited by insulin, resulting in an acute inhibitory effect of insulin on hepatic VLDL-TG secretion to limit postprandial plasma lipid excursion. Hepatic apoB mRNA expression is stimulated by forkhead box O1 (FoxO1) and inhibited by insulin in cell systems [47]; moreover, hepatic FoxO1 activity is increased during fasting and inhibited in response to feeding [48]. FoxO1 is a transcription factor that plays a key role in regulating hepatic glucose metabolism during fasting by inducing the expression of genes involved in gluconeogenesis [49]. In addition, FoxO1 is expected to regulate lipid metabolism by inducing hepatic MTP expression, thus resulting in increased production and secretion of VLDL [47]. Under physiological conditions, this effect is reversed by insulin [47]: after insulin release, FoxO1 is phosphorylated and translocated out of the nucleus, thus resulting in the inhibition of FoxO1 transcriptional activity; in the absence of insulin, as well as under insulin-resistant conditions, FoxO1 localizes in the nucleus in a transcriptionally active form and induces the expression of MTP (Fig. 7.4). These observations suggest an additional mechanism by which the liver controls hepatic apoB production at the transcriptional level.

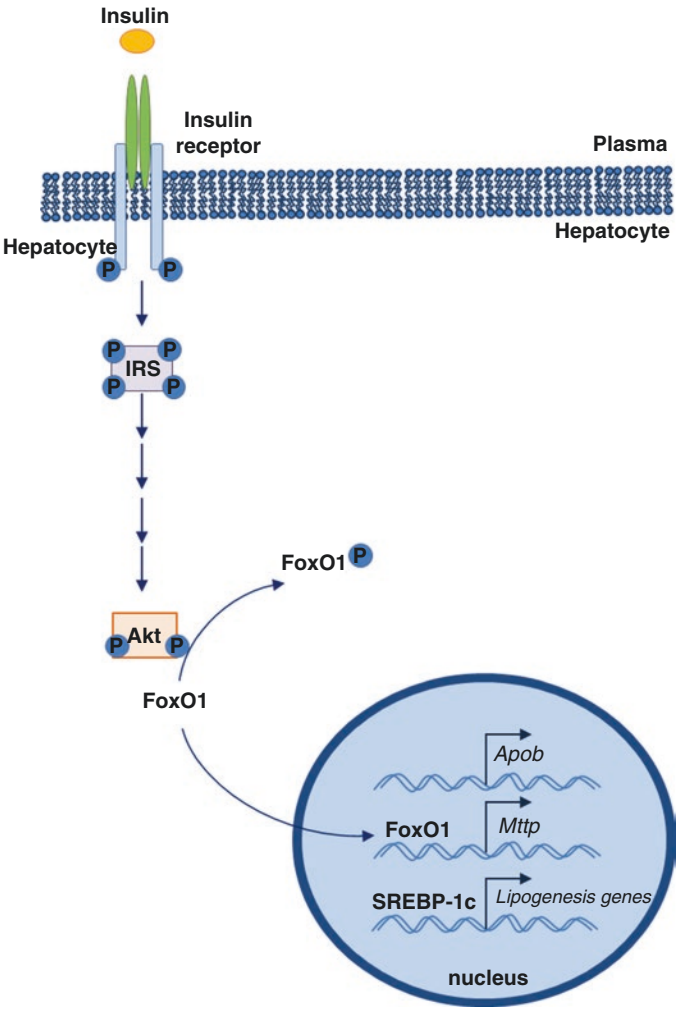

Fig. 7.4 Insulin regulation of FoxO1 activity

Interestingly, insulin promotes de novo fatty acid synthesis to improve the conversion of glucose to acetyl-CoA and fatty acids. The main transcription factor involved in this process is SREBP-1c (sterol response element binding protein 1c) [50], that in turn is regulated by insulin via LXR (liver X receptor) [51–53]; furthermore, insulin promotes the maturation of SREBP-1c independently of LXR [54]. This process is critical to maximize glucose metabolism and conversion to other substrates for storage.

In the liver, insulin also promotes the storage of glucose as glycogen and fatty acids as TG during feeding, thus limiting the hepatic VLDL secretion and glucose release. Notably, decreased VLDL secretion during feeding limits the contribution of VLDL to increased plasma TG during the postprandial phase, when intestinal fats are absorbed, packaged into CMs, and delivered to the adipose tissue.

Insulin Resistance

Diabetes is characterized by hyperglycemia as a consequence of defective insulin secretion and/or insulin response. Patients with insulin resistance are at high risk of developing diabetes and cardiovascular (CV) disease [55]. Insulin resistance is a condition of reduced responsiveness of tissues (liver, muscle, and adipose tissue) to normal circulating levels of insulin [56, 57] that can be observed under different conditions including type 2 diabetes [58], obesity, hypertension, and dyslipidemia [59]. When the concentration of blood glucose increases, the pancreas releases insulin into the circulation to maintain normal levels of blood glucose. In muscle and adipose tissues, insulin binds to cell surface receptors [60], resulting in the activation of several biochemical signals within cells, which promote glucose uptake and glycolysis to generate energy [61]. If the pancreas fails to produce enough insulin, or the insulin receptors do not function properly, cells cannot uptake glucose and the level of glucose in the blood remains high.

Several alterations can promote insulin resistance, including insulin receptor defects, insulin signaling defects [61, 62], mutations in insulin signaling molecules [63], or mitochondrial dysfunction [62]. In the early stages of insulin resistance, the pancreas partly compensates insulin dysfunction by increasing insulin production to control the increased levels of glucose in the blood. Patients present with higher blood glucose and insulin levels (a condition known as hyperinsulinemia) at the same time. If this condition is not treated, the islets of Langerhans (the insulin-secreting groups of cells) in the pancreas may eventually shut down and decrease in number. When an insulin-resistant subject cannot maintain the degree of hyperinsulinemia required to bypass the defective action of insulin, type 2 diabetes develops.

The Role of Insulin Resistance in TGRL Metabolism

In animal models of insulin resistance, hepatic MTP mRNA levels are significantly higher with simultaneous increase in VLDL levels [64–66]; on the other hand, treatments that improve insulin resistance and dyslipidemia reduce MTP expression and VLDL levels [48, 67, 68]. These observations suggest that, in insulin-resistant subjects, the increased MTP expression (due to the impaired insulin response) results in a VLDL overproduction.

Insulin resistance is considered mainly a carbohydrate metabolism disorder; however, lipid and lipoprotein abnormalities are common in individuals with insulin resistance [69], and include (1) increased plasma levels of VLDL-TG and apoB100, (2) reduced plasma levels of HDL and apoA-I, (3) relatively normal LDL-C levels with increase of small dense LDL particles.

Acute insulin infusion reduces production of TG-rich VLDL in healthy non-obese humans [20, 39, 70, 71]; this effect can result from several mechanisms, including inhibition of hepatic MTP expression [72], increased apoB degradation

[42], and inhibition of VLDL particle maturation [73]. This suppressive effect of insulin is however attenuated or even reversed [40, 74] when exposure to insulin is prolonged (such as in conditions of insulin resistance [70, 71]), where an increase in VLDL (mainly in the VLDL1 fraction) production is observed [75–77]. These observations suggest that chronic hyperinsulinemia plays a role in mediating the increased production of hepatic VLDL. In addition, insulin resistance of adipose tissue increases the levels of circulating FFA that can enter the liver, thus stimulating VLDL production [78].

Finally, loss of insulin inhibition of FoxO1 activity in insulin resistance increases the production of both glucose and VLDL-TG, contributing to the dual onset of hyperglycemia and hypertriglyceridemia in diabetes.

Hepatic TG in Insulin Resistance

Notably, fatty acid flux to the liver is increased during insulin resistance [79, 80], due to the failure of insulin to inhibit TG lipolysis in adipose tissue [81]. Increased levels of fasting and postprandial TG are thus features of insulin resistance [82]. The increase in postprandial TG is due both to defective lipolysis of VLDL and CM, as a consequence of reduced LPL activity and induction of apoC-III (an inhibitor of LPL) secretion [83], combined with increased VLDL and CM secretion [82, 84]. While under physiological conditions, CM production is inhibited by insulin, this inhibitory process is lost or reduced in the presence of impaired insulin response (hepatic insulin resistance). Another source of hepatic TG could be the de novo lipogenesis, which contributes significantly to VLDL and is increased in insulin-resistant subjects.

Diabetes and Hepatic Uptake of Remnant Lipoproteins

Diabetes impairs the hepatic uptake of remnant lipoproteins [28, 85, 86] via the impairment of HSPG activity. In type 1 diabetes, hepatic HSPGs exhibit sulfation defects [87, 88], due to the suppression of a crucial enzyme in HSPG assembly [89]; moreover, the farnesoid X receptor, a known inducer of HSPG expression [90], is suppressed [91]. In type 2 diabetes and other diseases characterized by insulin resistance, proteoglycans exhibit several defects, including decreased sulfation [92, 93]. Insulin resistance also induces the hepatic overexpression of the heparin sulfate glucosamine 6-*O*-endosulfatase-2 (SULF2), an enzyme that degrades both cell surface and matrix HSPGs, thus reducing the catabolism of remnant lipoproteins and contributing to postprandial dyslipoproteinemia in type 2 diabetes [94]. Notably, LDLR does not contribute significantly to remnant lipoprotein catabolism in diabetes [95, 96].

Triglyceride-Rich Lipoproteins and Vascular Dysfunction

Changes in TG-rich lipoprotein metabolism affect the metabolism of other lipoproteins. Increased plasma levels of TG modify both size and composition of LDL (the final product of VLDL intravascular remodeling), with the generation of small dense LDL enriched in bioactive pro-inflammatory lysophospholipids, suggesting an increased inflammatory potential per particle [97, 98]. Under this condition, typically observed in insulin-resistant states such as type 2 diabetes and metabolic syndrome, the LDL profile consists predominantly of small particles and remnant lipoproteins. The reduction of particle size and alterations in apoB conformation reduce the binding affinity to LDLR, thus resulting in an increased lifetime. Hypertriglyceridemia may also affect HDL metabolism, with major alterations in HDL proteome and lipidome, which in turn may negatively affect HDL functions [99–101].

While there is no evidence that TG directly exert pro-atherogenic effects, FFA generated during TG lipolysis (especially saturated fatty acids) can promote an inflammatory response in arterial wall cells. In fact, an increase in circulating FFA impairs endothelium-dependent vasodilatation [102], likely due to an enhanced oxidative stress [103].

In addition, the metabolism of TGRLs produces a heterogeneous population of remnant particles that are only partially lipolyzed; some of them (*transient remnants*) are further lipolyzed by lipases to produce LDL, whereas others undergo remodeling processes that render them resistant to further lipolysis (*end-product remnants*), increasing their half-life and remaining in the circulation until they are cleared by the liver [104]. A longer residence time in the circulation, that may be influenced by several factors (for example, the levels of apoC-III), leads to a substantial enrichment in cholesterol, so that end-product remnants may contain up to four-fold greater cholesterol content per particle than LDL [104].

Remnant lipoproteins induce several effects in the cells of the arterial wall, either directly or indirectly. CM and VLDL are too large to cross the endothelium layer, but their remnants are small enough to cross the endothelial barrier and enter the intima, where they are trapped by proteoglycans. Although both CM and VLDL remnants still contain more TG than cholesterol in proportion, their large size allows the deposition of a greater amount of cholesterol per particle than LDL; furthermore, remnants are directly taken up by macrophages without requiring modification (contrarily to LDL), leading to or exacerbating foam cell formation and atherosclerotic lesions, and they are hardly released back to circulation [105]. This remnant-mediated cholesterol accumulation favors macrophage M1 polarization in the intimal microenvironment, with an enhanced inflammatory response which promotes lesion development and destabilization. Thus, in condition of overproduction or delayed removal of remnant particles, an increased number of remnants is likely to enter the subendothelial space. Although LDL particles are more abundant in plasma and have a longer half-life, TGRLs and their remnants are typically elevated under particular conditions, such as the postprandial time, which can be as long as 8-h if the lipolytic capacity of the individual is compromised [106].

In this context, apoC-III, which is carried by VLDL, LDL, and HDL, plays a major role in lipoprotein metabolism. in fact, it dampens apoB-containing lipoprotein clearance by interfering with the binding to hepatic apoB/apoE receptors, leading to hypertriglyceridemia and production of small dense LDL [107]; furthermore, apoC-III at high concentrations inhibits the activity of LPL and enhances VLDL assembly and secretion [108–110]. The result of all these events is a marked hypertriglyceridemia. Beside to its role in impairing plasma lipoprotein metabolism, apoC-III associated with apoB-containing lipoproteins directly stimulates vascular inflammatory processes and triggers the activation of NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, which, in turn, promotes the release of interleukin (IL)-1ß and induces systemic inflammation [111]. Interestingly, also HDL contains apoC-III, but does not induce IL-1ß release, suggesting that the type of lipoprotein carrying apoC-III (and thus its property) matters. In agreement with this finding is the observation that elevated non-HDL apoC-III strongly associates with an increased risk of cardiovascular events [112].

The impact of TGRLs in the postprandial phase on endothelial function and inflammation is highly relevant; indeed, several in vivo studies have demonstrated that postprandial hypertriglyceridemia impairs endothelial function [113, 114]. Postprandial hypertriglyceridemia is also associated with an inflammatory state and enhanced levels of tumor necrosis factor (TNF)-α, IL-6, soluble intercellular adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1 [115–117]. In normolipidemic subjects, under fasting conditions, CM are rapidly metabolized, and thus the TGRL fraction is mainly composed of apoB100-rich particles, and remnants derive mainly from the catabolism of VLDL. In dyslipidemic individuals, CM are metabolized at a lower rate, resulting in the accumulation of CM remnants in the fasting state; in the postprandial phase, an enormous production of TGRLs containing both apoB48 and apoB100 occurs, thus leading to a large impairment of endothelial function.

TGRLs are lipolyzed by LPL, generating different biologically active products that may affect endothelial cell (EC) function [118]. Studies conducted in ECs indicate that VLDL can also activate nuclear factor (NF)-κB [119], a transcription factor that plays an important role in the phenotypic modulation of ECs in a pro-inflammatory condition. To date, plasminogen-activator inhibitor-1 is the only gene that has been shown to be consistently induced in ECs to a larger extent when comparing VLDL from patients with hyperlipoproteinemias type IV and type II versus VLDL from normolipidemic subjects [120]. Both in human umbilical venous ECs and human aortic ECs, TGRLs from hypertriglyceridemic subjects induce an increased mRNA expression of adhesion molecules, such as VCAM-1, platelet/endothelial cell adhesion molecule (PECAM)-1, and endothelial/leukocyte adhesion molecule (ELAM)-1, while TGRLs from normolipidemics induced VCAM-1 expression in both the cell lines and ELAM-1 selectively in the aortic ECs, but to a lesser extent [121]. Specific inhibition of p38 mitogen-activated protein kinase and NF-κB suggests a major involvement of these factors in adhesion-molecule expression induced by TGRLs in both NTG and HTG patients. Furthermore, TGRLs induced monocyte chemoattractant protein (MCP)-1 expression in ECs suggesting

that activation of the endothelium by TGRLs could support both adhesion and transmigration of leukocytes. In addition, TGRLs from hypertriglyceridemic patients induced IL-6 expression. Again, these effects are mainly dependent on NF-κB activation.

The composition of the TGRL particles plays a key role in determining the proinflammatory response to TGRLs [122]. A different composition of VLDL (fatty acid, lipids, and apoproteins) may be responsible for the differences observed between normolipidemic and hypertriglyceridemic TGRLs. TGRLs isolated following a meal enriched in saturated fatty acids induced E-selectin and VCAM-1 expression to a higher extent than TGRLs isolated after a meal enriched in monounsaturated and polyunsaturated fatty acids [122]. Furthermore, lipolysis products from TGRLs increase endothelial permeability, perturb zonula occludens-1 and F-actin, and induce apoptosis [118]. Although hypertriglyceridemia is an independent risk factor for coronary artery disease [123–125], accumulating evidence suggests that postprandial (hyper)lipidemia contributes to the development of atherosclerosis and coronary artery disease [126]. Several studies have demonstrated that postprandial hypertriglyceridemia impairs endothelial function, suggesting a role for TG in the initiation and further progression of atherosclerosis [113, 114]. Postprandial hypertriglyceridemia is associated with an inflammatory state and increased levels of TNF-α, IL-6, sICAM-1, and sVCAM-1 [115–117]. Although TGRLs isolated from fasting plasma samples of hypertriglyceridemic subjects induce an inflammatory response in ECs [121], ECs incubated with postprandial TGRLs demonstrated an increased mRNA expression of VCAM-1, ELAM-1, P-selectin, PECAM-1, and ICAM-1. Similarly, postprandial TGRLs increased ICAM-1 and VCAM-1 protein expression [127]. Also fasting TGRLs increase adhesion molecule expression, but the effect observed is less pronounced. Furthermore, ICAM-1 expression was induced solely upon incubation with postprandial TGRLs. Likewise, MCP-1 and IL-6 expression was induced upon incubation with postprandial TGRLs; again, this effect is more pronounced than that observed with fasting TGRLs, which may induce endothelial dysfunction. Notably, a single high-fat meal led to a significant elevation of endothelial microparticles, known to be a sensitive indicator of endothelial disturbance, in healthy normolipidemic subjects [114]. This observation suggests that endothelial microparticles may be an indirect marker of endothelial dysfunction or injury induced by postprandial TGRL.

TGRLs and their remnants have been detected in human and experimental atherosclerotic lesions [128–130]: CM remnants directly penetrate the endothelial cell layer and are entrapped within the subendothelial space, leading to focal accumulation [129] (Fig. 7.5). TGRLs may directly contribute to the atherosclerotic process by inducing endothelial dysfunction [131], enhancing monocyte adhesion [132], and triggering lipid accumulation within the artery wall [133]. Exposure to TGRLs, especially those of patients with type 2 diabetes [134], leads to the intracellular accumulation of triglycerides and/or cholesteryl esters in human monocyte-derived [134] and murine macrophages [133, 135]. Abnormal reverse cholesterol transport and low levels of HDL associated with hypertriglyceridemia

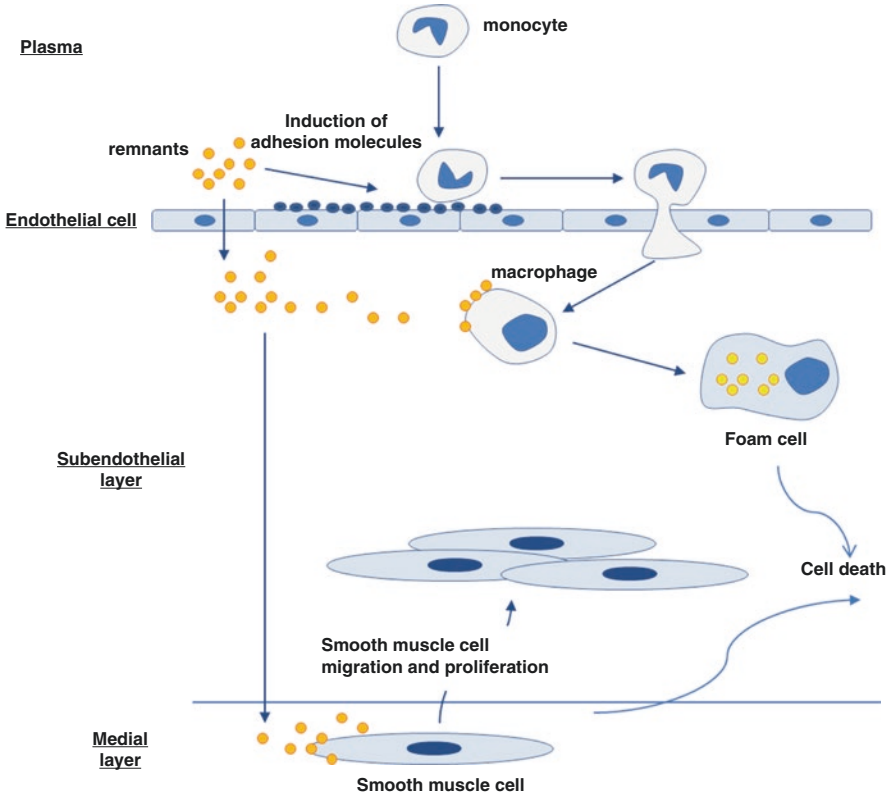

Fig. 7.5 Remnant contribution to the atherosclerotic lesion development

[136, 137] can accelerate lipid deposition process within arterial macrophages. The interaction of TGRLs with cholesterol-loaded human macrophages increases the cell lipid content, while compromising the subsequent efflux of cholesterol to lipid-poor apoA-I [138]. These aspects may contribute significantly to the generation of macrophage foam cells in vivo and might account for the accelerated atherogenesis observed in patients with type 2 diabetes. Finally, remnant lipoproteins induce smooth muscle cell activation and proliferation [139, 140].

Most of the available evidence suggests that in normolipidemic subjects either in the fasting or the postprandial phase TGRL may affect endothelial function only when a pro-inflammatory environment is already present and may perhaps contribute to accelerating the damage induced by other lipids and non-lipidic factors. However, in hypertriglyceridemic patients, TGRLs from the fasting state and postprandial phase can both induce endothelial dysfunction by promoting a pro-inflammatory activation of the endothelium. These findings are in line with the idea that these lipoproteins may play a relevant role in the early stages of atherogenesis.

The Role of ApoC-III in the Metabolism of TGRLs

ApoC-III is mainly produced by the liver and, to a lesser extent, the intestine [141]. ApoC-III is a key regulator of TG levels; this protein is, in fact, a potent inhibitor of LPL and therefore inhibits the LPL-mediated lipolysis of TGRLs, but also has relevant LPL-independent effects on lipid metabolism, as it impairs the hepatic clearance of TGRL remnants by displacing apoE (the major receptor ligand) from remnant particle surface and promotes TG and VLDL synthesis and secretion by the liver [142, 143]. Thus, elevated levels of plasma apoC-III contribute to hypertriglyceridemia. In agreement, targeting apoC-III with an antisense oligonucleotide reduces TG levels by promoting LPL activity in patients lacking apoE-mediated TGRL hepatic clearance (apoE2 homozygotes) [144], as well as in patients with hypertriglyceridemia genetically determined by LPL deficiency, supporting a role for apoC-III in modulating plasma TG levels also in an LPL-independent manner [145].

The causal link between apoC-III and TG levels is supported by the observation that individuals carrying loss-of-function mutations in *APOC3* (the gene encoding apoC-III) present with lower plasma TG levels and a reduced risk of cardiovascular disease [146, 147]. This lower risk appears to be mainly mediated by the substantial reduction in remnant cholesterol levels rather than changes in LDL-C: individuals carrying loss-of-function mutations of *APOC3* in heterozygosis have 3% lower LDL-C and 43% lower remnant cholesterol than non-carriers [148], suggesting *APOC3* and consequently remnant cholesterol as suitable targets for reducing CV risk.

Diabetic patients optimally treated with cholesterol-lowering therapies still present a substantial residual CV risk, which appears to be, at least in part, linked to elevated plasma levels of TG and abnormal metabolism of TGRLs and their remnants [149]. Diabetes mellitus can be associated with an altered apoC-III synthesis and secretion due to elevated levels of glucose and insulin resistance [150], with enhanced apoC-III promoting hypertriglyceridemia and inflammation in vascular cells. Increased serum levels of apoC-III are an independent predictor of type 2 diabetes; not only apoC-III is induced by insulin resistance, but also worsens insulin-resistant status [151].

Diet can modulate plasma levels of apoC-III. Glucose induces the expression of apoC-III, and the carbohydrate content of the diet correlates with plasma apoC-III levels. As an example, fructose increases the expression of apoC-III, but also stimulates de novo fatty acids synthesis and VLDL production in the liver, while it does not stimulate insulin secretion, thus resulting in an impaired clearance of TGRLs. Consumption of saturated fatty acids increases plasma apoC-III levels, whereas omega-3 polyunsaturated fatty acids reduce plasma apoC-III levels [152, 153].

The Role of ANGPTL3 in the Metabolism of TGRLs

ANGPTL3 belongs to the family of angiopoietin-like proteins and is involved in different biological processes, including lipid metabolism; ANGPTL3 is exclusively expressed in the liver, where it undergoes several posttranslational modifications, which prompt its activation [154]. ANGPTL3 inhibits the activity of LPL and EL, leading to a reduced hydrolysis of TG and increased TG plasma levels. Furthermore, ANGPTL3 induces lipolysis in the adipose tissue, resulting in the release of FFAs that, in turn, increase the hepatic synthesis of VLDL [154]. Accordingly, loss-of-function mutations in *ANGPTL3* cause familial combined hypolipidemia, characterized by lower plasma levels of TG, fatty acids, VLDL-C, LDL-C, and HDL-C due to decreased rates of VLDL-apoB production and increased fractional catabolic rates for LDL apoB [155]. ANGPTL3 deficiency is associated with protection from CAD [156].

Subjects with type 2 diabetes mellitus have higher levels of ANGPTL3 than nondiabetic subjects [157, 158], and obese nondiabetic subjects have higher ANGPTL3 levels than non-obese nondiabetic subjects [158]. On the other hand, ANGPTL3 deficiency is associated with increased insulin sensitivity, LPL activity, and lower serum FFAs [159]. These observations support the link between ANGPTL3 and insulin resistance and also suggest that ANGPTL3 might interfere with carbohydrate metabolism by several mechanisms. ANGPTL3 may deteriorate glucose metabolism by inducing the release of FFAs from the adipose tissue, which in turn induces peripheral and hepatic insulin resistance [160]; the increased plasma levels of FFAs determined by ANGPTL3-induced lipolysis also upregulate the expression of ANGPTL4, which is involved in the regulation of TG levels through the inhibition of LPL activity [160], and whose levels are increased in type 2 diabetes [161] as well as in obese subjects with altered glucose tolerance [162].

These observations suggest that ANGPTL3 inhibition might represent an alternative approach to reduce dyslipidemia and dysglycemia. Targeting hepatic ANGPTL3 with the antisense oligonucleotide vupanorsen in diabetic patients with hepatic steatosis and hypertriglyceridemia extensively reduced TG and apoB-containing lipoproteins levels, including remnant cholesterol and VLDL-C, thus improving lipid/lipoprotein profile [163]. Inactivation of ANGPTL3 substantially lowers also LDL-C, which likely explains the reduced CV risk associated with ANGPTL3 deficiency; this reduction was found to be independent of LDLR or LRP1, but related to a reduced hepatic VLDL-TG secretion (but not VLDL-apoB) [164], and required an EL-dependent VLDL clearance [165], with multiple remnant receptors likely contributing to VLDL removal when ANGPTL3 is inhibited [165]. This LDLR-independent mechanism of action makes ANGPTL3 inhibition a valuable approach for the management of patients with homozygous familial hypercholesterolemia, who commonly do not respond efficiently to drugs upregulating LDLR expression [166, 167].

TGRLs and Their Remnants: Novel Targets for Anti-atherosclerotic Therapy?

While LDL-C level control represents unequivocally the main approach for reducing the risk of atherosclerotic-related cardiovascular events, accumulating evidence from epidemiologic and genetic studies supports a causal relationship between elevated levels of TG-rich lipoproteins and their remnants and the risk of ASCVD [104]. This may explain, at least in part, the residual CV risk frequently observed in patients with LDL-C levels at goal, particularly in patients with diabetes. Despite that, there is limited evidence from randomized clinical trials that lowering TG or TGRLs reduce ASCVD risk, which, in turn, limits the recommendations contained in the current 2019 ESC/EAS guidelines for the management of dyslipidemias, which consider the use of drugs to lower TG levels only in high-risk patients having TG levels >200 mg/dL (>2.3 mmol/L) [168].

Drugs commonly used for the reduction of plasma TG levels include statins, fibrates, and omega-3 fatty acids; statins increase the clearance of apoB-containing particles, and were shown to provide a greater benefit on CV outcomes in patients with CHD having higher TGRL levels [169], thus clearly indicating that TGRL levels are a potential target for therapeutic intervention, and providing a rationale for the development of new agents that specifically reduce TG and TGRLs. These include drugs targeting ANGPTL3 or apoC-III, but also drugs with a different mechanism of action, including icosapent ethyl and PCSK9 monoclonal antibodies.

Evinacumab is a monoclonal antibody targeting ANGPTL3; it significantly reduces TG and VLDL-C levels (both by up to ~80%) in hypertriglyceridemic subjects [170, 171]. Similarly, vupanorsen, an *N*-acetyl galactosamine-conjugated antisense oligonucleotide that selectively inhibits ANGPTL3 protein synthesis in the liver, dose-dependently reduced TG (36–53%) and apoB-containing atherogenic lipoproteins (non-HDL-C: 18%) in patients with type 2 diabetes mellitus, hepatic steatosis, and hypertriglyceridemia [163]. At month-6, a significant proportion of patients receiving vupanorsen achieved TG levels <150 mg/dL (<1.7 mmol/L). Both apoC-III and remnant cholesterol levels were also significantly reduced in patients treated with vupanorsen (40–61% and 35–47%, respectively) [163]. Whether these reductions translate into a CV benefit remains to be assessed.

All commonly used drugs have modest effects on apoC-III levels, with reductions ranging from 10% to 30% for fibrates, fish oils, niacin, statins, and ezetimibe. Volanesorsen is a second-generation antisense oligonucleotide targeting apoC-III mRNA; compared with placebo, volanesorsen reduced significantly apoC-III carried by all classes of lipoproteins (mean percent reduction ~80%) in patients with hypertriglyceridemia [172]. Volanesorsen reduced apoC-III and TG levels also in patients with type 2 diabetes and hypertriglyceridemia by 88% and 59%, respectively, accompanied by a 57% improvement in insulin sensitivity [173]. Olezarsen (AKCEA-APOCIII-L_{Rx}) is an N-acetyl galactosamine-conjugated antisense oligonucleotide that selectively inhibits apoC-III protein synthesis in the liver; a phase 1/2a study in healthy volunteers with TG levels ≥90 or ≥200 mg/dL showed

dose-dependent reductions in apoC-III (up to 80%) and TG (up to 77%) levels, with an overall improvement in the atherogenic lipid profile [174]. A recent randomized, double-blind, placebo-controlled, dose-ranging study showed that treatment with olezarsen dose-dependently reduced triglyceride levels, ranging from 23% with 10 mg every 4-weeks up to 60% with 50 mg every 4-weeks, compared with increase by 6% for the pooled placebo group in patients with moderate hypertriglyceridemia (200–500 mg/dL; 2.26–5.65 mmol/L) at high for or with established CVD [175].

Icosapent ethyl is a highly purified ethyl ester of eicosapentaenoic acid that was shown to significantly reduce the risk of cardiovascular events by 25% in patients with elevated TG levels on statin therapy [176]. It is currently indicated at a dose of 2 g twice daily for patients at high cardiovascular risk who have fasting TG levels ≥135–499 mg/dL (1.5–5.6 mmol/L) despite maximally tolerated statin treatment [168]. The ANCHOR study showed that, in statin-treated patients with persistently high TG levels, icosapent ethyl 4 g/day reduced TG levels by 21.5%, non-HDL-C by 13.6%, VLDL-TG by 26.5%, VLDL-C by 24.4%, remnant cholesterol by 23.0%, and apoC-III by 16% [177]. An exploratory analysis in patients from the MARINE and ANCHOR studies showed that icosapent ethyl 4 g/day significantly reduced remnant cholesterol (−29.8% and −25.8%, respectively) compared with placebo; the reduction was observed in all patients, but was greater in those with higher versus lower baseline TG levels, in those receiving statins versus no statin, and in those receiving medium/higher-intensity versus lower-intensity statins [178].

Finally, PCSK9 inhibition has been shown not only to reduce LDL-C levels and the risk of CV events [179, 180], but also to positively affect other lipids. In a real-world study population, inhibition of PCSK9 with evolocumab resulted in an increased VLDL size (estimated as VLDL-TG/apoB ratio) and reduced VLDL-associated apoproteins, suggesting a higher clearance of small atherogenic VLDL remnant particles [181], in line with two previous studies reporting a reduction in the levels of small VLDL and an increase in the average VLDL particle size with alirocumab or evolocumab [182, 183]. Moreover, evolocumab treatment improved postprandial response of TGRLs in subjects with type 2 diabetes; indeed, the postprandial rise in total TG and VLDL1-TG was significantly lower (by 21% and 15%, respectively), as were TGRL-cholesterol, remnant cholesterol, apoC-III, and apoB48, whereas the increment in CM-TG was not significantly affected [184]. A post hoc analysis of phase 3 BANTING and BERSON trials showed that evolocumab added to statin therapy reduced atherogenic lipids and lipoproteins in patients with type 2 diabetes with or without atherogenic dyslipidemia, with a significant reduction in non-HDL-C, remnant cholesterol, TG, and VLDL-C levels [185].

Whether the reductions in TGRL levels also translate into a clinical benefit remains to established. Furthermore, a correct quantification of TGRLs still represents a major issue, and new specific methods for their quantification would appreciably improve the understanding of their biology and their role in promoting atherosclerosis in diabetes and other disorders.

Conclusions

Triglyceride-rich lipoproteins (TGRLs) are pathogenic, inducing endothelial activation, vascular inflammation, foam cell formation, and atherosclerosis and contribute to residual vascular risk in people with diabetes even after optimal LDL-C management. TGRLs and their metabolism are modulated by many factors, including genetics, obesity, glucose, and insulin resistance, with bidirectional relationships between TGRLs, glycemia and insulin resistance and other lipoproteins. Many existent lipid drugs impact TGRL metabolism, and several aspects of TRGLs are novel therapeutic targets undergoing evaluation.

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Chapter 8
Triglyceride- and Cholesterol-Rich Remnant Lipoproteins in Risk of Cardiovascular Disease in Diabetes Mellitus

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Introduction

During the last decades, much progress has been made in atherosclerotic cardiovascular disease prevention and treatment in high-income countries. Much of the progress can be attributed to increased awareness and treatment of established risk factors such as: smoking, hypertension, and hypercholesterolemia due to elevated low-density lipoprotein (LDL) cholesterol.

In addition to LDL cholesterol, current evidence indicates that elevated remnant cholesterol (cholesterol held in remnant lipoproteins) also causes atherosclerotic cardiovascular disease [1]. Furthermore, high levels of triglycerides, the other major lipid component of remnant lipoproteins, are a known cause of acute pancreatitis [2].

Elevations in remnant lipoproteins usually result from a combination of factors, but the main contributor is the metabolic syndrome, characterized by adiposity, insulin resistance, and often, ultimately, type 2 diabetes [1]. Crucially, the metabolic syndrome pandemic is still growing, and the number of people with diabetes mellitus worldwide is bound to surpass 500 million within the coming years [3]. Statins and other lipid-lowering drugs have made it possible to achieve very low LDL levels in patients with and without diabetes mellitus, but the effect on remnant lipoproteins is smaller. This means that hypercholesterolemia due to elevated remnant lipoproteins relative to LDLs is increasing, with important implications for cardiovascular disease prevention and treatment. Indeed, multiple drugs for lowering remnant lipoproteins are currently in development, and more therapeutic options could therefore become available in the years ahead.

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Familiarity with remnant lipoproteins in diabetes mellitus is now important for clinicians in many different specialties, including endocrinology, cardiology, primary care, and clinical biochemistry. We hope to offer the interested reader a thorough overview, focused on easy translation into clinical practice; the aim is to support patient treatment and to provide a solid foundation for understanding the clinical guidelines.

Clinical Signs

Lipoprotein levels in plasma are usually determined by biochemical analysis. However, severely elevated remnant lipoproteins also produce clinical signs which are visible to the naked eye. These signs are relatively rare but can give a helpful visual impression of the mechanisms involved.

Blood Sample Characteristics

Figure 8.1 shows plasma samples from three different individuals. To the left is shown a plasma sample from an individual without diabetes mellitus, but with increased concentration of two established and well-known risk factors for atherosclerotic cardiovascular disease, LDL cholesterol, and lipoprotein(a) [4, 5]. This sample exhibits normal yellow coloration and transparency of plasma. However, in individuals with diabetes mellitus, dyslipidemia is more often characterized by a higher concentration of remnant lipoproteins and triglycerides in plasma. In case of moderate hypertriglyceridemia (triglycerides between 2 and 10 mmol/L [177–886 mg/dL]), the plasma loses much of its transparency (Fig. 8.1, middle sample). The turbidity increases with increasing concentration of triglycerides because remnant lipoproteins increase in size, and the color changes from yellowish to white. The increased turbidity is caused by large triglyceride-rich remnant lipoprotein particles

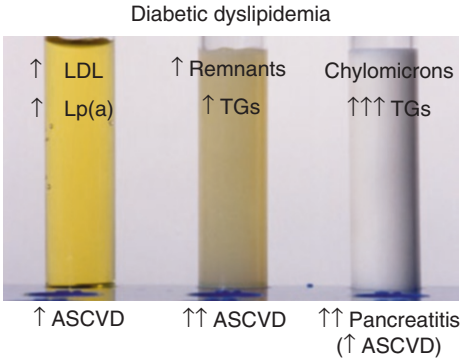


Fig. 8.1 Visual inspection of plasma from patients with different levels of plasma triglycerides. *LDL* low-density lipoprotein, *Lp(a)* lipoprotein(a), *TGs* triglycerides, *ASCVD* atherosclerotic cardiovascular disease

which scatter the light transmitted through the plasma sample. In the most extreme cases, accumulation of triglyceride-rich remnant lipoproteins or simply triglyceride-rich lipoproteins in the blood stream results in plasma samples that are milky white, as seen in the plasma sample to the right in Fig. 8.1. It can appear as strawberry pink before centrifugation [6]. Plasma samples with this appearance will likely have a concentration of triglycerides around 100 mmol/L (8860 mg/dL). In these individuals, the first presentation to the physician, although rare, can be eruptive xanthomas.

Physical Examination

Figure 8.2 shows a patient with eruptive xanthomas caused by an extreme accumulation of triglycerides and triglyceride-rich lipoproteins in plasma (Fig. 8.1, right panel). In this case, plasma triglycerides were 129 mmol/L (11,400 mg/dL). Eruptive xanthomas are reddish-yellowish papules about 1–5 mm in size. They are often located at extensor surfaces of the extremities, buttocks, and the back. The papules consist of macrophages with high lipid content, also called foam cells [7]. If the underlying cause of hypertriglyceridemia is treated and triglyceride concentration is lowered, the xanthomas disappear. Clinical recognition of lipemic blood samples and eruptive xanthomas is important, as they can be manifestations of unrecognized or dysregulated diabetes mellitus [8], but also because hypertriglyceridemia is a risk factor for acute pancreatitis [2, 9].

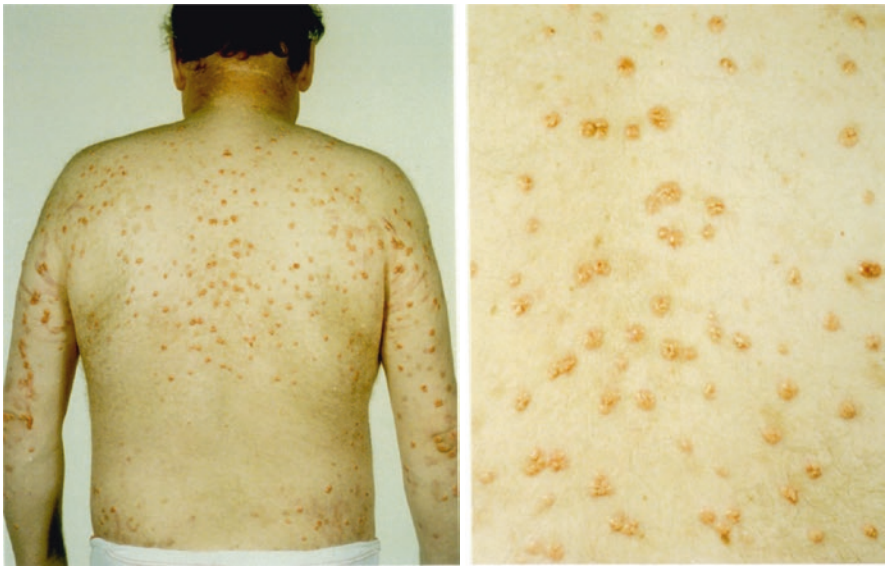


Fig. 8.2 Patient with eruptive xanthomas at extreme high triglycerides and therefore at high risk of acute pancreatitis. Plasma triglycerides were 11,352 mg/dL (129 mmol/L)

Lipids and Lipoproteins

Lipids, that is triglycerides and cholesterol, are carried by lipoproteins in the bloodstream. The metabolism of lipids and lipoproteins in the diabetic state are discussed in detail elsewhere in this book. In this chapter, we take a closer look at lipids and lipoproteins in individuals with diabetes mellitus from a clinical and epidemiological view. In the clinic, lipids and lipoproteins are most often evaluated using a standard lipid panel including plasma triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol.

Remnant lipoproteins are often called triglyceride-rich lipoproteins; lipoproteins which, as the name suggests, have a relatively high triglyceride content. These lipoproteins are the lipoproteins not characterized as LDLs or high-density lipoproteins (HDLs) and thus include (1) chylomicrons from the intestine and their remnants and (2) very low-density lipoproteins (VLDLs) from the liver and their remnants including intermediate-density lipoproteins (IDLs) [10]. This definition is easy to apply in clinical practice, the cholesterol content of remnant lipoproteins can simply be calculated as total cholesterol minus LDL cholesterol minus HDL cholesterol [11].

Composition of Lipoproteins

Besides the outer phospholipid monolayer with unesterified cholesterol and apolipoproteins embedded within, remnant lipoproteins consist of a core of triglycerides and cholesterol esters. The relative content of triglycerides and cholesterol (that is cholesterol esters) in the remnant lipoproteins is determined by the exact type of remnant lipoprotein in question, and how it has been metabolized. In individuals with dysregulated diabetes mellitus, lipoprotein metabolism can be deranged to a degree causing severe hypertriglyceridemia. This is due to both increased production and secretion of VLDL particles from the liver and decreased activity of lipoprotein lipase. Lipoprotein lipase is the enzyme responsible for converting triglycerides into glycerol and free fatty acids at the vessel wall, leading to slower metabolization of chylomicrons (in the non-fasting state), VLDL particles, and their remnants [12–14], and decreased removal of these particles by the liver. These triglyceride-rich remnant lipoproteins therefore accumulate in the bloodstream, which results in higher concentrations [15, 16].

Standard Lipid Profile

As shown in Fig. 8.3, the standard practice is to measure the total concentration of plasma triglycerides and cholesterol, pooled from all the lipoproteins carried in the blood. Besides this, the standard lipid profile includes lipoprotein specific direct measurement of HDL cholesterol, while LDL cholesterol is most often calculated

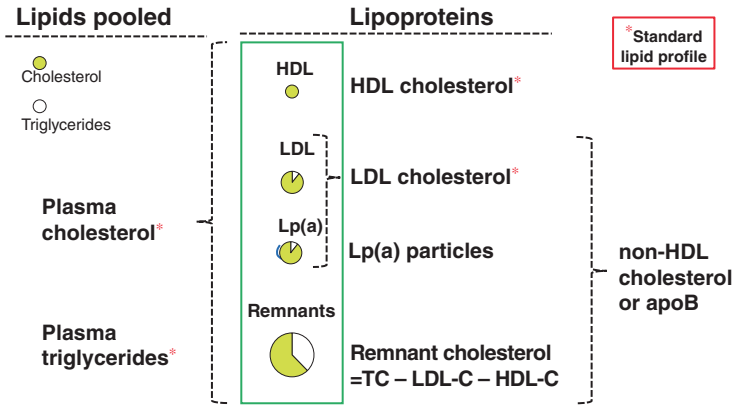


Fig. 8.3 Lipids and lipoproteins included in a standard and expanded lipid profile. *ApoB* apolipoprotein B, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *Lp(a)* lipoprotein(a), *TC* total cholesterol

using the Friedewald equation [17] from 1972, as LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/2.2 (when concentrations are in mmol/L), or triglycerides/5 (when concentrations are in mg/dL). This approach is considered valid for triglyceride concentrations below 4.5 mmol/L (400 mg/dL). When the plasma triglyceride concentration is above 4.5 mmol/L, direct measurement of LDL cholesterol is required to attain a valid LDL cholesterol concentration. However, as the ratio of triglycerides to VLDL cholesterol varies across the range of plasma triglyceride concentration, other methods of estimating the LDL cholesterol concentration have recently emerged in addition to Friedewald equation, namely the Martin-Hopkins equation [18] and the Sampson-NIH equation [19]. Furthermore, both calculated and directly measured LDL cholesterol include cholesterol carried in lipoprotein(a) particles, an LDL-like particle with an apolipoprotein(a) particle bound to the apolipoprotein B (apoB) particle [20].

Remnant Cholesterol and Non-HDL Cholesterol

From the measurement included in the standard lipid panel, it is also easy to calculate the concentration of remnant cholesterol as total cholesterol − LDL cholesterol − HDL cholesterol, and the concentration of non-HDL cholesterol as total cholesterol − HDL cholesterol; the latter includes all cholesterol carried in LDL, remnant lipoproteins, and lipoprotein(a) [21, 22] (Fig. 8.3). Some would suggest measuring of apoB instead of calculation of non-HDL cholesterol as one apoB molecule is present in each non-HDL lipoprotein particle. However, measurement of apoB is not yet common standard in lipid profiles. Remnant cholesterol calculated as described above includes cholesterol in chylomicrons, chylomicron remnants,

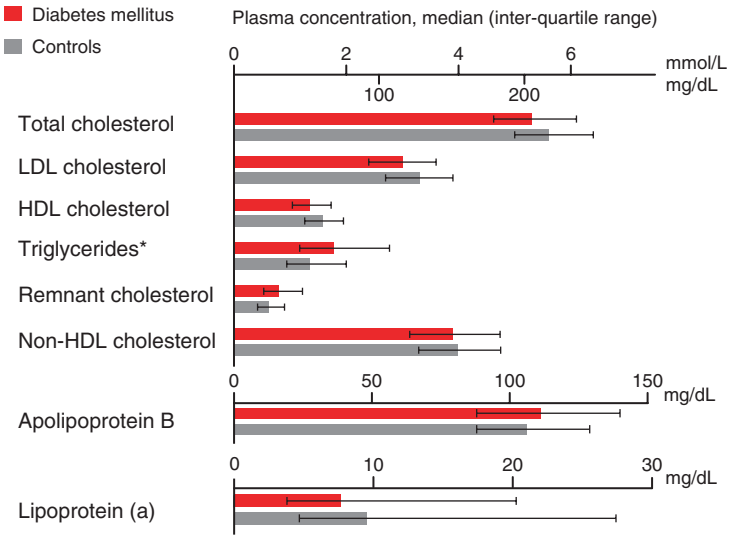
VLDL, and some IDL (some IDL cholesterol is also included in LDL cholesterol). This differs slightly from the definition of remnant cholesterol as either (1) cholesterol held in triglyceride-rich lipoproteins, which excludes cholesterol held in IDLs, which are relatively triglyceride-poor [23, 24] or (2) cholesterol held in remnant lipoproteins, which excludes cholesterol held in nascent chylomicrons and VLDL particles. However, the above calculations of remnant cholesterol and non-HDL cholesterol allow for the quantification of two relevant measures from a standard lipid profile without any extra costs.

Lipoprotein Composition in Diabetes Mellitus

From Fig. 8.3, it is also possible to get a rough idea of the relative amounts of triglycerides and cholesterol in the different lipoproteins. HDL particles carry almost exclusively cholesterol and very little triglycerides, and LDL particles also carry much more cholesterol than triglycerides. For remnant lipoproteins, the distribution is different and depends on the exact type of remnant lipoprotein. For large VLDLs, triglycerides constitute more than 50% of the total lipid content, while for small VLDLs, triglycerides constitute 20–40%. For IDLs, triglycerides only constitute around 10% of the total lipid content, comparable to the triglyceride content in LDL particles [23].

The lipid metabolism is altered in individuals with diabetes mellitus. Insulin increases uptake and storage of triglycerides from the blood, and decreases the lipolysis of triglycerides in adipose tissue, leading to lower plasma concentration of triglycerides [25]. Consequently, the opposite is the case in individuals with diabetes mellitus, where production of triglyceride-rich VLDL is increased. Due to either low or absent production of insulin, as seen in type 1 diabetes, or low insulin sensitivity, as seen in type 2 diabetes, plasma triglycerides and remnant cholesterol in individuals with diabetes mellitus are elevated compared to individuals without diabetes mellitus. This is illustrated in Fig. 8.4 which shows median lipid levels in individuals with or without diabetes mellitus in a large study of the general population of Copenhagen, Denmark: the Copenhagen General Population Study. The individuals with diabetes mellitus include both type 1 and type 2 diabetes. Most individuals with diabetes mellitus in the figure were on antidiabetic medications, but individuals receiving lipid-lowering medication (most often a statin) were excluded from the data. The median concentrations of total cholesterol, LDL cholesterol and HDL cholesterol were slightly lower in individuals with diabetes mellitus compared to individuals without diabetes mellitus.

By contrast, in the Framingham Offspring Study concentrations of total cholesterol and LDL cholesterol were not lower in individuals with diabetes mellitus [26]. Plasma triglycerides, on the other hand, were moderately elevated in individuals with diabetes mellitus in both the Copenhagen General Population



*Values can not be read on the mg/dL-scale. To get triglycerides in mg/dL, multiply values in mmol/L by 89.

Fig. 8.4 Lipids and lipoproteins in individuals with and without diabetes mellitus not on lipid-lowering therapy. Based on 105,000 individuals from the Copenhagen General Population Study. *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

Study, as shown in Fig. 8.4, and in the Framingham Offspring Study [26]. The higher concentration of triglycerides is also reflected in higher remnant cholesterol. In the Framingham Offspring Study, remnant lipoprotein cholesterol and remnant lipoprotein triglycerides were elevated in individuals with diabetes mellitus; although it must be noted that the definition of remnant lipoproteins in the Framingham Offspring Study did not include nascent VLDL particles and nascent chylomicrons [26].

Metabolomic Profiling of Lipoproteins

In support of, and further elucidating the role of triglycerides and remnant lipoproteins in diabetes mellitus, new studies have used nuclear magnetic resonance (NMR) spectroscopy metabolomics to characterize lipoprotein particles. These studies have identified a higher concentration of medium, large, and very large remnant (=triglyceride-rich) lipoprotein particles in individuals with type 2 diabetes compared with individual without diabetes mellitus [27]. Also, increased size of VLDL particles as well as higher relative and absolute concentration of triglycerides in VLDL and LDL particles has been associated with increased risk of type 2 diabetes [28]. Regarding the observation of low HDL cholesterol in

individuals with diabetes mellitus, NMR studies have found HDL particle number, size, and cholesterol content to be inversely associated with risk of type 2 diabetes [28–30]. Finally, LDL particle concentration was slightly higher in individuals at higher risk of diabetes mellitus, while their LDL particles were slightly smaller [27, 30].

In individuals with diabetes mellitus, the concentration of non-HDL cholesterol is lower than in individuals without diabetes mellitus, while the opposite is the case for apoB (Fig. 8.4). Both are measures of the total amount of atherogenic lipoprotein particles and cholesterol in blood, but the difference in direction of the association in individuals with diabetes mellitus points to the fact that individuals with diabetes mellitus have a larger number of atherogenic particles, but each particle has a lower cholesterol content. This goes well in hand with the above-mentioned observation of smaller LDL particles in individuals with diabetes mellitus [27, 30].

Figure 8.4 also illustrates lower concentration of lipoprotein(a) in individuals with diabetes mellitus. This is in line with a number of studies which show that low concentrations of lipoprotein(a) are associated with increased risk of type 2 diabetes [31–34].

Low High-Density Lipoprotein as a Marker of Elevated Remnant Lipoproteins

The principal findings in Fig. 8.4 are, however, the elevated concentrations of triglycerides and remnant cholesterol, and lower concentration of HDL cholesterol in individuals with diabetes mellitus. The lower concentration of HDL cholesterol is expected as concentrations of triglycerides and remnant cholesterol are well known to be inversely associated with HDL cholesterol [10, 35, 36]. Measured plasma concentration of triglycerides in each individual shows some degree of fluctuation over time, so-called within-subject biological variation, in part due to variance related to time since last meal and the fat content of that meal. Within-subject biological variation for HDL cholesterol is much lower, reflecting the fact that HDL cholesterol concentration is almost unaffected by an oral fat load [36]. This has led to the suggestion that low HDL cholesterol could be used as a marker for long-term monitoring of elevated plasma levels of triglycerides and remnant cholesterol, much the same way as elevated HbA1c is used as for long-term monitoring of elevated plasma glucose [36] (Fig. 8.5).

In this case, low HDL cholesterol would represent a high average concentration of triglycerides and remnant cholesterol during a medium to long period, bypassing issues with short-term fluctuation of plasma triglycerides and remnant cholesterol. This suggestion is coherent with observations that HDL cholesterol is an excellent marker of cardiovascular risk [4], while clinical trials [37] and genetic Mendelian randomization studies [38, 39] have failed to establish causality.

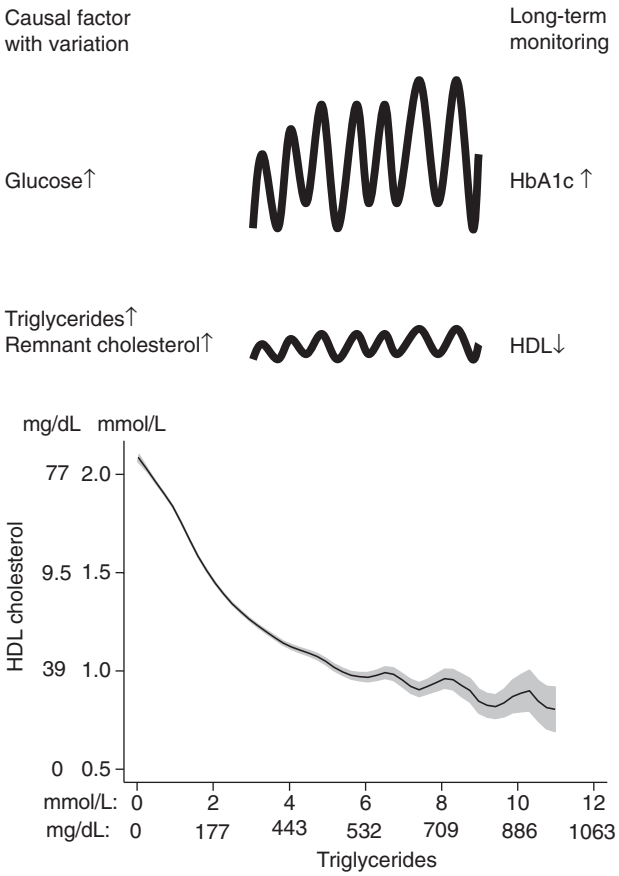


Fig. 8.5 Long-term monitors of high levels of plasma glucose and triglycerides/remnant cholesterol (upper panel) and inverse relationship between plasma levels of plasma triglycerides and HDL (high-density lipoprotein) cholesterol (lower panel). Low HDL cholesterol can be viewed as a long-term monitor for average elevated plasma triglycerides and remnant cholesterol, exactly as elevated hemoglobin A1c (HbA1c) is a long-term monitor of elevated plasma glucose. While plasma triglycerides and remnant cholesterol vary relatively fast in response to different intakes of fat, just like plasma glucose varies fast in response to different intakes of glucose, HDL cholesterol and hemoglobin A1c are more stable markers over time

Atherosclerotic Cardiovascular Disease

Atherosclerosis is characterized by plaque formation, which narrows blood arteries and promotes plaque rupture with formation of thrombi. In turn, this leads to chronic or acute ischemia. Atherosclerosis is as such a root cause of two of the most common causes of death and disability: ischemic heart disease and ischemic stroke. In addition, atherosclerosis can lead to debilitating and deadly conditions such as peripheral artery disease.

While classic risk factors are shared between the different types of atherosclerotic cardiovascular disease, their relative importance varies between these. For instance, smoking is known to be a particularly strong risk factor for peripheral artery disease [40], while hypertension is especially important for ischemic stroke [41]. Elevated LDL cholesterol seems to be especially harmful for the coronary arteries, as illustrated by familial hypercholesterolemia, a genetic disorder characterized by elevated LDL cholesterol levels, which leads to early development of ischemic heart disease [42]. Remnant lipoproteins have been less extensively studied than smoking, hypertension, and LDL cholesterol in relation to risk of atherosclerotic cardiovascular disease. Still, substantial evidence has been collected over several decades, mainly from epidemiologic studies, including Mendelian randomization studies, but also from studies of rare genetic conditions and from clinical trials of drugs which affect remnant lipoprotein levels.

Triglycerides

Elevated plasma triglycerides have been investigated as a cause of cardiovascular disease since 1959 [43]. However, it lost much of its attention in the 1980s, after findings that the observational associations often attenuated or disappeared after adjustment for factors like HDL cholesterol and plasma glucose [44]. We now know that the relationship between elevated triglycerides and increased risk of cardiovascular disease is likely explained by the cholesterol (remnant cholesterol) that is carried together with triglycerides in the remnant lipoproteins.

By considering elevated plasma triglyceride levels to be markers of elevated remnant cholesterol, inferences can be drawn from the plethora of studies that have been published on plasma triglycerides since 1959. A meta-analysis of 29 prospective studies found an adjusted odds ratio of 1.7 (95% confidence interval: 1.6–1.9) for coronary heart disease, comparing individuals with the highest third of triglyceride levels to individuals with the lowest third [45]. Likewise, analysis of more than 300,000 individuals from the Emerging Risk Factor Collaboration revealed that elevated levels of plasma triglycerides were associated with a stepwise increased risk of ischemic heart disease and ischemic stroke [4]. Similar results were also found in 90,000 individuals from the Copenhagen General Population Study and Copenhagen City Heart Study, and a similar pattern was also observed for risk of myocardial infarction and all-cause mortality [22] (Fig. 8.6).

Individuals with the rare disorder remnant (type III) hyperlipidemia or dysbetalipoproteinemia, in which plasma triglycerides and cholesterol are typically severely elevated, have long been known to be at increased risk of ischemic heart disease and peripheral artery disease [4, 46, 47]. We now know the cause of the disorder is a genetic variant which decreases removal of VLDL and IDL, which why it is also called remnant removal disease [48].

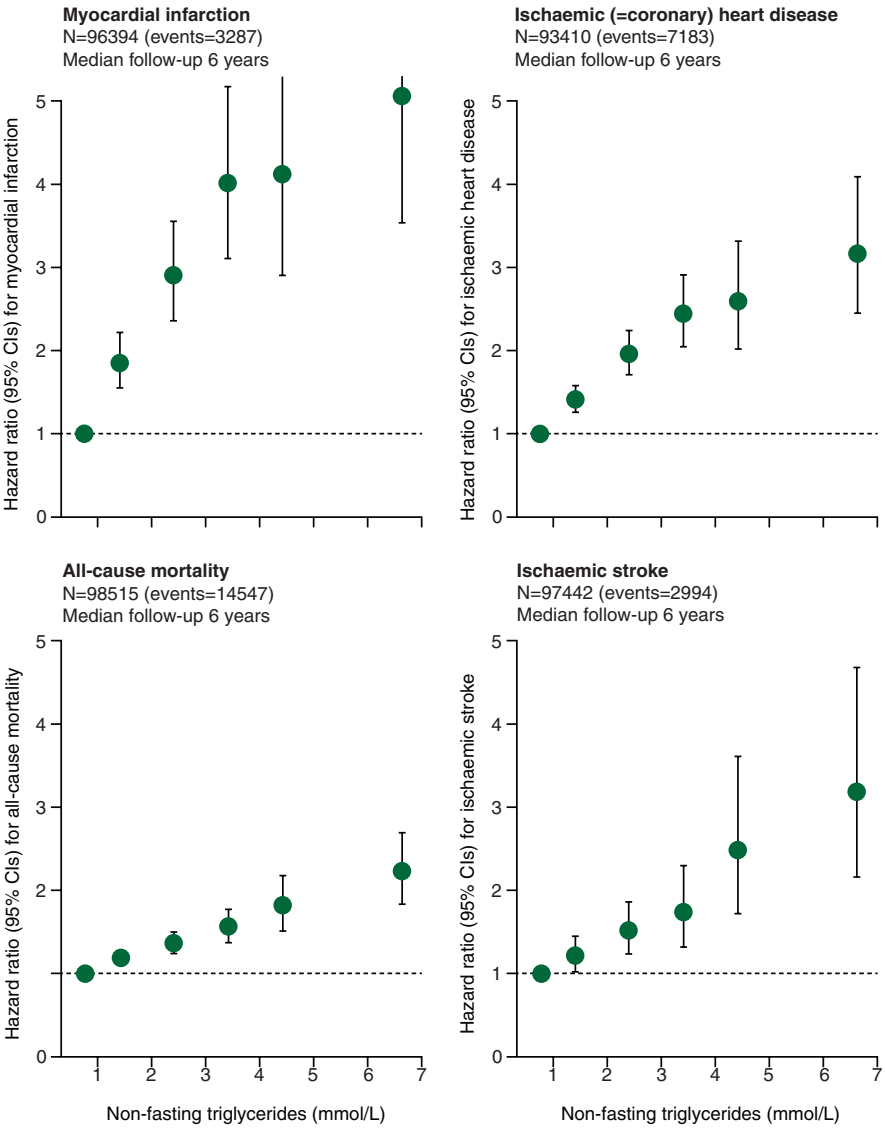


Fig. 8.6 Risk of myocardial infarction, coronary heart disease, all-cause mortality, and ischemic stroke as a function of increasing non-fasting plasma triglycerides. Hazard ratios were adjusted for age, sex, and study population (Copenhagen City Heart Study and Copenhagen General Population Study). *CI* confidence interval. (Reproduced with permission from [22])

Remnant Cholesterol

In newer studies, rather than elevated plasma triglycerides, elevated remnant cholesterol as calculated by use of the Friedewald formula [17] or other more modern

equations [18, 19] is studied. In this way, relationships with risk of cardiovascular disease can be estimated per unit of cholesterol increase. Observational findings are now also strengthened by evidence from Mendelian randomization studies, which support causality [1]. Mendelian randomization results have found associations between elevated remnant cholesterol and risk of myocardial infarction [49], ischemic heart disease [10], and aortic valve stenosis [50]. These results argue strongly in favor of a causal pathway from elevated remnant cholesterol to increased atherosclerotic cardiovascular disease, alongside elevated LDL cholesterol and lipoprotein(a) [51] (Fig. 8.7).

When studying associations with cardiovascular risk, it can be argued that direct measurements of remnant cholesterol, as the atherogenic component of remnant lipoproteins, would be preferred over remnant cholesterol calculated from a standard lipid profile. However, calculated remnant cholesterol is largely an accurate reflection of the cholesterol content and thus of the cardiovascular risk. Indeed, in the Copenhagen General Population Study, calculated and directly measured remnant cholesterol was concordant in approximately 90% of individuals [11]. In individuals with discordant measures, those with high directly measured remnant cholesterol and low calculated remnant cholesterol were at increased risk of myocardial infarction (Fig. 8.8). Inversely,

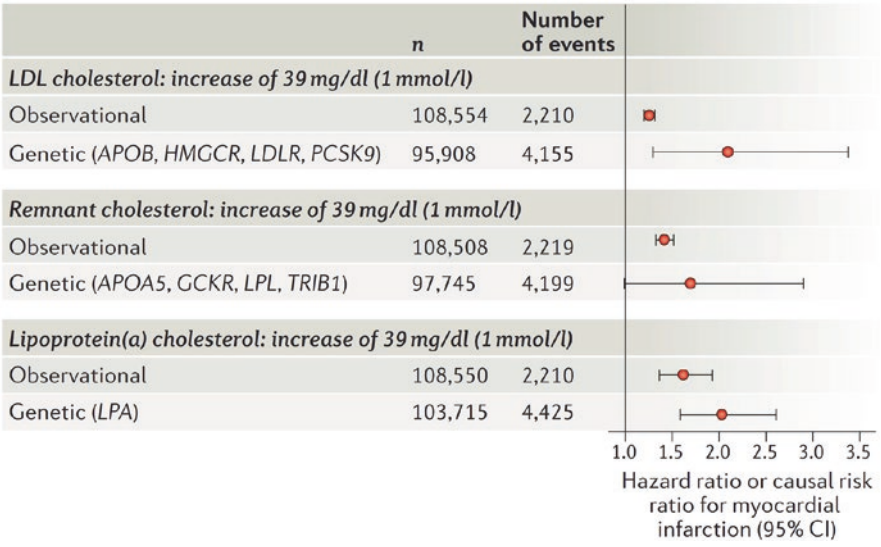


Fig. 8.7 Genetic and observational back-to-back comparison for risk of myocardial infarction for the same increase in cholesterol content of LDL, remnants, and lipoprotein(a). Observational analyses can be used to examine relative effect sizes for the three lipoprotein fractions. Genetic analyses show that all three lipoprotein fractions each are causally associated with increased risk of myocardial infarction. *APOA5* apolipoprotein A5, *APOB* apolipoprotein B, *CI* confidence interval, *HMGCR* 3-hydroxy-3-methylglutaryl-CoA reductase, *LDLR* low-density lipoprotein receptor, *LPA* lipoprotein(A), *PCSK9* proprotein convertase subtilisin/kexin type 9, *TRIB1* Tribbles pseudokinase 1. (Reproduced with permission from [51])

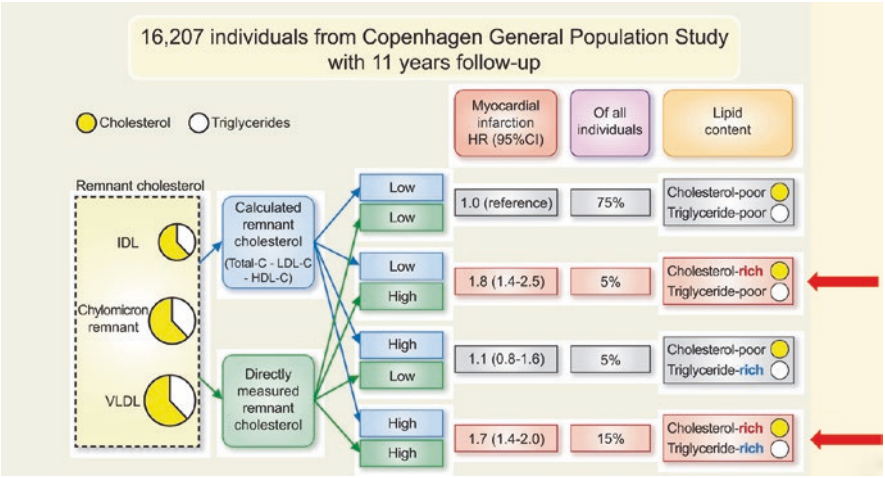


Fig. 8.8 Discordant and concordant remnant cholesterol calculated from a standard lipid profile or measured directly in relation to risk of myocardial infarction and content of cholesterol and triglycerides in remnant particles. High indicates concentration ≥80th percentile, and low indicates <80th percentile. *HR* hazard ratio, *C* cholesterol, *CI* confidence interval, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein, *VDLD* very low-density lipoprotein. (Reproduced with permission from [11])

individuals with low directly measured remnant cholesterol and high calculated remnant cholesterol were not. This finding is in line with the view that it is the cholesterol content of remnant lipoproteins that is atherogenic and not the triglycerides.

Epidemiologic evidence suggests that remnant cholesterol is a strong risk factor for atherosclerotic cardiovascular disease. In the Copenhagen General Population Study, remnant cholesterol ≥1.5 mmol/L (58 mg/dL), which is present in roughly 6% of individuals, compared to <0.5 mmol/L (19 mg/dL), is associated with a 4.2-fold increased risk of myocardial infarction, 1.8-fold increased risk of ischemic stroke, and 4.8-fold increased risk of peripheral artery disease [52]. These results were independently confirmed in the Copenhagen City Heart study (Fig. 8.9).

Lipoprotein Subclasses

Studies of individual lipoprotein subclasses can provide additional information about cardiovascular disease risk associated with high remnant cholesterol levels. This has been made possible by NMR spectroscopy for metabolomic profiling of lipoproteins, which is now feasible to use at large scale. Using NMR, 32% of total plasma cholesterol was found to be held in remnant lipoproteins (IDL, VLDL, and chylomicron remnants) in individuals from the Danish general population [23]; as

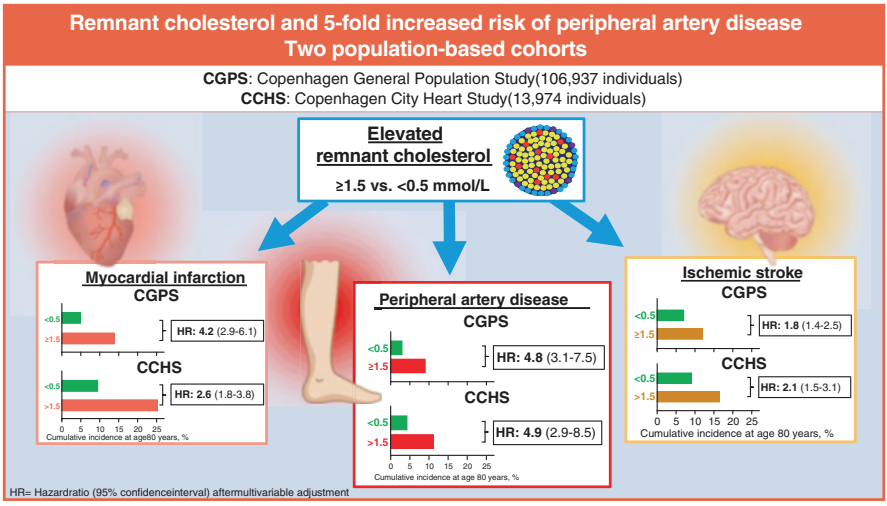


Fig. 8.9 Comparison of risk of peripheral artery disease, myocardial infarction, and ischemic stroke as a function of elevated remnant cholesterol in two studies of the Danish general population (Copenhagen City Heart Study and Copenhagen General Population Study). (Reproduced with permission from [52])

IDL cholesterol often is included in LDL cholesterol, the cholesterol content of VLDL and chylomicron remnants combined will be less than the 32% reported. Importantly, the percentage of cholesterol held in remnant lipoproteins is even higher in overweight and obese individuals, as observed in another study [53]. Here, it was observed that VLDL cholesterol, but not LDL cholesterol, was higher in overweight and obese individuals compared to individuals of normal weight. Interestingly, in that study VLDL cholesterol was estimated to mediate 40% of the increased risk of myocardial infarction associated with obesity.

NMR measurements of lipoprotein subclasses can be combined with genetics in a Mendelian randomization design for causal inference. A large study of this kind prioritized lipoprotein subclasses and found that extra small VLDL was the most important causal subclass for peripheral artery disease, while large LDL was most important for coronary artery disease [54]. Findings from other studies [52, 55, 56] also suggest that different lipoproteins might have varying importance for different atherosclerotic cardiovascular diseases; indeed, remnant lipoproteins could have an especially strong effect on the development of peripheral artery disease.

Evidence in Diabetes Mellitus

Remnant cholesterol could be of special importance in individuals with diabetes mellitus due to their high absolute risk of cardiovascular disease [57] and elevated remnant cholesterol levels (Fig. 8.4). Elevated remnant cholesterol therefore likely

explains part of residual cardiovascular disease risk in individuals with diabetes mellitus [58].

Naturally, studies specifically of individuals with diabetes mellitus are smaller than studies of the general population. Nonetheless, a few studies have been done. In over 2000 patients with diabetes mellitus and diabetic nephropathy, elevated remnant cholesterol was clearly associated with increased cardiovascular mortality [59]. An association with increased risk of major adverse cardiovascular events were also found in individuals with diabetes mellitus and prediabetes in a secondary prevention cohort [60]. The associations with cardiovascular disease risk mirror those observed in the general population. As such, there are no clinical or biological indications that the atherogenic potential of remnant cholesterol would be different in individuals with diabetes mellitus, compared to in individuals in the general population [16].

Clinical Interventions

Levels of remnant cholesterol can be lowered by many lifestyle changes which are known to decrease the risk of atherosclerotic cardiovascular disease [61], such as smoking cessation [62], exercise [63], diets high in vegetables and polyunsaturated fatty acids, and low in carbohydrate (e.g., the Mediterranean diet) [64, 65], and weight loss [66]. It is likely that lower remnant cholesterol explains part of the atheroprotective effect of these interventions, probably in combination with other mechanisms (e.g., decrease in blood pressure, LDL cholesterol, and oxidative stress).

Statins, which are used for their LDL cholesterol-lowering effect, also lower remnant cholesterol. Depending on the type of statin, the effect on remnant cholesterol varies from −3% to −31%, where the widely used atorvastatin and rosuvastatin are among the statins with the strongest triglyceride and thus remnant cholesterol-lowering effects [67–69]. It is therefore likely that the atheroprotective effect of at least some statins is partly mediated by decrease in remnant cholesterol. Meta-analyses of randomized controlled trials have assessed the association between amount of triglyceride lowering and decrease in risk of cardiovascular events for statins [70]. However, conclusions about reductions from high remnant cholesterol levels are difficult to draw, as most of the trials excluded individuals with elevated triglycerides.

Fibrates, also called peroxisome proliferator-activated receptor (PPAR) activators, have remnant cholesterol-lowering effects. In individuals not selected for high plasma triglycerides and remnant cholesterol, they decrease the risk of major cardiovascular events by a modest 10% and the risk of coronary events by 13%, according to a meta-analysis of multiple clinical trials [71]. However, in post-hoc analyses of participants with elevated plasma triglycerides (and with low HDL cholesterol) at entry, in all fibrate trials, risk reduction of atherosclerotic cardiovascular events were much larger than mentioned above [16, 22]. Nevertheless, for now safety

concerns and limited data for fibrates as an add-on to statin therapy have somewhat limited their use for cardiovascular disease prevention [72]. Additionally, the recent PROMINENT trial, testing whether a new generation of fibrates, pemafibrate, could reduce atherosclerotic cardiovascular disease when given on top of statins in individuals with diabetes, elevated plasma triglycerides, and low HDL cholesterol was stopped due to futility [73]. However, the decrease in remnant cholesterol was counteracted in this trial was counteracted by an increase in LDL cholesterol similar in magnitude, which likely explains the lack of effect.

Clinical trials of omega-3 fatty acids have historically shown conflicting results on whether they can reduce the risk of cardiovascular disease, but now there is renewed interest. In 2018, a highly purified omega-3 fatty acid called icosapent ethyl was shown in REDUCE-IT to decrease risk of ischemic cardiovascular events by 25% relative to a mineral oil placebo [74]. However, omega-3 fatty acids have several potential "atheroprotective effects," and the decrease in plasma triglycerides and remnant cholesterol does not appear to be the full explanation for the results [75, 76], particularly as the largely similar STRENGTH trial had a similar triglyceride reduction (19% in REDUCE-IT and 20% in STRENGTH) without reduction in cardiovascular events [77].

Mechanisms

The simple biological fact that most cells can degrade triglycerides, while no cells can degrade cholesterol, would explain why it is the cholesterol content in remnant lipoproteins that mainly causes atherosclerosis and subsequently atherosclerotic cardiovascular disease [22].

Both cholesterol-containing LDLs and remnant lipoproteins (except chylomicrons and very large particles of VLDL, which are all too large) can penetrate the arterial wall and enter the arterial intima, where they may get trapped within the connective tissue matrix, accumulate, and exert atherogenic effects (Fig. 8.10) [78–80]. The entrance into the arterial intima across the endothelium is thought to be through a process representing nonspecific molecular sieving with increasing intimal influx with increasing plasma lipoprotein concentration, decreasing lipoprotein size, and with arterial injury [1, 78, 81].

Due to their larger size and possibly through attachment to extracellular components such as proteoglycans, remnant lipoproteins are believed to be trapped preferentially to LDL [79, 82, 83]. Within the intima, macrophages are able to directly take up remnant lipoproteins without modification as opposed to LDL, which require oxidative or other modifications [84]. This may lead to excessive cholesterol ester accumulation in cytoplasmic lipid droplets resulting in foam cell formation and eventually atherosclerotic plaques [85]. Furthermore, upon hydrolysis of triglycerides in remnant lipoproteins by lipoprotein lipase both within the arterial

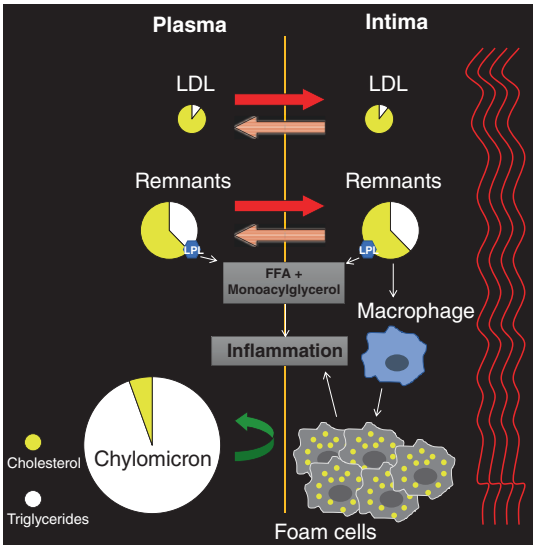


Fig. 8.10 Likely mechanism from elevated triglyceride-rich lipoproteins in plasma to development of atherosclerosis with an inflammatory component. *FFA* free fatty acids, *LDL* low-density lipoprotein. (Reproduced with permission from [22])

intima and at the vascular endothelium, tissue-toxic free fatty acids and other hydrolytic lipid derivatives are produced [22, 84]. These molecules cause local injury and inflammation, which may further facilitate plaque initiation, progression, and rupture [16, 86–88].

Translational Perspective

As previously described, individuals with diabetes mellitus are characterized by raised remnant cholesterol concentrations compared to individuals without diabetes mellitus (Fig. 8.4). Due to their larger size, remnant particles contain at least twice the amount of cholesterol per particle compared to LDL particles [89]. The higher cholesterol content, preferential intimal trapping of remnant particles, and rapid direct uptake by macrophages make it plausible that remnant particles are more atherogenic on a per particle basis than LDL particles. In fact, the association with risk of myocardial infarction may be more than twice as strong for VLDL particles (which constitute the majority of remnant particles) compared to LDL particles in individuals in the Danish general population [90]. Therefore, people with a higher proportion of remnant lipoproteins relative to LDL, such as many individuals with diabetes mellitus, potentially have a higher risk of cardiovascular disease compared to others with similar apoB concentrations.

Acute Pancreatitis

In individuals with diabetes mellitus, insulin resistance leads to liberation of free fatty acids from peripheral tissue, especially adipose tissue, which in turn results in increased synthesis and secretion of VLDLs from the liver, and impaired plasma clearance of VLDLs and chylomicrons. All these effects may result in elevated plasma triglycerides [15]. Hypertriglyceridemia is responsible for approximately 10% of acute pancreatitis cases overall and possibly more in individuals with diabetes mellitus [91, 92].

The exact mechanisms by which raised triglycerides may lead to acute pancreatitis are not fully elucidated, and different mechanisms have been proposed. If exposed through chylomicron or VLDL penetration into pancreatic tissue similarly to development of eruptive xanthomas (Fig. 8.2), it is believed that triglycerides are hydrolyzed by pancreatic lipase within the pancreas. This process liberates and accumulates tissue-toxic free fatty acids within the pancreatic tissue causing damage to acinar cells and the vascular bed resulting in ischemia and inflammation [93, 94]. Hyperviscosity due to high concentrations of chylomicrons enriched with triglycerides may also lead to ischemia and inflammation within the pancreatic tissue and ultimately to acute pancreatitis [93, 95].

Interestingly, some epidemiological studies show that the risk of acute pancreatitis increases stepwise with increasing concentrations of plasma triglycerides; an increased risk that is observed from concentrations as low as >1 mmol/L (>88 mg/dL), indicating that triglycerides may play a more important role as a risk factor for acute pancreatitis than previously thought [9, 96–101]. In a clinical setting this implies that the risk of acute pancreatitis increases already at mild-to-moderate hypertriglyceridemia (2–10 mmol/L [177–886 mg/dL]). However, it is important to note that in mild-to-moderate hypertriglyceridemia, the lipid pool is mainly dominated by elevated triglyceride-rich lipoproteins and their remnants, which are likely to also cause atherosclerotic cardiovascular disease. Inversely, in severe hypertriglyceridemia (>11.4 mmol/L [>1000 mg/dL]), the lipid pool is mainly dominated by chylomicrons and large VLDLs, which are more likely to cause acute pancreatitis. Atherosclerotic cardiovascular disease is much more prevalent than acute pancreatitis. This means that despite the increased risk of acute pancreatitis in mild-to-moderate hypertriglyceridemia, the absolute risk of atherosclerotic cardiovascular disease is many times higher and therefore a more pressing issue.

To prevent acute pancreatitis, American and European guidelines on the management of dyslipidemia recommend triglyceride-lowering interventions at concentrations above 5.7 mmol/L (500 mg/dL) and 10 mmol/L (880 mg/dL), respectively [92, 102]. Triglyceride-lowering interventions include dietary recommendations with restrictions of calories, fat content (≤15% of calorie intake), and refined carbohydrates [92, 102]. Furthermore, individuals are encouraged to increase physical activity, lose weight, and abstain from or at least reduce alcohol intake [92, 102].

In individuals with poorly controlled diabetes mellitus and markedly raised plasma triglycerides, triglyceride levels can be lowered efficiently by obtainment of glycemic control. For individuals with persistently elevated or increasing triglycerides,

triglyceride-lowering drugs may be indicated (e.g., high-intensity statins, fibrates, and high-dose omega-3 fatty acids) to prevent acute pancreatitis. In severe cases, lipoprotein apheresis and use of volanesorsen may also be considered [92, 103].

Guidelines

The most effective available therapies for lowering of remnant cholesterol (or triglycerides, a common proxy marker for remnant cholesterol in clinical guidelines) are dietary modifications and weight loss, which can lower levels by up to 70% [91]. These, in addition to physical exercise and smoking cessation, are recommended for all individuals with diabetes mellitus [104]. Fibrates are also recommended for people who have reached the LDL cholesterol goal, but have triglycerides above 2.3 mmol/L (200 mg/dL), according to European guidelines [105]. After a recent update of guidelines, icosapent ethyl, a highly purified omega-3 fatty acid which reduces remnant cholesterol levels in individuals with diabetes mellitus by 26% [106], should be considered in patients with either established atherosclerotic cardiovascular disease or additional risk factors [91, 104, 105].

As described previously, non-HDL cholesterol amounts to remnant cholesterol plus LDL cholesterol. Likewise, apoB concentration reflects the concentration of LDL plus remnant lipoproteins. This means that remnant cholesterol is implicitly included in guidelines also wherever non-HDL cholesterol and apoB are mentioned. The dyslipidemia in type 2 diabetes and insulin resistance is characterized by elevated remnant cholesterol, while LDL cholesterol levels could be normal. Non-HDL cholesterol, or apoB, is therefore recommended as treatment target especially for patients with diabetes mellitus or elevated plasma triglycerides [92, 107].

For secondary prevention, including patients with diabetes mellitus, the non-HDL cholesterol goal is <2.4 mmol/L (93 mg/dL) in Canada, <2.2 mmol/L (85 mg/dL) in Europe, and <2.6 mmol/L (100 mg/dL) in the United States (Table 8.1, top). ApoB goals are only specified in Canada and Europe, where they are <70 mg/dL and <65 mg/dL, respectively.

In Canada and Europe, there are also non-HDL and apoB goals for primary prevention of patients with diabetes mellitus at high cardiovascular risk. This means either older age (≥40 years in Canada, and ≥35 years for diabetes mellitus type 1 and ≥50 years for diabetes mellitus type 2 in Europe), long diabetes mellitus duration (≥15 years in Canada and ≥10 years in Europe), and/or at least one other risk factor. The goals for this group are the same in both Canada and Europe, at <2.6 mmol/L (100 mg/dL) of non-HDL cholesterol and <80 mg/dL of apoB. In the United States, there is only a specified goal for LDL cholesterol for this group, at <1.8 mmol/L (70 mg/dL).

The goals for patients with diabetes mellitus and young age, short diabetes mellitus duration, and without additional risk factors are for non-HDL cholesterol <3.2 mmol/L (124 mg/dL) in Canada and <3.4 mmol/L (131 mg/dL) in Europe (Table 8.1, bottom). There are no goals specified for this patient group in United

Table 8.1 Targets and goals for lipid lowering in adults with diabetes using statins or similar drugs according to cholesterol and dyslipidemia guidelines

Target	Canada 2021 CCS	Europe 2019 ESC/EAS	US 2018 ACC/AHA
Secondary prevention: patients with diabetes and atherosclerotic cardiovascular disease			
LDL cholesterol	<70 mg/dL <1.8 mmol/L	≥50% and <55 mg/dL <1.4 mmol/L	≥50% and <70 mg/dL <1.8 mmol/L
Non-HDL cholesterol	<93 mg/dL <2.4 mmol/L	<85 mg/dL <2.2 mmol/L	<100 mg/dL <2.6 mmol/L
Apolipoprotein B	<70 mg/dL	<65 mg/dL	
Primary prevention: Patients with diabetes + older age, diabetes duration ≥15/≥10 years or other risk factor			
LDL cholesterol	<77 mg/dL <2.0 mmol/L	≥50% and <70 mg/dL <1.8 mmol/L	≥30% or ≥50% and <70 mg/dL <1.8 mmol/L
Non-HDL cholesterol	<100 mg/dL <2.6 mmol/L	<100 mg/dL <2.6 mmol/L	
Apolipoprotein B	<80 mg/dL	<80 mg/dL	
Primary prevention: Patients with diabetes + young age and duration <15/<10 years and no other risk factors			
LDL cholesterol	<97 mg/dL <2.5 mmol/L	<100 mg/dL <2.6 mmol/L	<160 mg/dL <4.1 mmol/L
Non-HDL cholesterol	<124 mg/dL <3.2 mmol/L	<131 mg/dL <3.4 mmol/L	
Apolipoprotein B	<85 mg/dL	<100 mg/dL	

Data taken from [102, 105, 107]
ACC/AHA American College of Cardiology/American Heart Association, *CCS* Canadian Cardiovascular Society, *ESC/EAS* European Society of Cardiology/European Atherosclerosis Society, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

States guidelines for any of the lipid measurements, why the primary prevention LDL cholesterol goal for "selected patients" is implied, at <4.1 mmol/L (160 mg/dL).

Statins, ezetimibe, and PCSK-9 inhibitors also lower remnant cholesterol, although to a lesser degree than LDL cholesterol. In order to reach non-HDL or apoB goals, these drugs can therefore be used [92].

Future Perspectives

There is a global growth in the prevalence of diabetes mellitus [108]. It is well known that most of the morbidity and mortality in diabetes mellitus are due to lifestyle risk factors, such as high body mass index, a diet high in processed foods, low physical activity, and smoking. Still, the prevalence of these risk factors continues to rise globally, which also drives the increasing incidence of diabetes mellitus [109]. Strikingly, global childhood obesity prevalence has increased more than

seven-fold since 1975 and is now at over 10% in most high-income countries, while many middle-income countries also see high levels [110]. This is a worrying sign that diabetes mellitus incidence will only continue to increase. Much more must be done on a societal level to encourage lower caloric intake, more physical activity, and a healthier diet through childhood and up. However, given the current trends, prevalence of diabetes mellitus will continue to increase, and cardiovascular disease prevention in individuals with diabetes mellitus will become ever more important for clinicians worldwide.

The failure of the PROMINENT trial to lower remnant cholesterol without simultaneusly increasing LDL cholesterol underscores the importance of remnant cholesterol-lowering drugs without this adverse effect [73]. In this context, several gene therapeutics with effects on triglyceride-rich lipoproteins are in different stages of development, and some have been approved for clinical use. One of the gene therapeutics is volanesorsen, a new antisense oligonucleotide which inhibits apolipoprotein C-III synthesis. It decreases triglycerides by 77% and non-HDL cholesterol by 46% in patients with the familial chylomicronemia syndrome [111]. Volanesorsen is currently approved by the European EMA to decrease the risk of acute pancreatitis in these patients, but is also being investigated for use in other forms of hypertriglyceridemia, including in patients with diabetes mellitus [112, 113]. The US FDA has rejected approval of volanesorsen because of related incidents of thrombocytopenia, however, a newer antisense oligonucleotide inhibiting apolipoprotein C-III, olezarsen, is currently being tested with similar effects on triglycerides (reduction of 60%) and remnant cholesterol (reduction of 58%) [114]. Vupanorsen is another antisense oligonucleotide which inhibits angiopoietin-like 3 (ANGPTL3). In a phase II trial including patients with type 2 diabetes, hepatic steatosis, and triglycerides >1.6 mmol/L (150 mg/dL), remnant cholesterol was lowered by 38% and non-HDL cholesterol by 18% at the highest dose [115]. Evinacumab is a monoclonal antibody targeting ANGPTL3, which lowers both LDL cholesterol and triglycerides by 50% at the highest dose [116, 117]. It is currently approved only for use in homozygous familial hypercholesterolemia, but the potent effect and so far, few reported adverse events show promise for an expansion of the indications for evinacumab or other ANGPTL3 reducing drugs.

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Chapter 9
HDL Function in Diabetes

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder which is characterized by disturbed insulin secretion and diminished tissue sensitivity to insulin [1]. These disturbances result in impaired cellular glucose transport and hyperglycaemia. The presence of hyperglycaemia is associated with an enhanced inflammatory response and mitochondrial oxidative stress, leading to vascular endothelial dysfunction [2, 3]. Hyperglycaemia is a metabolic feature characteristic for diabetes which over time can cause major health complications [1]. The prevalence of diabetes mellitus is constantly growing worldwide due to the high prevalence of obesity, unhealthy eating habits, and lack of physical activity. In 2017, globally there were 22.9 million new cases and 1.37 million diabetes-related deaths [4]. According to the most recent 9th edition of the International Diabetes Atlas, 463 million adults are suffering from DM and over 4 million people aged between 20 and 79 years died from complications related to DM in 2019 [5]. Furthermore, by 2030 more than 4 million people (20–79 years) will have been affected by DM, while by 2045, it is estimated that there will be 700 million diabetic patients in the world. Based on estimations, at least 68% of people above the age of 65 years who suffer from diabetes die from some type of heart disease, while 16% die of stroke [6]. The increased cardiovascular risk in type 2 diabetic patients can be partly explained by lower levels of HDL and the occurrence of compositional and functional alterations in the various

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molecules that comprise it [7]. In a population of people aged over 60 years, the U-shaped association was found between HDL-C and glycosylated haemoglobin, while in those below the age of 40, an inverted U-shaped distribution between HDL-C and glycosylated haemoglobin is observed.

There are different approaches to diabetes management, but their main goal is to achieve appropriate glycaemic control in order to forestall the development of diabetes-related complications, including micro- (proliferative retinopathy, diabetic neuropathy) and macrovascular disorders (atherosclerotic cardiovascular disease), cardiovascular diseases (heart failure, restrictive cardiomyopathy, diastolic dysfunction), renal insufficiency and failure, cerebrovascular diseases, dementia, and some malignant neoplasms and peripheral neuropathy [8, 9]. This chapter focuses on the impact of diabetes on compositional alterations of HDL, its function and clinical relevance.

Type I Diabetes Mellitus and Its Impact on Lipoproteins Levels

Type 1 diabetes (T1DM) is a chronic autoimmune metabolic disease. It is characterized by an absolute insulin deficiency caused by T-cell-mediated autoimmune damage of the pancreatic beta cells which results in the impairment of glucose metabolism [10–12]. T1DM is diagnosed in 7–12% of the diabetic population [13]. This form of diabetes is most prevalent during childhood and adolescence; however, it can also occur in adulthood [14]. The insufficient supply of insulin in patients with this type of diabetes is associated with increased glucose concentrations or hyperglycaemia and subsequent complications that considerably worsen quality of life and decrease the lifespan [13]. Hyperglycaemia in patients with T1DM triggers enhanced oxidative stress and a low-grade inflammatory response [15]. According to estimates, a 1% increase in HbA1c translates into a 37% higher risk of advanced diabetes microvascular complications, including diabetic neuropathy and retinopathy [16]. In addition to the risks posed by elevated HbA1c, the presence of serum glycated albumin formed as a product of early and advanced glycation further augments the risk of macrovascular complications [17]. Patients with T1DM face an increased risk of premature cardiovascular disease (CVD), which is a frequent cause of mortality [14].

Better insulin replacement therapies and careful glycaemic control have reduced the occurrence of microvascular complications in T1DM patients, but the impact of the latter on cardiovascular disease risk and its complications is far less pronounced [18]. In patients with T1DM, considerable risk of CVD events exists despite normal or even increased HDL levels. This paradox may be associated with the qualitative changes in HDL (glycation and/or oxidation) which alter its functional properties in T1DM patients. Increased levels of HDL-C in T1D have been suggested to be due to higher lipoprotein lipase (LPL) activity related to peripheral hyperinsulinemia resulting from systemic insulin administration, which promotes the hydrolysis of

triglyceride-rich lipoproteins thus enriching HDL with cholesterol [7]. Apart from changes in HDL levels, patients with a long history of T1DM frequently also manifest with macroalbuminuria, higher plasma TG, and a shifted balance between very low-density lipoprotein (VLDL) and HDL [19]. Hypertriglyceridemia occurring in diabetic dyslipidaemia may be associated with both augmented production and diminished clearance of triglycerides [20]. In T1DM patients, the presence of hypertriglyceridemia is primarily associated with impaired VLDL removal as a result of the inability of the liver to respond to increased free fatty acid (FFA) under absolute insulin deficiency. Appropriate control of glycaemia mirrored by circulating glucose levels close to the physiological range was suggested to have a positive impact on lipid metabolism in this group of patients [10, 21]. Some studies indicate that lipid profiles of patients with type 1 diabetes mellitus and good glycaemic control with insulin are comparable with the general population, however, poorly controlled diabetes is associated with hypertriglyceridemia, lower HDL, and increased low-density lipoprotein cholesterol (LDL) [22, 23]. Improved glycaemic control was found to correlate not only with ameliorated lipid profile but also with improved survival of T1DM patients [24, 25]. However, in some T1DM patients with dyslipidaemia, proper glycaemic control may be insufficient to normalize lipids [26]. Finally, Ahmed et al. [27] observed sex-specific differences in HDL biology in type 1 diabetes. Maintained levels of HDL as well as greater ABCA1-independent efflux were demonstrated in men, but not in female diabetic patients compared to non-diabetic individuals.

Type II Diabetes Mellitus and Its Impact on Lipoproteins Levels

Characteristic features of type 2 diabetes mellitus (T2DM) involve an increase in blood glucose due to the progressive worsening of insulin resistance and incapacity of pancreatic to meet the increasing demand for insulin production as well as increased fractional secretion rate (FSR) of de novo synthesized insulin compared to non-diabetic family members [1, 28, 29]. The impairment of pancreatic beta cells is the critical element intricated in the pathogenesis of T2DM. The dysfunction of pancreatic beta cells is associated with glucotoxicity and high levels of free fatty acids as well as a heightened inflammatory response. Moreover, the presence of hyperglycaemia enhances the oxidation of lipoproteins, thus stimulating the development of atherosclerosis. Both T2DM and insulin resistance are well-known factors significantly enhancing cardiovascular (CV) risk. Indeed, in patients with T2DM, an increase in mortality from coronary artery disease by 2–4% is observed [30].

Dyslipidaemia is found in 60–70% of patients with T2DM [31]. Dyslipidaemia in the course of T2DM is characterized by a highly pro-atherogenic lipoprotein profile involving decreased concentrations of HDL cholesterol (HDL-C), the prevalence of small dense HDL particles, increased levels of plasma triglycerides (TG)

(fasting and post-prandial), rise in apolipoprotein B (ApoB)-containing lipoproteins, particularly small and dense low-density lipoproteins (LDLs), and large very-low-density lipoproteins (VLDLs) [22, 32]. Total cholesterol (TC) and LDL may be normal or slightly raised [33]. Decreased levels of HDL have been suggested to be due to the elevation in plasma TGs in the presence of insulin resistance, which stimulates increased activity in cholesteryl ester transfer protein (CETP)-mediated lipid exchange resulting in the formation of HDL particles largely depleted of cholesteryl esters (CE). Following the replacement of CE in high-density lipoprotein (HDL) with TG from VLDL1, TG-rich HDL is generated; however, due to the fact that it is thermodynamically unstable, it undergoes accelerated catabolism in the kidneys which leads to low serum levels of HDL_2 [34, 35]. Apart from the reduction in their concentration, HDL particles also become abnormally enriched in TGs as well as deprived of ApoA-I [1, 36]. A case-control study demonstrated lower levels of larger HDL particles in the T2DM group in comparison with non-diabetic individuals (28.20% versus 30.40%; $P = 0.016$) [37]. However, a higher adiponectin level in this group of patients was associated with a lesser negative impact of T2DM on HDL functionality through the increase in ApoA-I, particle size, and cholesterol content, thus increasing antioxidant capacity.

Insulin resistance (IR) is associated with dysregulated, constitutive hormone-sensitive lipase (HSL)-mediated lipolysis, which releases free fatty acids (FFA) from visceral adipose tissue, thus raising hepatic triglyceride (TG) production [38]. Increased levels of hepatic TG are associated with diminished degradation of apolipoprotein (Apo) B100 and facilitates the generation of VLDL [32]. In IR, VLDL1 rich in TG, ApoC-III and ApoE are preferentially produced compared to TG-poor VLDL2 [22]. Cholesteryl ester transfer protein (CETP) replaces cholesteryl ester (CE) in low-density lipoprotein cholesterol (LDL) with TG from VLDL1 which results in the formation of TG-rich LDL undergoing preferential hydrolysis to form small dense LDL (sdLDL) [39]. Prolonged circulation of sdLDL enables the development of atherogenic modifications of sdLDL particles in the plasma [40].

According to some studies, the presence of low HDL-C levels and high TG before the onset of diabetes may promote the development of diabetes and its complications as a result of decreased protection of pancreatic beta cells and endothelial cells [41, 42]. However, the causal association between HDL cholesterol and the onset of diabetes remains inconclusive. The results of epidemiological studies have indicated an inverse correlation between low plasma levels of HDL-C and the risk of T2DM development [43, 44]. Moreover, low HDL appears to be an independent risk factor of diabetes complications, including diabetic nephropathy, the amputation of lower extremities, and wound-related mortality [45, 46]. Some genetic studies imply that genetic predisposition to low HDL may predict an increased risk of T2DM, while others fail to find such a relationship [44, 47]. It has been also suggested that high levels of TG accompanied with decreased HDL may play a role in the promotion of T2DM onset due to the fact that the presence of increased TG is associated with higher FFA levels, the disruption of the cascade linking insulin receptors with glucose transporters, as well as subclinical inflammation. This chain of events results in IR and beta (β)-cell dysfunction [48].

HDL Particle Structure and Function

HDLs are a very heterogeneous class of lipoproteins with densities ranging from 1.063 to 1.210 g/mL. According to most classifications, human plasma contains large, less dense (1.063–1.125 g/mL), lipid-enriched HDL_2 and small, dense (1.125–1.210 g/mL), protein-enriched HDL_3 [49]. In an HDL particle's structure, triglycerides (TGs) and cholesterol esters (CEs) form a hydrophobic central core, which is surrounded by an envelope made up of amphipathic molecules, including free cholesterol (FC), phospholipids (PLs), and apolipoproteins (Apos) [50, 51]. The HDL molecule carries hundreds of different compounds, some of which are biologically active, while others are passive cargo constituents (cholesterol) [52, 53]. HDL comprises various components, including apolipoproteins (apolipoprotein ApoA-1, ApoA-2, ApoC-3, Apo-IV, ApoC-I, ApoC-II, ApoC-III, ApoC-IV, ApoD, ApoE, ApoF, ApoH, ApoJ, ApoL-I, ApoM), enzymes (e.g. cholesterol ester transfer protein (CETP), lecithin–cholesterol acyltransferase (LCAT) and phospholipid transfer protein (PLTP) participating in HDL maturation, paraoxonase-1 (PON) and platelet-activating factor acetyl hydrolase (PAF-AH)), as well as lipids [49, 54, 55]. HDL particle population may contain over 85 different proteins which can stimulate haemostasis (α-2-HS-glycoprotein), proteolysis (α-1-antitrypsin), inflammation (haptoglobin-related protein), complement activation (i.e. complement C3), and immunity (i.e. the acute-phase reactant serum amyloid A-4 protein precursor [SAA4]) [56]. ApoA-I is a crucial protein component of HDL that forms pre-β HDL following its binding to ATP-binding cassette transporter A1 (ABCA1), which enables the transfer of free cholesterol and phospholipids to ApoA-I leading to the formation of nascent-discoidal HDL. The maturation of HDL also involves the binding of HDL particles to ABCG1 resulting in the transformation of smaller particles (HDL_3) into larger HDL_2 particles. The process of maturation requires the action of lecithin-cholesterol acyltransferase (LCAT) (located on the surface of HDL particles) which transforms free cholesterol into CE enabling its translocation to the core of the particle [57].

The structural complexity of HDL allows for HDL's functional diversity; thus, this lipoprotein is involved in numerous biological activities. HDL promotes cellular cholesterol efflux and interacts with numerous cell surface receptors [53, 58]. A central role of HDL is associated with its involvement in reverse cholesterol transport (RCT) which is believed to exert atheroprotective effects [49]. The role of HDL in RCT includes the stimulation of cholesterol efflux from peripheral cells (also lipid-laden foam cells within atherosclerotic lesions) and returning this acquired lipid to the liver [59]. This transfer of cholesterol occurs following HDL binding to scavenger receptor class BI (SR-BI) expressed in hepatocytes or via the translocation to low-density lipoprotein (LDL)/VLDL particles mediated by cholesteryl ester transfer protein (CETP). Moreover, functional HDL prevents ox-LDL-mediated endothelial dysfunction from developing, thereby supporting endothelial integrity and function, limits endothelial adhesion and leukocyte and platelet activation, inhibits transmigration of leukocytes via endothelium and macrophage activation,

hampers lipid oxidation, inactivates oxidized lipids, limits inflammation via the reduction of myelopoiesis and immune activation at the site of atherosclerosis [60–65]. The principal antioxidant, anti-inflammatory, antiapoptotic, and antithrombotic properties of HDL are responsible for its potent anti-atherogenic effects [49]. The antithrombotic activity of HDLs is associated with its impact on the prostacyclin signalling pathway, due to the fact that prostacyclins interact with nitric oxide (NO) to prevent platelet activation and aggregation [49]. HDL has been also demonstrated to exert protective functions not only on the normal function and survival of pancreatic beta cells, but also on the sensitivity of target cells to insulin [63, 66, 67].

Many epidemiological studies have indicated that high plasma HDL-C levels are associated with a decreased risk of atherosclerosis, however, new evidence has emerged that (1) this may not be true for diabetic patients and (2) increased levels of modified lipoproteins may hasten the development of CV complications in diabetes [10]. The failure in clinical trials of the therapies targeted at increasing the concentration of HDL-C, focus attention to the quality and functionality, not the level of HDL as the more important factor. It has been suggested that hyperglycaemia and oxidative stress can cause irreversible post-translational modifications affecting HDL composition and function. In an inflammatory environment, HDL becomes transformed into “acute phase HDL” enriched in free fatty acids, triglycerides, serum amyloid A (SAA); it is also depleted of such anti-inflammatory enzymes as PON1 [68–70]. Moreover, under such conditions, the secretion of myeloperoxidase (MPO) is enhanced which is associated with oxidative modifications of apolipoprotein A-I and consequent impairment of its ability to accept cholesterol [71]. Hyperglycaemia induces the formation of advanced glycation end products, triggering of protein kinase C isoforms, and amplifying flux through the hexosamine pathway. All these factors may contribute to enhanced oxidative stress [72]. Alterations in HDL structure and size, which result in changes in cellular cholesterol homeostasis and signalling, are associated with the proliferation, survival, and the functioning of many cells, such as endothelial cells, adipocytes, macrophages, myocytes, and pancreatic β cells [58]. The aforementioned enhancement of oxidative stress in diabetes exerts an impact on both the vascular wall and lipoproteins in the circulation; thus, it may promote atherogenesis. Impaired HDL-C function is associated with the activation of local inflammation and endothelial thrombosis, enhanced endothelial cell apoptosis, and hampered vascular repair processes [73]. The presence of inflammation and low HDL-C facilitates the development of immune system disorders and the progression of atherosclerosis [74].

The Impact of Type 1 Diabetes on HDL Particle Composition and Function

The physiological heterogeneity of HDL is even higher in patients with some disease states, including diabetes or CVD as a result of the loss/acquisition of some components and/or the modification of other HDL constituents [58]. Numerous

studies have indicated that in diabetic individuals, normal (within the normal range) or elevated levels of high-density lipoproteins (HDLs) are not equivalent to appropriate functionality [1, 75]. Post-translational modification of HDL, especially the oxidative modification of HDL particles and ApoA-I, appears to be the main reason for the appearance of its dysfunctional form [68, 76, 77]. Compositional modifications occurring within HDL in T1DM patients have been found to affect its functionality [78, 79]. Such alterations are reported even in those T1DM individuals whose lipid profiles are normal [80]. The results of studies confirm the damage of important constituents of HDL in T1DM as a result of enhanced oxidative stress during insulin deprivation [13].

The following changes in HDL composition are observed in type 1 diabetic patients: (1) increased particle size, (2) lower content of cholesterol esters in small HDL_3 particles, (3) diminished HDL triacylglycerol content, (4) elevated levels of phospholipid transfer protein (PLTP), (5) CETP and lecithin-cholesterol acyltransferase (LCAT), (6) damage of ApoA-I protein, and (7) altered PL and sphingolipid profiles [10]. Reduced cholesteryl ester (CE) content in small HDL_3 is associated with diminished TG cargo in HDLs isolated from T1DM, which make these particles more vulnerable to the action of CETP [81]. All these abnormalities have been suggested to result from glycoxidative stress, relative peripheral hyperinsulinism, and the absence of physiological regulation of insulinemia in T1D [81]. Moreover, Lassenius et al. [82] observed enhanced activity of some HDL-associated proteins (PLTP and CETP) as well as reduced activity of others (e.g. PON1). The changes in the activity of PLTP and CETP may suggest alterations of anti-inflammatory, antioxidant, and RCT HDL-mediated functions in T1DM individuals. The ability of HDL to remove cholesterol from peripheral cells by the RCT pathway, which involves cholesterol efflux from cells to extracellular acceptors, appears to be among its most important atheroprotective functions [83].

In T1DM patients, the greater size of HDL particles was demonstrated to independently correlate with higher total CEC compared to closely matched participants without diabetes, even after the adjustment for elevated HDL cholesterol and HDL particle size. According to the authors, both ABCA1-dependent and ABCA1-independent CEC pathways were enhanced in type 1 diabetes [10]. Also, Ahmed et al. [27] found that diminished levels of small- and medium-sized HDL particles in type 1 diabetes did not affect ABCA1-dependent CEC which was found to be higher compared to non-diabetic participants. Usually, ABCA1-dependent efflux is associated with lipid-poor pre-β-1 HDL; this is in disagreement with the results obtained by Ahmed et al. [27] which may suggest that larger HDL particles in type 1 diabetes might enable greater storage of non-esterified cholesterol resulting in the change in the relationship between ABCA1-dependent efflux and particle size. Despite the enhancement of CEC in type 1 diabetes, the observed shift towards larger HDL particles may decrease access to interstitial fluid, thus diminishing this important route of RCT [27]. Furthermore, it has been suggested that in women, marked lowering of small HDL particles could result in loss of cholesterol flux via the lymphatic RCT pathway.

The results of some other studies analysing HDL fractions revealed higher content of acute-phase inflammatory protein serum amyloid A (SAA) in HDL_2 and HDL_3 in type 1 diabetes which translated into decreased CEC (which is in contrast with the aforementioned studies) and impaired anti-inflammatory properties of the HDL particles [84–86]. The enrichment of HDL with serum amyloid A (SAA) which is an acute-phase protein was demonstrated to promote the transformation of this lipoprotein into its dysfunctional form [70]. Such HDL particles lack atheroprotective effects due to decreased capacity to efflux cholesterol, but also it undergoes rapid catabolism which results in unfavourable changes in lipid metabolism [87]. Also, the formation of glycated albumin in patients with type 1 diabetes and poor glycaemic control was shown to diminish the capacity of HDL_2 and ApoA-I to remove cell cholesterol in RCT via reducing ABCA1 expression leading to intracellular lipid accumulation [17]. Also, Manjunatha et al. [13] reported diminished HDL CEC in subjects with type 1 diabetes compared to matched non-diabetic counterparts. This decrease in efflux capacity of HDL was here irrespective of glycaemic control. Moreover, they observed no difference in antioxidant activity of HDL between patients with good vs. poor glycaemic control [13]. The fact that both diabetic groups had considerably lower CEC and HDL antioxidant activity compared to non-diabetic subjects may imply a lack of impact of glycaemic control (HbA1c $\leq$6.6%) itself on HDL functions. Manjunatha et al. [13] also encountered a considerably higher abundance of modified ApoA-4 in T1DM with poorly controlled glycaemia compared to controls. Further analysis of specific modifications revealed a significantly higher abundance of oxidation and deamidation of ApoA-4 in the T1D-PC group. However, also in the group with properly controlled glycaemia, greater oxidation (a trend for higher deamidation) of ApoA-4 was observed compared to the matched non-diabetic group, which may indicate reduced ApoA-4 functionally contributing to the limited CEC reported in both T1D-PC and T1D-GC individuals as well as lower antioxidative activity of ApoA-4 [13]. Also, a greater abundance of oxidatively modified ApoE in patients with T1DM and poor glycaemic control may contribute to both impaired CEC and antioxidative functions of HDL in this group [13]. Manjunatha et al. [13] observed the overexpression of not only ApoM and ApoA-4, but also alpha-2-antiplasmin in patients with poorly controlled T1DM, as well as the decreased expression of SAA2 and alpha-2-HS-glycoprotein. In turn, the expression of ApoC-1, haptoglobin, and hemopexin in this group of patients was lower.

The results of other studies also demonstrated increased CETP activity in T1DM patients with higher plasma glucose levels as well as impaired function of the CETP activity inhibitor apoC-I [88, 89]. Since CETP is involved in HDL metabolism, its higher activity reduces concentrations of HDL-C, which results in an enhanced incidence of macrovascular complications in T1D patients [90]. Also, the activity of PON1 was found to be reduced in T1DM patients compared to non-diabetic subjects which may contribute to vascular dysfunction and late diabetic complications [78, 91]. High glucose levels in T1DM may induce the dissociation of PON1 from HDL leading to its diminished antioxidant capacity and enhanced PON1 lactonase activity [92]. In T1DM patients, especially obese ones, higher levels of

lipoprotein-phospholipase A2 (Lp-PLA2) have been reported. Lp-PLA2 (which is also carried by HDL) hydrolyses oxidized LDL (Ox-LDL) which results in the formation of two pro-inflammatory mediators: oxidized free fatty acids and lysophosphatidylcholine. It is considered an independent risk factor for CVD [93, 94]. Kinney et al. [95] found lower activity of Lp-PLA2 in T1DM compared to non-diabetic subjects, which independently correlated with the progression of coronary calcification. Another study revealed the relationship between the increased activity of Lp-PLA2 and the greater risk of CAD in T1D (HR: 1.54, CI: 1.11, 2.12) [94]. According to studies, in patients with T1DM, the activity of lipoprotein lipase is enhanced which results in the improved hydrolysis of triglyceride-rich HDL particles and consequent assembling of surface components from these particles into larger HDL particles [7, 96]. Sphingosine-1-phosphate (S1P) is another HDL component carried by ApoM, which appears to play a vital role in the anti-atherogenic and anti-inflammatory functions of HDL [10]. Its decreased levels were found in both HDL_2 and HDL_3 in T1D. Lower concentrations of S1P may result from the general reduction in HDL plasma levels [78]. However, another study found that the concentrations of ApoM and S1P are stable, and the reduction in the ApoM/HDL-C and S1P/HDL-C ratios in T1D mirrors selective enrichment of cholesterol in HDL [96]. Numerous studies have confirmed that the anti-inflammatory capabilities of HDL deficient in ApoM and S1P are decreased compared to HDL containing ApoM/S1P complex [97, 98]. According to Frej et al. [96], HDL-associated ApoM/S1P complex shifts from dense to light density HDL particles in patients with T1DM. S1P inhibits vascular cell adhesion protein 1 (VCAM-1) via binding to its S1P1 receptor thus protecting against endothelial dysfunction in T1D [97, 98]. Moreover, the aforementioned authors have suggested that despite acting via the same receptor, G proteins regulating downstream signalling pathways are differently activated depending on HDL subfraction [96]. According to studies, HDL from individuals with diabetes can stimulate the enhanced synthesis of adhesion molecules (e.g. intercellular adhesion molecule 1 (ICAM-1), VCAM-1), thus promoting monocyte infiltration and vascular inflammation and it fails to decrease cytokine production from macrophages and LDL-induced monocyte chemotaxis [99]. Moreover, glycated ApoA-1 was demonstrated to trigger NF-κB signalling pathway and subsequent cytokine synthesis through its interaction with the AGEs receptor or via the upregulation of SRB1-mediated ox-LDL uptake from macrophages [100]. Greater glycation triggers oxidative stress and enhances the formation of reactive oxygen species (ROS), which may aggravate the oxidative damage of HDL proteins [13]. Glycation of HDL has also been hypothesized to limit the anti-inflammatory properties of HDL. Indeed, many studies have indicated that HDL from T1DM patients has a low capability to protect against lipid oxidation [91]. Manjunatha et al. [13] found that HDL from type 1 diabetic patients had diminished efficacy against lipid peroxides. However, at the beginning of T1DM, the anti-inflammatory and immunoregulatory effects of HDL appear not to be altered since such HDL was shown to be able to control the activity of macrophages and immunoinflammatory response [10]. The co-culture of HDL with macrophages from recent-onset T1D patients induced the reduction in an individual and a combined ratio of pro-inflammatory/

anti-inflammatory cytokines compared to co-cultures of HDL with macrophages from healthy controls [101]. In the course of T1DM, poor glycaemic control enhances oxidative damage to apolipoprotein A-I, and advanced glycated albumin impairs the anti-inflammatory properties of HDL [102, 103]. However, according to Kjerulf et al. [104] HDL obtained from subjects with T1DM, and poor glycaemic control possessed normal, unaltered anti-inflammatory capacity in adipocytes. The discrepancies between in vitro and in vivo studies of HDL from T1DM and glycated HDL may suggest that artificial in vitro alterations of systems may not always reflect changes observed in vivo [105].

The Impact of Type 2 Diabetes on HDL Particle Composition and Function

Chronic hyperglycaemia in diabetic patients promotes the formation of reactive aldehydes, and subsequently AGEs and/or ALEs, whose detection has been found to correlate with diabetes-related complications [103, 106]. In the course of T2DM, both the HDL composition and functions become altered resulting in the formation of dysfunctional lipoprotein. It has been suggested that the alterations in HDL size may occur before the onset of diabetes since small HDL particles were found to positively correlate with future T2DM risk, while the presence of large HDL particles was associated with reduced risk [25]. It appears that the phenotype of HDL in this group of patients results from a diminished number of circulating particles and altered composition of its molecule [1]. The studies of HDL composition with the use of ultra-centrifugation demonstrated alterations in HDL size, including the reduction in large and very large HDL_2 accompanied by the increase in the amount of small HDL_3 which are rich in TGs and poor in cholesterol [1, 107]. In turn, the analysis of HDL with two-dimensional gel electrophoresis revealed diminished levels of large α-1, α-2, and pre-α-1 particles as well as increased amounts of lipid-poor α-3 HDLs in diabetic patients [108]. Finally, the results of nuclear magnetic resonance (NMR) spectroscopy showed decreased levels of large (11.5–18.9 nm) and medium (9.0–11.5 nm) HDL particles and elevated levels of small (7.8–9.0 nm) in patients with diabetes mellitus compared with controls [109]. It becomes apparent that in the course of diabetes, levels of large and very large HDL_2 are reduced, and a shift towards small HDL_3 is observed. The accelerated formation of small HDL particles and the decreased HDL-C levels in type 2 diabetic patients was suggested to be associated with either a higher catabolic rate or impaired maturation and decreased biogenesis [110]. The core of the HDL particle in T2DM becomes enriched in triglycerides instead of cholesteryl ester (CE) which is associated with enhanced renal elimination [35, 111]. The analysis of lipid content revealed the rise of 77% in TGs and diacylglycerol (DG) and an 8% reduction of CEs in diabetic compared to control particles [112]. Greater activity of CETP activity favours the formation of TG-enriched HDLs which are used as substrates by endothelial and hepatic lipases for the production of small, dense HDLs [113]. Apart from the

described mechanisms, also the impairment of LCAT activity resulting from high levels of glycated HDL (which are not appropriate substrates) can contribute to the shift in HDL size [114, 115]. The changes in particle architecture and fluidity are due to the decrease in surface lipids, including phosphatidylcholine, sphingomyelin, ether-linked phosphatidylcholine, ceramides, and free cholesterol, reported in 10–50% of individuals with T2DM and dyslipidaemia [112]. HDL particles from diabetic patients have reduced protein content. According to studies, 17 proteins were increased while 44 were decreased in diabetic patients compared to healthy subjects [1]. The amounts of serum amyloid A (SAA), apolipoprotein ApoC-II and ApoC-III, and fibrinogen are increased, while the levels of other apolipoproteins, including ApoA-I, ApoA-II, ApoE, ApoM, and paraoxonase-1 (PON-1) are markedly diminished [1]. The reduction in ApoA-I level has been suggested to be associated with its lower affinity towards the small HDL particles (typical of T2DM) which results in the dissociation of ApoA-I and the subsequently enhanced clearance by the kidneys [116]. Also, the expression of ApoA-I may be hampered in the presence of insulin resistance and synthesis can be diminished as a result of high glucose levels-induced inhibition of transcription factors. Finally, the attachment of pro-inflammatory protein serum amyloid A (SAA) to HDL particles forces the removal of ApoA-I [117]. In turn, ApoC-III transcription (apoC-III is an inhibitor of lipoprotein lipase) was found to be stimulated by glucose, which provided a mechanism linking hyperglycaemia and hypertriglyceridemia in patients with T2DM [118]. Increased plasma ApoC-III levels have been demonstrated to be associated with an enhanced risk of diabetes. Thus, it appears that ApoC-III negatively affects HDL functions, possibly via impairing glucose homeostasis and increasing circulating triacylglycerol levels [119]. The presence of hyperglycaemia and oxidative stress in diabetic patients promotes the formation of various glycosylated proteins [120]. The state of hyperglycaemia and oxidative stress observed in diabetic patients is associated with the non-enzymatic glycation of several macromolecules, including HDL [121]. Indeed, the in vitro incubation of HDLs with high concentrations of glucose markedly enhanced glycation, particularly of the protein component, including ApoA-I, paraoxonases, and malondialdehyde [120]. Finally, HDL from T2DM individuals have been suggested to be enriched in polyunsaturated phosphatidylethanolamines (PE) and deficient in phosphatidylcholines (PCs) and phosphatidylinositol (PI) species that contain monounsaturated fatty acids (PC 36:2, PC 34:2, PI 36:2, and PI 34:2) compared to HDL from healthy subjects [52]. The presence of T2DM was also inversely associated with lysophosphatidylcholine LPC 22:5 and, less strongly with LPC 18:0. LPC 18:0, LPC 18:2, and LPC 22:5. The loss of LPC 22:5 with T2DM is important due to the anti-inflammatory effects of LPCs containing polyunsaturated fatty acids [122]. The analysis of HDL isolated from T2DM in the DIWA cohort also revealed increased levels of ether-PCs in HDL from T2DM patients [112]. All the aforementioned modifications significantly decrease anti-atherogenic functions of HDL due to weakened antioxidant, anti-inflammatory, and vasodilator properties, accompanied by impaired HDL cholesterol efflux capacity in patients with T2DM [104, 123, 124].

Cholesterol Efflux Capacity

HDL from subjects with type 2 DM (T2DM) was found to be less efficient at driving cholesterol efflux from macrophages than HDL isolated from healthy individuals [75]. CEC is an important property of HDL cholesterol, whose impairment has been linked with the development of atherosclerosis and the incidence of cardiovascular disease (CVD) [125, 126]. The results of many studies have shown decreased HDL-related CEC in T2DM compared to healthy individuals [127]. A study involving 640 types 2 diabetic patients with or without cardiovascular disease (CVD) and 360 non-diabetic controls matched for serum HDL cholesterol levels demonstrated markedly reduced plasma pre-β1 HDL levels in diabetic patients which correlated with diminished cholesterol efflux mediated by ABCA1 [128]. The glycosylation of ApoA-I was found to be negatively associated with ABCA1-dependent cholesterol efflux in macrophages [129]. Since oxidative modifications and glycation of ApoA-1 impair its stability and compromise the interaction with ATP-binding cassette subfamily A member 1 (ABCA1), impaired cholesterol efflux has been reported in the majority of individuals with type 2 diabetes [68, 130]. Diabetic patients positive for anti-apoA-1 IgG have 5.7 times increased CVD risk [131]. The occurrence of anti-apoA-1 IgG was associated with markedly increased serum concentrations of LDL-C in diabetic patients. In diabetic patients, the macrophage expression of ABCA1 and ATP-binding cassette G1 (ABCG1) is decreased which suggest a double impairment of reverse cholesterol transport (RCT). Diminished release of cholesterol from cells of the arterial wall is accompanied by a lower capacity of extracellular particles to discharge excess cholesterol which negatively affects atherogenesis in diabetic patients [132, 133]. He et al. [134] suggested that the impairment of HDL CEC was associated with the loss of serpin family A member 1 (SERPINA1). SERPINA1 contains amphipathic α-helices which enable the binding of phospholipid to apolipoproteins and the stimulation of ABCA1 activity. It appears that diminished ABCA1 activity of small HDL particles lacking in SERPINA1 could enhance cardiovascular disease risk in subjects with diabetes mellitus.

Antioxidant Properties

The compromised function of HDL in type 2 diabetes does not allow for full antioxidant protection to LDL and is responsible for the failure to counteract ox-LDL-induced vasoconstriction [135]. HDLs obtained from diabetic patients failed to exert a significant inhibitory effect on the formation of endothelial cell superoxide or on nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, which translate into the loss of HDL antioxidant effects on the endothelium [1]. Nosecount et al. [136] demonstrated up to 52% decrease in protection against LDL oxidation by small and dense subfractions HDL_{3B} and HDL_{3C} fractions prevalent in diabetic patients. However, according to Gowri et al. [36], it is large HDL_2 from

diabetic subjects that displayed reduced defense potential against LDL oxidative modifications compared to healthy controls.

The results of functional studies have found lower susceptibility of HDL_3 subfraction to oxidation as a result of higher PON1 activity as well as greater capability to inhibit low-density lipoprotein oxidation (compared to HDL_2) [137, 138]. Moreover, the activity of HDL-bound antioxidant enzyme PON1 is diminished in individuals with T2DM [139]. Reduced activity of HDL PON1 is associated not only with the development of microvascular alterations but also with enhanced lipid peroxidation, thus favouring diabetic complications and increasing mortality [140]. The decrease in PON1 level was suggested to be associated with its displacement with SAA, as a result of inflammation and oxidative stress. Since the interaction between PON1 and ApoA-I is crucial for optimal PON1 activity/stability, also the loss of this apolipoprotein in diabetic patients may decrease the antioxidant properties of HDL [141]. Finally, diminished activity of PON1 may result from enhanced glycation [142]. Indeed, the glycation of HDL is responsible for a 65% decrease in PON1 enzymatic activity [120]. Apart from the impact on PON1 actions, HDL glycation is also associated with ROS-mediated pro-atherogenic activity on vascular smooth muscle cells (VSMC) [1]. The results of studies have confirmed that both HDL molecules which were glycated in vitro, and HDL isolated from patients with T2DM stimulated the proliferation and migration of VSMC and this effect was limited following the suppression of ROS [143]. Some studies have also indicated that impaired antioxidant action may also be related to decreased platelet-activating factor-acetylhydrolase (PAF-AH) in the HDL_{3C} fraction of diabetic patients [136, 144, 145]. HDL from subjects with T2DM displayed impaired protection of isolated cardiomyocytes from oxidative stress [96].

Anti-inflammatory Properties

Apart from the loss of antioxidant properties, in diabetic individuals, HDL anti-inflammatory capabilities are impaired [1]. Numerous studies have confirmed reduced inflammatory response in patients with T2DM, even in those with appropriate metabolic control [105, 139]. An in vitro study demonstrated enhanced expression of vascular cell adhesion molecule 1 (VCAM-1) mRNA in endothelial cells exposed to HDLs from diabetic participants compared to non-diabetic ones [139]. Ebtheai et al. [139] also found higher levels of high sensitivity C-reactive protein (CRP) and tumour necrosis factor-α (TNF-α). Moreover, they suggested that glycation may underlie the relationship between HDLs, inflammation, and diabetes [139]. Indeed, in vitro glycation of HDL was found to decrease its anti-inflammatory effect on adipocytes with palmitate-triggered inflammation [105]. Furthermore, glycation of this lipoprotein reduced its ability to limit the production of TNF-α and interleukin-1β (IL-1β) by lipopolysaccharide (LPS)-stimulated macrophages [1, 146]. In turn, Nobércourt et al. [103] reported higher expression of adhesion molecules VCAM-1 and ICAM-1 in human coronary endothelial cells that were

pre-incubated with reconstituted glycated HDL and challenged with TNF-α compared to control reconstituted HDL. Another reason for the loss of anti-inflammatory properties by HDL is the aforementioned enrichment of the particle with SAA [105, 139]. Mao et al. [147] demonstrated that HDL from patients with diabetic nephropathy had a lower capacity to limit the secretion of TNF-α from LPS-treated monocytes due to much higher levels of SAA. Such HDL was also unable to suppress TNF-α dependent activation of the nuclear factor κB (NF-κB) in endothelial cells as well as to deactivate oxidized LDLs and consequent greater monocyte adherence to endothelial cells [43, 120]. Small HDL particles (abundant in diabetic individuals) have been also shown to be the principal carriers of ceramides which are considered to be strong activators of the nuclear transcription factor NF-κB [112]. Furthermore, elevated LPC was demonstrated in HDL from dyslipidemic, diabetic subjects. In these individuals, LPC was associated with arachidonic acid which suggests that HDLs may be a biomarker of the inflammatory milieu in diabetes [112]. Moreover, the glycation of HDL protein components (i.e. ApoA-1 and PON1) was found to negatively influence endothelial cell survival, proliferation, and migration capacity. These effects are associated with the stimulation of vascular inflammation and endoplasmic reticulum stress and direct activation of pro-apoptotic pathways [148]. The analysis of reconstituted HDL with in vivo glycated-APOA1 revealed a considerable reduction in anti-inflammatory properties in endothelial cells due to impaired intracellular signalling and pro-oxidative changes [103].

Vasodilatory Properties

In a normal, healthy state, HDL protects endothelium via the stimulation of nitric oxide (NO) synthesis by endothelial nitric oxide synthase (eNOS) [1]. However, in pathological states including diabetes mellitus, this property of HDL can be significantly impaired. A case-control study nested in the Cooper Centre Longitudinal Study revealed nearly 40% lower ability of HDL from patients with T2DM to stimulate eNOS activity ($P < 0.001$) despite similar plasma HDL-C concentrations as well as 80% decreased capability of limiting TNFα-dependent NF-κB-mediated inflammatory response in endothelial cells ($P < 0.001$) compared to non-T2D controls. Vaisar et al. [43] found also that reduced capacity to stimulate eNOS activity negatively correlated with plasma levels of P-selectin (a marker of endothelial dysfunction) ($r = -0.32$, $P < 0.001$) and was associated with diminished sphingosine-1-phosphate (S1P) levels in this group of patients. Diabetic HDLs are also not able to promote endothelium-dependent vasodilation [123]. Again, glycation was found to be at least partly responsible for decreased vasodilation ability of HDL through decreased S1P binding to HDL and subsequent lack of the S1P receptor-mediated activation of eNOS and NO release [149, 150]. Indeed, the results of studies reported the decrease in S1P content of HDLs in diabetic patients [151]. In a normal,

physiological state ApoM stimulates is S1P in HDLs which leads to the enhanced synthesis of endothelial NO, however, in T2DM patients, levels of ApoM are decreased contributing to the pro-atherogenic actions of dysfunctional HDL. In this group of patients, the reduced S1P concentration intensifies the pro-atherogenic effects via diminishing NO production [30, 152]. Reduced ability of diabetic HDL to prevent the inhibition of endothelium-dependent vasorelaxation induced by oxidised LDL implies that HDL becomes less atheroprotective in type 2 diabetic patients compared to the control group [135].

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Chapter 10
Lipoprotein(a): Metabolism, Pathophysiology, and Impact on Diabetes Mellitus

Karam Kostner and Gerhard M. Kostner

Introduction

Lipoprotein(a) [Lp(a)] was originally described by K. Berg in 1963 as a genetic variant of ß-lipoprotein [1]. Despite intensive research into its physiological functions, comprehensive characterization of Lp(a) remains elusive. Today, there is little doubt that elevated plasma Lp(a) levels are causally related to cardiovascular diseases and myocardial infarction [2, 3]. First reports of the last century were mainly based on case control studies with only a few patients and on observational reports of single families. Based on the results from several more recent prospective longitudinal studies with large sample sizes, it became clear that the risk for developing coronary artery diseases (CAD) in the Western population is more than two times higher in individuals with increased plasma Lp(a) levels [3, 4]. There are still many gaps in our understanding of Lp(a) physiology and pathophysiology. This is one of the reasons why no specific Lp(a) lowering medications were available. Now it appears that with the antisense therapies that are currently in phase III clinical trials such medications will be soon available. These studies should ultimately answer the question whether Lp(a) lowering may be able to reduce the at least some "residual risk" for cardiovascular events. In this chapter, the major focus is directed at the pathophysiology of Lp(a) in general, with specific emphasis on diabetes mellitus.

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The Structures and Composition of Lp(a) and Apo(a)

Lp(a) is composed of an LDL-like core lipoprotein and of the glycoprotein apo(a) complexed to each other by a disulfide bridge (Fig. 10.1). The disulfide bridge links Cys 4326 in apoB-100 with the only free Cys 4057 in apo(a), that is located in kringle four (K-IV) Type-9. The lipid core of Lp(a) is almost indistinguishable from classical LDL of density 1.019–1.063. Table 10.1 shows the average composition of Lp(a) in comparison to LDL isolated from the same individuals.

Apo(a), the characteristic glycoprotein component of Lp(a) has a unique structure. It consists of repetitive protein segments, so-called kringles (K) that are highly homologous to K-IV in plasminogen. K-IV's contain approximately 110 amino acids, forming a secondary structure, which resembles "Danish kringles" [5]. The N-terminal part of apo(a) consists of various numbers of unique or repetitive copies of these kringle IVs. Apo(a) in addition has one copy of a K-V like kringle and a protease-like domain similar to plasminogen, yet the protease domain in apo(a) is non-functional. In humans, there exist probably 30 and more genetically determined apo(a) isoforms giving rise to great size heterogeneity. The smallest apo(a) isoform consists of the protease domain, one copy of K-V, and 11 K-IVs of which K-IV Type-1 (T-1) and T-(3-10) are unique in their primary structure, whereas K-IV T-2 is present in 2 identical copies. Larger isoforms differ by the number of K-IV T-2's; the largest apo(a)'s described so far had 52–54 K-IVs. The K-IV domains are connected by linker regions, that are highly glycosylated by N- and O-linked sugars. Although the majority of apo(a) is complexed to LDL, there are small and variable amounts of free apo(a) in plasma [6] that are found in the bottom fraction after ultracentrifugation at d. 1.21. Free apo(a) is prone to proteolytic degradation, and the generated fragments are secreted into urine (see below).

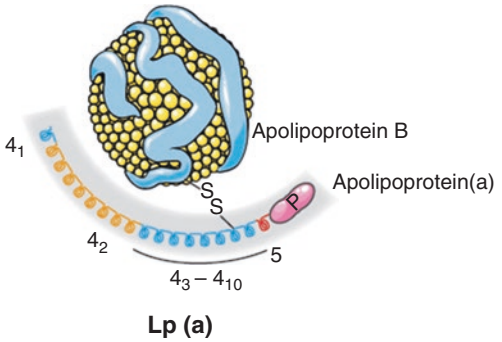

Fig. 10.1 Schematic view of an Lp(a) particle: Lp(a) is composed of an LDL-like core lipoprotein with apoB-100 as main protein component. The characteristic glycoprotein apo(a) is covalently linked to the core lipoprotein by a single disulfide bridge. Apo(a) is highly polymorphic and consists of numerous so called kringles that are homologues to the kringles found in plasminogen. There are ten unique kringles of K-IV 1 Type 1–10 (in blue) and several repetitive kringles K-IV Type 2 (in yellow). Apo(a) has one kringle K-V and the protease domain, all homologues to the counterparts in plasminogen

Table 10.1 Chemical composition of Lp(a) and LDL (%)

	Lp(a)	LDL
Protein	30	21
Carbohydrates	10	1.3
Cholesteryl ester	31.5	42
Free cholesterol	7	9
Phospholipids	16	20.7
Triglycerides	5.5	6

Lp(a) Metabolism

To get a better insight into the pathophysiology of Lp(a), a closer look into its metabolism is appropriate.

The Assembly of Lp(a)

Apo(a) is biosynthesized only in humans and Old World monkeys but not in conventional laboratory animals which poses some problems in studying its metabolism in detail. Apo(a) expression takes place primarily in the liver, yet small amounts of APOA mRNA have also been detected in testis and brain [5]. The functional role and metabolism of Lp(a) in the two latter are unknown. Hepatocytes from primates have been found to synthesize a pre-form of apo(a) that is not fully glycosylated [7]. Upon maturation, intracellular apo(a) reaches the Golgi apparatus and is secreted in mature form as a glycoprotein, most probably without attached LDL. The genetically determined size of apo(a) reflecting the number of K-IV repeats correlates with the intracellular residence time and, thus, small isoforms are secreted much faster compared to larger isoforms. This is probably one reason for the negative correlation between apo(a) size and plasma Lp(a) concentration.

We and others have found that the assembly of Lp(a) from apo(a) and LDL is a two-step process [8]. In a first step, specific K-IVs of apo(a), mostly K-IV T3–6, bind non-covalently to Lys groups on apoB of LDL. This binding is reversible and may be dissociated by Lys analogues such as epsilon amino caproic acid, tranexamic acid, and others [9]. It has been argued that by interfering with the first step of Lp(a) assembly plasma Lp(a) levels may be reduced, as free apo(a) is degraded faster than LDL-bound apo(a). In in vivo and in vitro experiments, however, this assumption has been refuted and plasma apo(a) and Lp(a) levels move in opposite directions, i.e., were at least two-fold elevated [9]. The reason for these observations may be explained by the fact that free apo(a) binds to the surface of liver cells and upon contact with LDL forms a preliminary Lp(a) complex. Apo(a) which does not find

its way to LDL is bound and internalized by various organs and degraded. We actually could demonstrate that cell bound apo(a) dissociates upon treatment with Lys analogues and assembles with LDL more efficiently.

When recombinant apo(a) was mixed with LDL in vitro, a final assembly took place that is characterized by stable disulfide bridging. Similar mechanisms most probably also occur in vivo that do not need any enzyme as a cofactor. A schematic view of the assembly of Lp(a) is shown in Fig. 10.2. Interestingly, apo(a) has a preponderance for binding apoB-100 from humans and few animal species, yet apoB-100 from rodents, in particular from mice hardly form any intact Lp(a) upon incubation with apo(a). When studying the metabolism in mice, it is therefore advisable to use double transgenic human apo(a):apoB-100 mice.

The question of whether the Lp(a) assembly occurs in vivo outside or inside the hepatocyte is not yet fully resolved yet. Although the evidence for an extracellular assembly is favored by many investigators, there are still experimental data that may be only interpreted if an intracellular assembly of Lp(a) is assumed [10]. Further experiments will be needed to reach a definite conclusion.

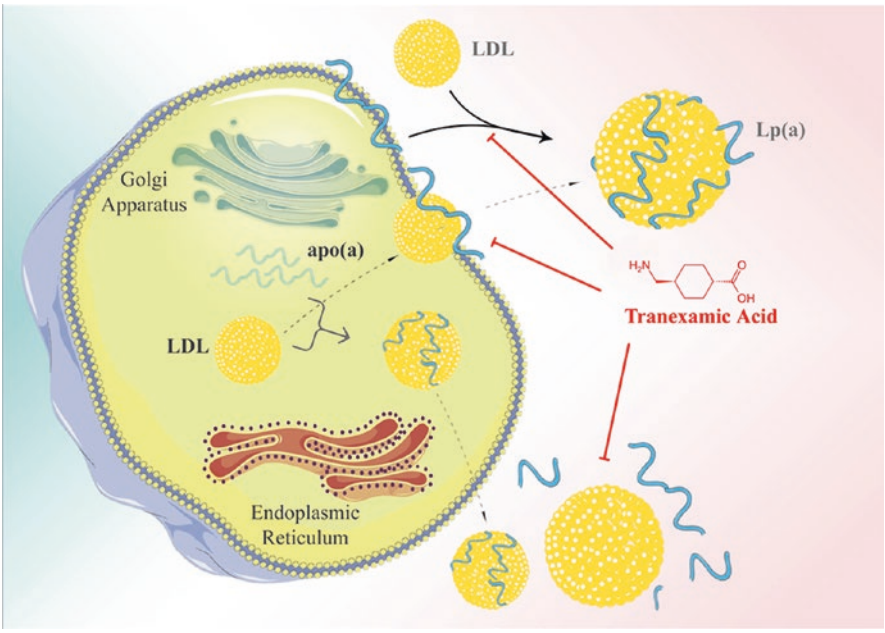

Fig. 10.2 Assembly of Lp(a). The assembly of Lp(a) from LDL and apo(a) is a two-step procedure: in the first step, apo(a) associates with LDL by binding of the Lys groups of LDL to the kringles in apo(a) in a similar way as plasmin binding to fibrin. In the second step the disulfide bridge is formed and stabilizes the Lp(a) complex. Lys analogues such as tranexamic acid interferes with the first step of assembly. There is no full consensus concerning the site of assembly—intracellular in the parenchymal liver cell or outside—probably on the cell surface. (Reproduced from Fig. 5 published in K. Kostner and G. Kostner, J. Lipid Res. 2017. 58: 1–14)

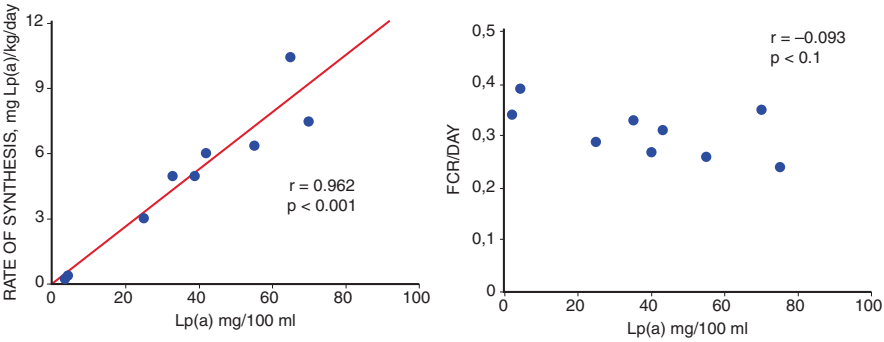

Fig. 10.3 Biosynthesis and catabolism of Lp(a). The rate of Lp(a) biosynthesis determines to a major degree the plasma concentration in healthy individuals. The fractional catabolic rate (FCR) does not correlate with plasma Lp(a) levels under normal conditions

In Vivo Metabolism of Lp(a)

As common laboratory animals do not express apo(a), we studied in early days Lp(a) metabolism in humans. We actually were first to demonstrate that plasma Lp(a) concentrations highly significantly correlate with the production rate, yet the Lp(a) catabolism has little impact on plasma Lp(a) levels [11]. This is demonstrated in Fig. 10.3, where we studied nine probands with Lp(a) levels ranging from 5 to 75 mg/dL. Our results have been confirmed subsequently by other investigators using radioactively labeled Lp(a) tracers or stable isotope precursors [12].

Concerning the catabolism of Lp(a), the liver appears to be the major organ of Lp(a) degradation. This has been proven by turnover studies in several animal species including rats, rabbits, mice, and hedge hogs. The latter animal model has been chosen since it synthesizes a lipoprotein that resembles Lp(a) [13]. In these in vivo studies, we found that approximately 50% of Lp(a) is taken up by the liver, followed by kidney, spleen, and muscle. Unfortunately, the exact mechanism of in vivo cellular uptake of Lp(a) is mostly unknown. In in vitro experiments, Lp(a) has been shown to bind to the LDL-receptor—yet with a greatly reduced affinity as compared to LDL. There have been also reports suggesting an in vitro binding of Lp(a) to other receptors such as the VLDL-receptor, Gp-330 (megalin), to scavenger receptors and to the asialoglycoprotein receptor [14], yet their relevance for in vivo metabolism remains to be established.

The Transcription of Apo(a)

The transcription of genes involved in lipid and lipoprotein metabolism is heavily influenced by nuclear receptors including PPAR's, RXR, CAR, PXR, LXR, FXR, in addition to others [15]. These nuclear receptors in concert with known

transcription factors coordinate pathways controlling lipid absorption, de novo biosynthesis, lipid excretion from different organs, and conversion of cholesterol to steroid hormones and bile acids. For bile acid metabolism, FXR is of major importance as it prevents their overproduction and toxification of liver and cells from the biliary tract. FXR is also involved in glucose homeostasis, intestinal bacterial infection, and tumorigenesis of liver [16]. Although the molecular mechanism of FXR action is not elucidated in full detail, it is fair to say that FXR that is mainly expressed in liver, intestine, kidney, and adrenals binds to response elements in the promoter region of genes as heterodimer together with RXR, thereby transactivating or trans repressing cognate target genes. Of key importance is the transactivation of small heterodimer partner (SHP) and of FGF-15/19. SHP is a transcriptional repressor that has no DNA binding capability but rather interacts with the DNA binding and/or activating factor-domain of numerous nuclear receptors, among them HNF-4, LRH-1, estrogen receptor (ER), and RXR, thereby interfering with gene transcription [17]. Mouse FGF-15 that is expressed almost exclusively in the intestine and its human orthologue FGF-19 expressed in the small intestine but also in the liver are also transactivated by FXR-RXR heterodimers. It has been demonstrated that FGF-15/19 signals from intestine to liver repress the transcription of key enzymes of bile acid biosynthesis. Inagaki et al. [18], for example, provided evidence that FGF-15 represses CYP7A1 in wild-type mice but did not affect CYP7A1 mRNA levels in SHP−/− mice. It was suggested that in the FGF15/19 pathway post-transcriptional activation of SHP may take place. It was also shown that FGF binding to its receptor, FGFR4, signals via the MAP-kinase pathway and interferes with CYP7A1 transcription [19]. In a recent report, Song et al. [20] published that the MAPK-ERK1/2 pathway is a major trigger of FGF19 mediated inhibition of CYP7A1. Binding of FGF19 to FGF4-receptor led to Tyr phosphorylation of the latter and in turn to a phosphorylation cascade of RAS, c-RAF, MEK1/2, and MAPK/ERK1/2 and finally to the transcriptional inhibition of CYP7A1. This pathway was independent of SHP.

In our recent studies, we made the observations that patients suffering from obstructive cholestasis with high plasma bile acid concentrations had comparatively low plasma Lp(a) levels. When patients were treated by surgery, plasma bile acid levels normalized and plasma Lp(a) rose significantly to levels compatible with their individual isoform [21] (Table 10.2). This led us to hypothesize that FXR might be responsible for these observed changes in plasma Lp(a) in a similar way as for bile acid biosynthesis. We therefore performed a series of in vivo studies with transgenic mice expressing apo(a) under the control of its genuine human promoter, in addition to in vitro studies using cultured primary hepatocytes from these mice aimed at elucidating the role of FXR ligands in apo(a) transcription. In a first report [21] we show that the apo(a) promoter contains at nucleotides 814–826 upstream to the transcription initiation site a direct repeat (DR-1) that binds HNF-4α with high affinity thereby transactivating apo(a) transcription. FXR upon activation by bile acids or synthetic ligands is transported from the cytosol to the nucleus and competes with the HNF-4α binding to the DR-1 and, in turn, downregulates apo(a) transcription. This pathway was

Table 10.2 Plasma Lp(a) levels of patients with obstructive cholestasis before and after surgical treatment

Patient	Sex m/f	Age (years)	Diagnosis	TBA (μmol/L) Before therapy	TBA (μmol/L) After therapy	Lp(a) number of K-IV repeats	Lp(a) (mg/dL)[a] Before therapy	Lp(a) (mg/dL)[a] After therapy
1	m	52	PHC	135.4	12.8	26	0.0	14.9
2	m	66	PHC	121.6	5.7	16/22	9.6	64.4
3	f	49	PHC	177.3	9.6	20	0.0	35.8
4	m	61	PHC	85.3	5.5	22/26	0.0	5.3
5	m	55	PHC	96.7	11.4	25	0.0	11.5
6	f	69	PHC	135.1	6.6	27	2.4	17.6
7	m	72	PHC	186.8	8.9	17/19	5.6	48.6
8	f	58	GBC	78.6	4.3	17/30	0.0	5.0
9	m	52	GBC	56.4	5.2	22/28	0.0	11.9
10	m	70	GBC	145.9	8.7	28/37	0.0	6.4
11	m	39	GBC	79.5	6.3	17	13.3	54.0
12	f	73	BDC	67.8	10.0	29/40	2.2	11.8
13	f	62	BDC	93.3	5.3	23/28	0.0	8.2
14	m	67	BDC	117.5	6.1	24	0.0	20.4
15	f	4w[b]	CBA	45.8	2.8	22	0.0	4.7
16	m	42	CLL	65.9	4.2	20/29	4.4	11.3
17	m	39	CLL	111.3	5.0	20	0.0	6.7
18	f	51	CLL	48.2	3.9	26	0.0	3.8
19	m	48	CLL	59.6	11.4	18/28	15.7	57.3
20	m	44	CLL	70.3	8.7	22	0.0	6.3
Mean				**98.9**	**7.1**		**2.7**	**20.3**
±s.d.				**41.3**	**2.9**		**4.8**	**19.9**

PHC pancreas head carcinoma, *GBC* gall bladder carcinoma, *BDC* bile duct carcinoma, *CBA* congenital biliary atresia, *CLL* choledocholithiasis, *TBA* total bile acids; control values males: mean 7.6; range 3.3–12.5; females: mean 6.2; range 2.8–10.5

[a] Values below the accuracy limit of the Lp(a) assay (1 mg/dL) were set as 0

[b] Newborn child at 4-weeks of age

further proven by reporter assays using a 2kB promoter sequence of apo(a) in front of the luciferase gene.

Feeding mice with a diet containing 0.2% cholic acid, the mouse ligand for FXR, led to a reduction of plasma apo(a) to almost zero. The pathway described above, however, accounted for only approximately 60% of the downregulation of apo(a) transcription. We therefore performed additional experiments exploring the possibility that SHP and/or FGF-15/19 might be responsible for the remaining 40% repression of apo(a) transcription. Overexpression of SHP in primary hepatocytes from transgenic apo(a) mice did not downregulate apo(a) biosynthesis. These findings were backed up also by promoter studies using a luciferase reporter assay. On the other hand, we showed that the addition of FGF-19 to primary hepatocytes of the apo(a) transgenic mice downregulated

apo(a) transcription and protein biosynthesis. Knock-down of the FGF-15/19 receptor (FGFR4) on the primary hepatocytes by a specific siRNA abolished the effect of FGF15/19. We also could prove that in this pathway FGF15/19 binding to FGFR4 activates a phosphorylation cascade involving RAS-RAF-MEK1/2 ERK1/2 that leads to ELK-1 phosphorylation and translocation to the nucleus. Using luciferase reporter assays in combination with site directed mutagenesis, we finally identified an ETS domain at not −1615 to −1630 that was responsible for P-ELK-1 binding and repression of APOA transcription [22]. These two pathways are displayed in a cartoon of Fig. 10.4. We believe that the clarification of these pathways may serve as the basis for developing new medications to treat individuals with elevated plasma Lp(a) levels and are at high risk for CAD.

Although bile acids are capable of downregulating apo(a) transcription almost completely by the two pathways described above, there are numerous additional regulatory sequences in the apo(a) promoter. In order to elucidate their possible importance, additional studies are currently being conducted.

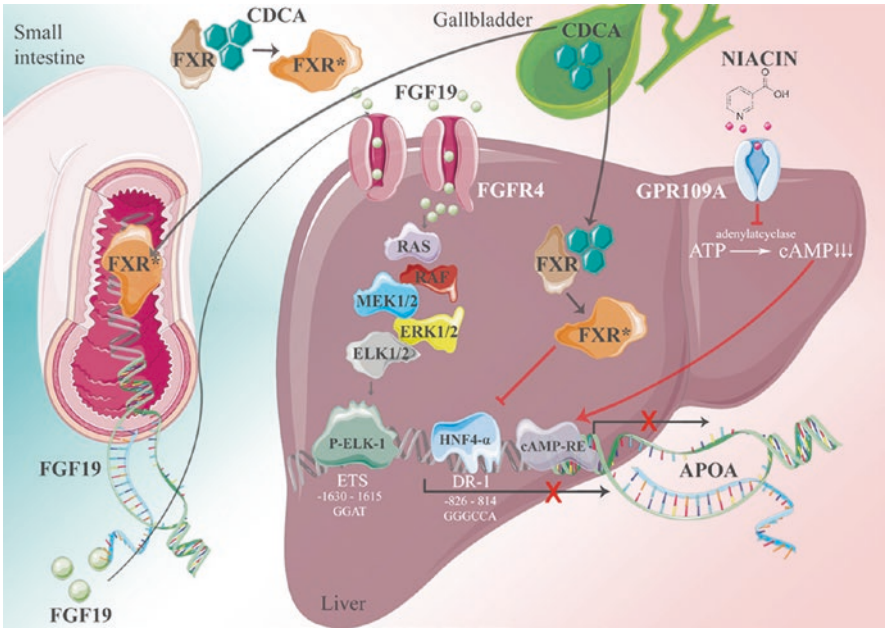

Fig. 10.4 Regulation of Apo(a) transcription. There are approximately 70 response elements for transcription factors and binding sites for nuclear receptors in the apo(a) promoter. The most significant ones are the ETS, DR-1 that bind P-ELK-1 and HNF-4α respectively that are most sensitive to farnesoid-X receptor (FXR). In addition, there is a cAMP-RE that is sensitive to nicotinic acid. For details see the description in the text. (Reproduced from Fig. 6 published in: K. Kostner and G. Kostner, J. Lipid Res. 2017. 58: 1–14)

Genetics of Lp(a)

The plasma Lp(a) concentrations in humans are genetically determined by >90%. Plasma concentrations range from almost 0 to >250 mg/dL and the apo(a) gene is located on chromosome 6q26-q27. Utermann et al. were first to recognize that apo(a) shows a tremendous size heterogeneity that is based on the number of K-IV repeats [23]. This size heterogeneity correlates with the levels of plasma Lp(a). Individuals with large isoforms are characterized by low plasma Lp(a) levels and vice versa. The molecular mechanism of these findings is based partly on the fact that large apo(a) isoforms are trapped and degraded during biosynthesis in the rough endoplasmic reticulum and in the Golgi compartment to a much greater extent than small isoforms [24].

The promoter region of apo(a) contains a variable number of tandem repeats (VNTR) with the penta-nucleotide TTTTA in addition to a +93 C/T polymorphism in the untranslated region of the apo(a) gene. Further mutations and polymorphisms are abundant in the apo(a) gene that may explain many, but not all, variations in plasma Lp(a) levels. Ichinose [25], for example, identified two additional functional SNPs in the distal enhancer region 20 kB upstream of the apo(a) gene. As mentioned above, the proximal apo(a) promoter region contains numerous regulatory sequences including binding sites for HNF-1 and -4, IL-6, SREBPs, CREB, and many more. It will be interesting to study the influence of genetic variations in these transcription factors in addition to their DNA binding sites in apo(a) in view of their impact on genetically determined variations of plasma Lp(a) levels.

More recent studies of Coassin et al. identified numerous polymorphisms in the K-IV domains of the apo(a) gene that showed significant impacts on apo(a) plasma concentrations [26]. These polymorphisms are quite abundant but variable among ethnic groups and may cause a two- to three-fold lowering effect on plasma Lp(a) levels. These findings are highly relevant for Lp(a) analysis with commercial assays and make any algorithm obsolete that corrects Lp(a) concentrations in relation to the number of K-IV repeats.

Another point that needs attention is the fact that large variations of plasma Lp(a) levels exist among various ethnic groups. Africans and African Americans, for example, have much higher Lp(a) levels than the white population even accounting for the individual size polymorphism. The opposite is the case with Chinese individuals and other Asian populations. The reason for these differences is to a major extent caused by a disproportional distribution of mutations and polymorphisms in different populations and need to be considered when calculating the cardiovascular risk on the basis of cut-off concentrations. This has been quite impressively documented in a report of Pare et al. [27] who assessed the risk for myocardial infarction in Africans, Arabs, Chinese, Europeans, Latin Americans, and South Asians and found quite characteristic differences in the association of myocardial infarction with the particular plasma Lp(a) concentrations.

Factors Affecting Plasma Lp(a) Concentrations

There are numerous factors that have been described to permanently or transiently modulate plasma Lp(a) concentrations [28]. A comprehensive list of most of these factors is shown in Table 10.3.

In addition to mutations and polymorphisms in the apo(a) gene, several other genes involved in lipid metabolism such as apoE, LDL-R, and HNF-1 and -4 have variable effects. Moreover it has been reported that other apolipoproteins than apo(a), for example apo CIII, are complexed to Lp(a) or to oxidized Lp(a) (Lp(a)-ox) and strongly affect their atherogenicity [29].

Among the secondary factors, renal and liver diseases appear to be the most striking ones. In kidney diseases, Lp(a) is elevated two- to three-fold and it appears that nephrotic syndrome and end-stage renal disease have a different etiology for elevating Lp(a). While in nephrotic syndrome, the rate of Lp(a) biosynthesis was found to be increased, end-stage renal disease is characterized by a reduced Lp(a) catabolism.

Since the liver is the only organ that synthesizes Lp(a), it is not surprising that liver diseases are characterized by a gross reduction of plasma Lp(a). This was observed at first instance in patients with cholestasis, yet their Lp(a) reduction is transient if the patients are successfully treated. Other substances that are liver toxic, including alcohol and several drugs, have been also shown to significantly reduce Lp(a).

Steroid hormones including estrogens, progesterone, testosterone in addition to synthetic sex-hormone analogues reduce plasma Lp(a) up to 40% yet rebound effects have been observed upon treatment with these compounds. In particular, anabolic steroids have a great reducing effect on plasma Lp(a), but the mechanism behind for this has not been elucidated to date.

The paradox of Lp(a) in diabetes mellitus: as will be shown in a later paragraph, patients with T2DM exhibit lower plasma Lp(a) concentration as compared to control non-diabetic individuals matched for the number of K-IV repeats. Nevertheless, T2DM patients have a higher incidence for coronary artery disease as compared to healthy controls [30]. The reasons for that have not been fully explored, yet there are strong indications that they might relate to pathophysiological effects of insulin signaling.

In addition to effects of Lp(a) lowering drugs that will be described in a paragraph below [31], there are dietary compounds and functional food that have a significant but rather small effect on plasma Lp(a) concentrations. In this group, for example, are omega-3 polyunsaturated fatty acids, L-carnitine, ascorbic acid, berberine, flavonoids, CoQ-10, gingko nuts, acetyl-salicylic acid, and others. Unfortunately, most of these compounds exhibit only marginal or transient effects.

Table 10.3 Factors affecting plasma Lp(a) concentrations

Genes	*Effect*	
APOA	Size polymorphism (50%); other 40%	
APOE	Variable	
LDL-R	Increase	
MODY (HNF1/4a)	Variable	
Diseases		
Acute phase	Increase	
Renal disease	Increase	
Diabetes mellitus	Increase	
Cancer: different forms	Increase	
Gout	Increase	
Anti-phospholipid antibodies	Increase	
Liver disease	Decrase	
Hyperthyroidism	Decrease	
Hypothyroidism	Increase	
Others		
Alcohol	Decrease	
Menopause	Increase	
Hormones		
Compound	*Effect*	*Comment*
Estrogens	Up to 35% reduction	Rebound effects have been observed
Progesterone	30–40% reduction	Only few studies reported
Tamoxifen	35% reduction	Anti-estrogen
Tibolone	35% reduction	Synthetic steroid hormone
Raloxifene	18% reduction	Estrogen-R modulator
Testosteron	30–40% reduction	Only short observations
Pregnancy	Two-fold increase	Normalized post-partum
Anabolic steroids	60–70% reduction	Not for clinical use
ACTH	30–40% reduction	Few observations
Conventional drugs		
Compound	*Effect*	*Comment*
Niacin	20–30% reduction	Currently most recommended
Fibrates	<20% reduction	Large study with Gemfibrozil
Statins	Inconsistent	Significant increases in Lp(a) reported
Neomycin (2 g/d)	24% decrease	Interferes with release of apo(a) from liver cell surface
N-Act-Cys	Controversial	Antioxidant, reduces –S–S– bonds
L-Carnitine	10–20% decrease	Mitochondrial FA transport
ASA	10–20% reduction	Even at low dose efficient

(continued)

Table 10.3 (continued)

Apheresis	50–80% reduction	Independent of the system except for AB-column
New medications under investigation		
Compound	*Effect on Lp(a)*	*Mechanism*
Mipomersene	>30% reduction	siRNA against apoB
Eprotirome	Up to 40% reduction	Thyroid-mimetic
PSK-9 inhibitor	Up to 30% reduction	PSK-9 antibody
Lomitapide	Up to 30% reduction	MTP-inhibitor
Anacetrapib	30–50% reduction	CETP-inhibitor
Different factors		
HGH treatment	2× increase	
Smoking	10–20% reduction	
Obesity	10–20% reduction	
Omega-3 FA	10–20% reduction	
Stearic acid	Up to 25% increase	
Trans-FA	Up to 25% increase	
Conjugated linoleic acid	Up to 25% increase	
Cyclosporine	2× increase	

The Role of the Kidney in Lp(a) Metabolism

As mentioned above, detailed knowledge on the site and mechanism of Lp(a) catabolism are still missing. When we infused radiolabeled Lp(a) into hedge hogs that express a human orthologue of apo(a), approximately 50% of the radioactivity was found in liver and bile, and 20% in the kidney. The role of kidney in Lp(a) metabolism in humans was studied by Kronenberg et al. [32] who reported on a 10% arteriovenous difference in Lp(a) plasma concentrations studied in healthy individuals. If these findings can be backed up by additional work, the kidney may turn out as an important organ in humans for Lp(a) catabolism.

Another interesting point is that apo(a) immune reactivity is found in urine [33]. As Lp(a) is by far too large to pass the glomerular cells of the kidney, only apo(a) fragments of various sizes are found in urine. Irrespective of the apo(a) isoform present in patient's plasma, consistently more than ten distinct apo(a) bands in urine with molecular masses between 50 and 160 kDa have been reported. These secreted apo(a) fragments are glycosylated and not complexed to apoB. Most importantly there is a highly significant correlation between urinary apo(a) concentrations and plasma Lp(a) levels. We also observed that diurnal urinary apo(a) levels normalized to creatinine remained constant over months within individuals. It is not fully clear where and how these fragments are formed, but it appears that a large portion might be formed extra-renally, followed by excretion by the kidney in a possible selective pathway. Urinary apo(a) excretion is rapid and depends on plasma Lp(a) levels: Reduction of plasma Lp(a) by LDL-apheresis leads to an immediate reduction of urinary apo(a) fragment concentration [34]. Studies from our laboratory showed

that under normal physiological conditions, a constant amount of apo(a) is excreted into urine, depending on the plasma Lp(a) level. The excretion rate of apo(a) into urine was not altered by changes in glomerular filtration rate and renal plasma flow in healthy males [28]. Urinary apo(a) was significantly decreased in a large study of patients with nephropathy, and this was even more pronounced in patients with a creatinine clearance of <70 mL/min [35]. Because urinary apo(a) excretion is highly dependent on plasma Lp(a) levels, patient and control groups were matched for plasma Lp(a) levels and it was found that with increasing plasma Lp(a) levels, kidney patients excreted significantly less apo(a) into urine as compared to controls [36]. Whether or not this mechanism might be responsible for the grossly increased Lp(a) values in kidney patients remains to be established. Above a plasma Lp(a) concentration of 30 mg/dL, urinary apo(a) excretion was highly diminished; there was a reduction of apo(a) excretion in patients to one fifth in comparison to controls. Interestingly, 30 mg/dL is the cut-off level for Lp(a) that best discriminates coronary heart disease patients from controls.

Subjects with small apo(a) isoforms in addition to higher plasma Lp(a) levels also exhibit significantly higher urinary apo(a) excretion as compared to patients with large apo(a) isoforms [33]. This observation and the fact that a good correlation of plasma Lp(a) and urinary apo(a) in both proteinuric patients and healthy controls exists led us to believe that urinary apo(a) excretion highly depends on plasma Lp(a) levels but not on apo(a) isoforms. Our earlier observation that after rapid reduction of plasma Lp(a) by LDL-apheresis urinary apo(a) is also rapidly reduced, supports this hypothesis.

Because of the significant correlation between plasma and urinary apo(a) concentrations, it should be possible to discriminate coronary artery disease patients (CAD) from healthy individuals by measuring urinary apo(a). In one study, 225 patients and controls were analyzed for plasma and urinary apo(a) and urinary apo(a) turned out to be a slightly better discriminator for CAD than plasma Lp(a) [37]. Since the analysis of these kringle IV fragments found in urine is not biased by the apo(a) phenotype, it might be appropriate to include the measurement of apo(a) fragments in future studies. In this regard, it is noteworthy that free apo(a) in serum, which consists mostly of these fragments, as measured by a recently described new ELISA [38], reportedly had a better diagnostic test performance than total Lp(a).

Another interesting observation was reported recently by Xuan et al. who studied more than 6200 adult Chinese individuals and correlated their renal function with plasma Lp(a) levels [39]. The individuals in the highest tertile of plasma Lp(a) concentration were at a 1.61-fold risk for reduced renal function compared to the lowest tertile. The association of high Lp(a) with a lowered renal function was by far more pronounced in the group of diabetic patients (odds ratio 4.4) and patients with hypertension. Similar findings were reported from a Japanese group who studied >6000 patients with chronic kidney disease. Independent of traditional risk factors they found by multiple regression analysis an odds ratio of 1.11 for elevated Lp(a) [40].

Also of relevance is the publication of Lippi et al. [41] who followed Lp(a) concentrations and several other serum parameters in 50 COVID-19 patients in relation

to kidney disease. The authors concluded that the Lp(a) concentration was significantly associated with the severity of the acute kidney failure. Unfortunately, the studies mentioned above could not clarify the question whether elevated plasma Lp(a) was the cause or a consequence of kidney dysfunction.

Lp(a) and the Risk for Atherosclerotic Diseases

In the original work from the laboratory of Berg, Lp(a) was described as "sinking-pre-ß" lipoprotein [42]. A semi-quantitative relation of sinking-pre-ß with coronary artery disease was suggested. Our laboratory in fact was among the first who quantified Lp(a) immunochemically by rocket electrophoresis, and by this method a cut-off level of 30 mg/dL for patients at an increased risk for MI was postulated [43]. We also showed in this report that individuals in this collective with elevated LDL-C and in particular those with a phenotype IIA hyperlipoproteinemia were at a ten-fold or higher MI risk. Later studies also demonstrated that the combination of high Lp(a) plasma levels with low HDL-C strongly increases the risk for CAD. In a prospective population study involving almost 800 male participants of the PROCAM cohort, von Eckhardstein et al. [44] reported that Lp(a) increases the coronary risk particularly in men with high LDL-C and low HDL-C in addition to high blood pressure and high global CAD risk. Even more importantly it has been found that polymorphisms in the apo(a) promoter were associated with an increased risk for MI [3]. In the following years, several thousand reports appeared in the literature dealing with one or the other aspect of atherosclerosis including myocardial infarction, stroke and peripheral vascular diseases in relation to elevated plasma levels, or in relation to low-molecular weight isoforms of apo(a). The majority of them strongly suggested that Lp(a) in fact is a severe risk factor—in several studies even the best discriminator for the MI risk. As Lp(a) metabolism is quite distinct from that of other plasma lipoproteins, it is not surprising that the atherogenicity of Lp(a) is independent of other lipids and lipoproteins.

It should be mentioned at this point that several prospective studies in the past, such as the Physicians Health Study, resulted in contrasting results [45]. In some of these reports, Lp(a) was measured in long-term frozen samples with insufficiently evaluated test kits. Moreover, due to the extremely wide range of plasma Lp(a) levels ranging from almost zero to more than 300 mg/dL, and the highly skewed distribution, studies that include a small number of cases/controls are prone to random deviations. Another reason for the controversial outcome of studies on Lp(a) is the fact that it is difficult to standardize the routine measurement procedures. This is mainly due to the great heterogeneity of Lp(a) structure and composition. There is in fact a great demand to harmonize commercial high-throughput assays to reach a better comparability of results from different laboratories. Cobbaert from University of Leiden (NL), therefore, established a working group with the help of IFCC to standardize the quantitation of human serum apolipoproteins with special emphasis on Lp(a) https://www.ifcc.org/ifcc-scientific-division/sd-working-groups/

wg-apo-ms/. The group currently establishes not only a reference method using LC-MS but also aims to prepare a reference material for Lp(a) analysis on the basis of fresh frozen serum that should be available soon to the public [46].

Starting in 2009, a series of papers were published that demonstrate beyond any doubt that Lp(a) not only is a risk indicator for atherosclerotic diseases but even more so that a causal relationship exists between elevated Lp(a) and CAD. The first report of this series was published by Tregouet et al. who studied 2700 CAD patients and >4500 control individuals by a SNP analysis using the 500 K Affymetrix chip [47]. In this haplotype association study, the authors identified the LPA gene cluster as a strong susceptibility locus for CAD. Kamstrup et al. [48] published in the same year their data of the Copenhagen Heart study comprising >40,000 individuals. There was a significant correlation between plasma Lp(a) levels, KIV-2 genotype, and the risk for myocardial infarction which they interpreted as proof for causality. Erqou et al. [49] finally performed a meta-analysis including 40 studies with >58,000 participants and found that individuals with smaller isoforms are at an >2-fold risk for coronary heart diseases.

More recent studies revealed that the APOA gene is by far more polymorphic than originally believed. These polymorphisms in fact have a great impact on the expression of apo(a) and in turn affects its atherogenicity. One example is the splicing variant KIV-2 4733G>A on Lp(a) that is quite abundant in the population [50]. The authors concluded that carriers of this variant not only lowers plasma Lp(a) significantly (13.6 mg/dL) but also translates to a noticeable risk reduction for coronary artery disease. A recent review by Langsted and Nordestgaard summarizes the genetic risk of atherosclerotic cardiovascular diseases with respect to elevated Lp(a) and aortic valve stenosis but also emphasizes the urgent need of results from intervention studies such as the HORIZON phase 3 trial to further back up the causality of Lp(a) for such diseases [51].

Another important finding is that Lp(a) may play a role in acute coronary syndrome. Shindo et al. [52] found significantly higher apo(a) and PAI-1 stainable areas in atherectomy specimens of patients with unstable than in those with stable angina. Cerebral vascular disease, peripheral vascular disease, and more recently carotid atherosclerosis have also been associated with elevated Lp(a) levels. Finally it appears that Lp(a) may also be involved as a cofactor in essential hypertension [53].

A key question relating to the causality of Lp(a) in atherogenesis obviously is the underlying pathophysiology. There is a great number of hypotheses published so far but it is beyond the scope of this review to mention all of them. One of the most plausible theory relates to Lp(a) and oxidation. It has been demonstrated that Lp(a) is a sink for oxidized phospholipids that are known to trigger the expression of inflammatory cytokines and in turn the proliferation of smooth muscle cells, hallmarks for the development of atherosclerotic plaques. In fact, it could be demonstrated that oxidized phospholipids on apoB containing lipoproteins are strongly associated with angiographically documented CAD [54]. Some of the oxidized phospholipids in Lp(a) have a similar structure as platelet activating factor (PAF), one of the strongest triggers of platelet aggregation. Interestingly we could also

show that Lp(a) binds a large amount of PAF-acetyl hydrolase, the enzyme known to inactivate PAF [55].

Impact of Lp(a) on Hemostasis

Apo(a) has a striking homology with plasminogen suggesting that Lp(a) interferes with fibrinolysis in several ways [56]. Findings from the past reported that Lp(a) competitively inhibited plasminogen binding to fibrinogen and fibrin that Lp(a) interferes with plasminogen conversion to plasmin, and that plasminogen activator inhibitor (PAI-I) biosynthesis in endothelial cells is stimulated by Lp(a). Lp(a) up-regulated PAI-2 expression in blood monocytes [57]. Another link between Lp(a) and thrombosis is its binding and inactivation of tissue factor pathway inhibitor (TFPI) [58]. On the other hand, there is evidence that Lp(a) binds platelet activating factor acetyl hydrolase (PAF-AH) with high affinity and specificity [55] and in turn inactivates one of the strongest triggers for platelet aggregation, PAF. PAF-AH appears to be beneficial as it hydrolyzes short chain phospholipids which are generated during lipid peroxidation [59], thereby possibly provoking anti-inflammatory actions. Lp(a) finally attenuates collagen-mediated platelet aggregation and in turn reduces thromboxane secretion. These latter findings are in line with several publications that question the thrombogenic effect of Lp(a). In one study, EDTA plasma was harvested from 27 volunteers and blood clotting lysis time was assayed with a new thromboelastometric method and correlated with Lp(a) concentrations. No delayed clot lysis was observed at elevated Lp(a) concentrations [60]. In another study comprising >100,000 individuals from the Copenhagen City Heart study and the Copenhagen General Population study, it was observed that high Lp(a) concentrations are associated with protection from major bleeding in the brain [3].

Taken together it appears that many of the proposed prothrombotic properties of Lp(a) are counter-weighted off by some quite significant anti-thrombotic effects and it remains to be determined which effect prevails under different pathophysiological situations.

Lp(a) and Diabetes Mellitus

Reflecting the high interest in this topic, there are several hundred reviews, reports, and abstracts published in this field. Two such reviews that summarize our current knowledge of this topic are found in [61, 62]. An early survey has been published by Haffner [63] who compiled the literature available until 1993 on Lp(a) and diabetes mellitus. The conclusions from his review of the literature were as follows: (1) Lp(a) concentrations in patients with insulin dependent diabetes mellitus (IDDM) are mostly elevated and related to metabolic control. (2) Patients with microalbuminuria have elevated plasma Lp(a). (3) Patients with non-insulin dependent

diabetes mellitus (NIDDM) have no elevation of Lp(a), and Lp(a) does not change with metabolic control. (4) There was no evidence that Lp(a) is a risk factor for CHD in the setting of diabetes mellitus.

Since then, a great number of studies on Lp(a) have been published on the role of diabetes mellitus as a causal modulator of plasma Lp(a) levels, yet the results are partly inconsistent [64]. This probably has three reasons: (1) there was for a long time—and still is—no standardized methodology for Lp(a) quantitation, (2) many studies did not dissect diabetes patients with or without impaired kidney function, the latter being known to strongly impact Lp(a) metabolism, and (3) the metabolic control of diabetes has not been fully accounted for in all studies.

In the following discussion, we try to summarize the current situation for patients with Type-1 (IDDM) and Type-2 (NIDDM) separately.

Lp(a) in Type-1 Diabetes Mellitus (T1DM) Patients

The first question to be answered is: Do Type-1 DM patients show differences in Lp(a) concentrations compared to matched controls and, if so, is that a primary (genetic) or secondary effect. Unfortunately, newer studies on this topic are missing and also a serious answer to this question cannot be provided taking all available literature into account. Our interpretation from available studies is that:

1. There is no inherent effect of T1DM on plasma levels of Lp(a)
2. Well-controlled T1DM patients have comparable Lp(a) levels to controls
3. T1DM patients with microalbuminuria—and even more so with impaired kidney function have increased Lp(a) levels
4. Physical activity and healthy lifestyle do normalize elevated Lp(a) levels in T1DM patients with normal kidney function

Lp(a) in Type-2 Diabetes Mellitus (T2DM) Patients

The Lp(a) situation in T2DM patients is by far more complex as compared to T1DM [28]. Of note is the existence a "diabetes paradoxon" as we call it (i.e., Lp(a) appears to be lower in this group of patients as compared to control individuals despite the fact that T2DM patients are at an increased CHD risk compared to non-diabetic patients.) It must also be kept in mind that T2DM is frequently accompanied by hypertension, elevated plasma triglycerides, hyperuricemia, hyperinsulinemia, parameters of inflammation, genetic polymorphisms in glucose transporters, transcription factors, and more. All of them may influence to a different degree the expression of Lp(a). Most of us consider T2DM as a very heterogenous mixture of metabolic diseases, and this probably also explains the conflicting study results. There are several interesting findings.

T2DM Paradox of Lp(a)

In our very first report from 1981 where we proposed that Lp(a) is a risk factor for myocardial infarction, we actually found that distinct from hyperlipoproteinemias caused by elevated plasma cholesterol, individuals with the classical Type-IV hyperlipidemia characterized by low LDL and high VLDL and elevated Lp(a) were at a significantly reduced risk for MI as compared to Type-IV individuals with low Lp(a) [43]. A plausible explanation for this phenomenon could not be provided, a retrospective look at these data however indicates that most of these Type-IV patients had impaired glucose tolerance and/or T2DM. In 2013, Kamstrup and Nordestgaard [65] published a Mendelian Randomization study on close to 80,000 individuals where they measured, in addition to plasma Lp(a) concentrations, the number of KIV-2 repeats and the rs10455872 SNP in order to answer the question whether the low plasma Lp(a) levels in T2DM might be causal or not. T2DM patients had lower Lp(a) concentrations with an odds ratio of 1.26. Individuals with high numbers of KIV-2 that correlates with high plasma Lp(a) concentrations showed a higher risk for T2DM. On the other hand, carriers of the rs10455872 SNP connected with elevated Lp(a) concentrations did not show a different risk of T2DM. The authors concluded that low Lp(a) concentrations by themselves might be not causal for an increased T2DM risk, yet this might be different for individuals with a high number of KIV-2. In the following years, several other groups measured plasma Lp(a) concentrations in T2DM patients and most of them reported lower Lp(a) levels as compared to control individuals (reviewed in [51]).

The obvious question now is what causes the reduced Lp(a) levels in T2DM. As indicated above the situation is complex due to the numerous factors that influence the phenotype of T2DM and the multiple etiologies, many of them related to mutations or polymorphisms of genes involved in lipid and lipoprotein metabolism. A good example for this complex situation is found in the article of Shih et al. [66] who studied the Q268X mutation in the MODY gene in relation to plasma apo AII, apo CIII, and Lp(a) levels. MODY stands for maturity onset diabetes of the young, and MODY genes are nuclear receptors (HNF-1α and HNF-4α), known as master regulators of genes expressed in the liver that are involved in lipid metabolism. As mentioned in the paragraph "transcriptional regulation of apo(a)," the expression of apo(a) is highly dependent on the binding of HNF-4α to DR-1 in the promoter. Thus, any mutation in HNF-4α that affects the transactivation of genes must have an influence on plasma lipid and lipoprotein levels. In fact, it was found that carriers of the Q268X mutation not only develop MODY but also have reduced plasma concentrations of Lp(a), apo AII, and apo CIII. There are other mutations and polymorphisms known in the MODY genes that may have similar effects on plasma Lp(a). Also of relevance are the findings, that T2DM patients show aberrations in hormones other that insulin such as testosterone, IFG-1, or thyroid hormones that are known to impact APOA expression [67].

Taken together it might be safe to say that T1DM patients have Lp(a) concentrations that are not different from healthy persons if they are well controlled and free

of renal dysfunction. T2DM patients on the other hand may have reduced Lp(a) due to mutations or polymorphisms in genes that affect the expression of the APOA gene on the one hand and the phenotype of diabetes mellitus.

Lp(a) as a Risk Factor for CAD in Patients with Diabetes Mellitus

In theory, Lp(a) should be at least as atherogenic, if not more, in diabetic patients than in non-diabetic patients.

Lp(a) contains a large amount of oxidized phospholipids that are a hallmark for atherosclerosis. Due to its longer residence time in blood as compared to LDL [11], Lp(a) is probably glycated to a larger extent than LDL, contributing to its atherogenicity. That this is actually real is supported by the findings of Kotani et al. [68] who demonstrated impaired endothelial function likely related to oxidized Lp(a) from T2DM patients. The theoretical considerations mentioned above have been corroborated in patient studies in vivo.

In 2006, Kollerits et al. [64] asked the question as to what extent Lp(a) might be an independent predictor of CVD in T1DM patients. More than 400 T1DM patients were followed over an observation period of 10.7 years. Since renal disease is a risk factor for CAD, patients with impairments of kidney function were excluded from the study. Although this study did not answer the question per se whether or not T1DM patients have increased Lp(a) levels, it was concluded that Lp(a) values >30 mg/dL contribute significantly to the CAD risk in T1DM. Similarly, calcified aortic valve disease was found in T1DM patients with high Lp(a) levels [69].

There are numerous reports documenting that the situation in T2DM patients with respect to the atherogenicity of Lp(a) is very similar to that of T1DM. To mention only one of them, Saeed et al. [70] examined the association of Lp(a) with the risk for CVD in close to 10,000 male and female participants, 1543 of them had been diagnosed with diabetes or pre-diabetes. From the results, the authors concluded that "*Adding lipoprotein(a) to traditional risk factors improved ASCVD risk prediction*."

Taking all current knowledge together it is fair to say that elevated plasma Lp(a) levels in T2DM patients positively correlate with the incidence of atherosclerotic cardiovascular diseases. Despite the Lp(a) paradox in T2DM, there is no indication that lowering Lp(a) might negatively affect cardiovascular outcome of this disease.

Treatment of Elevated Lp(a) Levels

Apart from LDL-apheresis therapy, it is currently not clear whether lowering of Lp(a) reduces hard cardiovascular endpoints. Several phase II and III trials with antisense and Si RNA targeted therapies are exploring this currently. Most

lipidologists and clinicians recommend to lower LDL-cholesterol more aggressively to levels below 100 mg/dL in case of elevated Lp(a) levels, even though the hard evidence for this is also lacking.

Diet

Dietary influences on plasma Lp(a) levels are variable and moderate, yet measurable. Polyunsaturated fatty acids and saturated fatty acids found in palm oil, have a mild, although significant, reducing effect. Dietary intake of omega 3 fatty acids has shown to decrease plasma Lp(a) levels in some studies. A diet rich in coconut oil has also been shown to reduce plasma Lp(a) levels [71]. In a similar way trans fatty acids were suggested to have a lowering effect on Lp(a). Taking all published studies on dietary treatment of hyper-Lp(a) patients together, it is fair to say that the effects are moderate and transient in many cases and appear to vary among individuals depending on their type of hyperlipoproteinemia. Long-term studies on this topic in fact are lacking.

Statins

Statin treatment may have a variable effect on plasma Lp(a) concentrations. In most studies, Lp(a) remains unchanged after treatment with HMG CoA reductase inhibitors. Treatment of hypercholesterolemic patients for 6-weeks revealed that approximately one-third responded with a reduction of plasma Lp(a), in one-third there was no change and in the remaining third Lp(a) was significantly increased [72]. Some studies have shown lowering of Lp(a) by long-term treatment of familial hypercholesterolemia (FH) patients with statins [73]. Importantly, aggressive LDL reduction with statins removes some of the risk associated with elevated Lp(a) levels.

Ezetimibe

In a metanalysis, ezetimibe alone or in combination with statins did not show a significant effect on Lp(a) levels [74].

Nicotinic Acid

Nicotinic acid and its derivatives can reduce Lp(a) levels by up to 35% [75]. Niceritrol, a nicotinic acid derivative has also been shown to reduce plasma Lp(a) levels in patients with chronic renal disease and hyperlipidemia. From all lipid-lowering drugs described so far, nicotinic acid and its derivatives appear to be the most efficient Lp(a) lowering agent. However, to date, the use of nicotinic acid in combination with a statin has failed to impact risk for cardiovascular events.

Fibrates

There are numerous reports in the literature concerning the influence of fibrates, which include clofibrate, fenofibrate, and gemfibrozil on plasma Lp(a) levels. In essence, it appears that there is no uniform response as part of the treated patients respond with approx. 25% decreases in plasma Lp(a), in some there are no changes and there are also numerous individuals whose plasma Lp(a) increases upon fibrate therapy. The latter group of patients is characterized by rather high plasma triglycerides and VLDL and respond upon fibrate therapy with elevations of LDL in addition to elevations of Lp(a). The pathomechanism of this phenomenon remains to be elucidated.

Other Agents

All ACE inhibitors in monotherapy lower elevated Lp(a) plasma concentrations in proteinuric patients by reversing proteinuria and in turn enhanced Lp(a) production by the liver [76]. Fosinopril seems to be the only ACE inhibitor to reduce Lp(a) concentrations also in non-proteinuric patients, probably by increasing apo(a) fragmentation and excretion into the urine (Kostner et al. unpublished results).

Lp(a) lowering steroid hormones are not indicated for treatment due to side effects. Likewise, tranexamic acid is able to lower Lp(a) plasma concentrations in vivo but cannot be used in the majority of patients due to possible side effects. The anti-estrogen tamoxifen also has an interesting Lp(a) lowering effect [77]. The synthetic steroid tibolone reportedly reduced Lp(a) by about 35%, however, this was accompanied by a concomitant reduction of H HDL-C by about 20%. Raloxifene is a selective estrogen receptor modulator and an alternative to estrogen replacement as it obviates the need for a progestin and does not increase C-reactive protein levels. In a recent study, it was reported that raloxifene significantly reduced Lp(a) by 18% [78].

As mentioned previously, ACTH has been found to decrease Lp(a) by more than 50% and also resulted in lower total cholesterol, LDL and apoB levels in hemodialysis patients, and steroid treated healthy and hyperlipemic individuals.

Recently L-carnitine was shown to reduce elevated Lp(a) levels by about 10% in patients with and without DM [79]. There are also reports indicating that aspirin and vitamin-C lower elevated Lp(a) levels.

Apheresis

One of the more effective therapies for lowering Lp(a) is apheresis. LDL-apheresis and selective Lp(a)-apheresis using antibody coupled columns, precipitation, and complex formation at low pH, double filtration, and direct absorption have been demonstrated to lower plasma Lp(a) to the same extent as LDL-cholesterol (up to 80%). However, these treatments are expensive and accessible only to a small

number of high-risk patients [80, 81]. A study of 154 patients with baseline Lp(a) of 108 mg/dL showed apheresis reduced Lp(a) by 68% and reduced CVD events by 81% [82].

Novel Lipid Lowering Compounds

ApoB Antisense and MTP Inhibitors

ApoB antisense inhibits only production of ApoB-100-containing lipoproteins such as Lp(a) which are found in the liver, whereas MTPi generally reduce both hepatic ApoB-100-containing lipoproteins as well as ApoB-48 lipoproteins which are produced in the intestine and transport dietary fat via chylomicrons. Even though MTP inhibitors have been shown to reduce apoB containing lipoproteins in humans, the future of systemic MTP appears uncertain because of their poor tolerability, transaminase elevations, hepatic steatosis, and significant negative impact on ApoA-1 lipoprotein and HDL-C.

The most advanced Apo B antisense drug in clinical use is mipomersen. Phase 2 studies in patients on statins and other lipid-lowering agents showed mipomersen dose-dependently reduced LDL-C, TG, and Lp(a) mainly by reducing the fractional catabolic rate of Lp(a) [83].

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

In 2003, Abifadel and colleagues described a new form of autosomal dominant hypercholesterolemia (ADH), which was not associated with mutations in the genes coding for the receptor or its ligand, apoB. They reported two mutations in the gene encoding proprotein convertase subtilisin/kexin Type 9 (PCSK9) that were responsible for hypercholesterolemia [84]. PCSK9 inhibitors have shown to reduce LDL and Lp(a) in several trials such as FOURIER and ODYSSEY OUTCOMES. The FOURIER trial showed significant reductions in Lp(a) levels with evolucomab, with the largest reduction seen in those patients with the highest baseline Lp(a) levels. Patients with Lp(a) levels of more than 80 mg/dL showed an almost 50% reduction in their Lp(a) and >30% in CV events [85]. The FOURIER trial also showed a greater absolute risk reduction in patients with diabetes and no worsening of glycemia and no increased risk of new-onset diabetes with evolucomab [86].

The ODYSSEY OUTCOMES trial with another PCSK9 inhibitor alirocumab also showed significant reductions in Lp(a) and interestingly the % reduction in Lp(a) predicted the clinical event reduction with alirocumab [87]. Alirocumab also had no effect on glycemia, even in patients with pre-diabetes in pooled analysis from the ODYSSEY trial programme [88].

Inclisiran, a small interfering ribonucleic acid molecule targeting apoB RNA and administered as a 6-monthly subcutaneous (sc) injection can also lower Lp(a) similar to other PCSK9 inhibitors by 15–25% and is currently in phase III clinical trials [89].

While PCSK9 inhibitors are very safe and effective in reducing LDL in diabetic patients, they are not indicated or reimbursed for primary Lp(a) reduction.

Cholesteryl ester transfer protein (CETP) inhibitors such as anacetrapib have been shown to lower Lp(a) by up to 25%, which may be due to a reduction in apo(a) production [90]. While the earlier CETP inhibitors are not available due to poor trials results, several new agents such as TA8995 which was associated with a 37% reduction in Lp(a) are still in clinical trials [91].

Eprotirome, a thyroid hormone analogue, induces up to 40% reductions of Lp(a) in statin treated dyslipidemic patients, but is currently not available for clinical use [92].

The currently most promising Lp(a) lowering therapies are antisense oligonucleotides and small interfering RNA therapies. Pelacarsen, is a ligand-conjugated, antisense oligonucleotide that targets hepatic apo(a) mRNA. The drug has shown median Lp(a) reductions of 80% with good tolerability of monthly subcutaneous injections and is currently being tested in a large placebo-controlled outcome trial (https://clinicaltrials.gov/ct2/show/NCT04023552). Two small interfering ribonucleic acid molecules targeting apo(a) RNA (AMG890 (Olpasiran) and SLN 360) are in phase II and III trials [93].

Diabetes Therapies and Lp(a)

Metformin, one of the oldest diabetes therapies, has not shown consistent results in reducing Lp(a) in people with diabetes [94], even though one study suggested an effect in non-diabetic patients [30]. The effect of insulin, sulfonylureas, glucagon-like peptide 1 agonists, and sodium glucose co-transporter 2 inhibitors have not been studied in detail, but do not seem to significantly reduce Lp(a) levels in our clinical experience [62].

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Chapter 11
Lipoprotein Glycation in Diabetes Mellitus

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Introduction

The prevalence of both Type 1 and Type 2 diabetes mellitus is increasing in both advantaged and disadvantaged regions, and in spite of modern measures to control blood glucose, blood pressure, lipid levels, and thrombosis, the neurovascular complications of diabetes affect large numbers of people and also society as a whole [1]. Diabetes is conservatively associated with a two- to four-fold increased risk of coronary artery, cerebrovascular, and peripheral vascular disease [1, 2]. Diabetes usually accounts for over a third of all patients with end-stage kidney disease (ESKD), and in the Western world is the most common cause of blindness in working age adults [2]. Over 60% of people with diabetes will likely die of macrovascular disease [1–3], which is particularly common in those subjects with microvascular damage, in particular diabetic nephropathy. Multiple genetic, biochemical, and

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lifestyle risk factors are recognized, with hyperglycemia and dyslipidemia being major risk factors [4–7]. These two factors independently have deleterious effects, but together they result in lipoprotein glycation, which can aggravate lipoprotein dysfunction and adverse effects on tissues. There is generally more circulating glycated LDL than oxidatively modified LDL, yet the literature has mainly focused on lipoprotein oxidation. There is relatively little research related to glycated lipoproteins, with relatively few studies since the publication of the the first edition of this book, with there being a shift towards studies using lipidomics. Either directly or indirectly via effects on metabolism, oxidation, and inflammation, lipoprotein glycation has deleterious effects on lipoprotein function, thrombosis, and cellular function in many tissues prone to the chronic sequelae of diabetes.

Lipids and Lipoproteins in Diabetes

Dyslipidemia is a well-accepted risk factor for atherosclerosis in the diabetic and non-diabetic population and in both Type 1 and Type 2 diabetes and is also a risk factor for diabetic retinopathy and nephropathy. As both *quantitative* and *qualitative* changes occur in lipoproteins and can affect lipoprotein related apolipoproteins and enzymes, we prefer the more encompassing term of dyslipoproteinemia rather than dyslipidemia [4]. Hyperglycemia and therefore dyslipoproteinemia, including lipoprotein glycation, also occurs in gestational diabetes and secondary forms of diabetes (such as iatrogenic (e.g., corticosteroid-induced or immune checkpoint inhibitor-induced diabetes or that secondary to pancreatitis or pancreatectomy), but research studies of lipoprotein glycation in these clinical settings are lacking [4]. In Type 2 diabetes, there is a characteristic lipid profile with increased triglycerides, normal to high Low Density Lipoprotein (LDL)-cholesterol, and reduced High Density Lipoprotein (HDL)-cholesterol levels. In people with Type 1 diabetes with moderate to good glycemic control, normal kidney function and the absence of other risk factors such as obesity, smoking, or coexistent familial dyslipidemia, the lipid profile is relatively normal, but vascular disease is still accelerated [5, 6]. Even with good glycemic control, which in clinical practice can be difficult to achieve, and with good lipid control, which often requires pharmacologic intervention, discussed in detail elsewhere in this book, residual vascular risk remains in people with diabetes. Residual risk is the remaining risk of vascular damage after optimal control of the known risk factors, such as related to glycemia, blood pressure, the traditional lipid profile, and smoking. Many factors may be contributory to residual risk, including qualitative changes in lipoproteins such as post-translational lipoprotein glycation. Other subtle lipoprotein abnormalities, such as oxidation, which can occur concurrently with glycation, alterations in lipoprotein composition, size, and immunogenicity, which are also discussed in other chapters in this book, may also contribute [4, 7]. Adverse biological effects of lipoprotein glycation may be direct and/or indirect via modulating coagulation, fibrinolysis, vascular tone, matrix binding, inflammation, altered susceptibility to oxidation and cellular and tissue responses, including angiogenesis.

The Chemistry of Lipoprotein Glycation

The glycation process can be divided into early and late glycation, summarized in Fig. 11.1. In 1912, French food chemist Louis-Camille Maillard first described the formation of brown-colored substances from non-enzymatic reactions between reducing sugars and proteins [8]. As well as in food, such chemical reactions also occur endogenously and are relevant to human health in people with and without diabetes. A simplified view of this complex chemistry is that carbonyl groups and amino groups react to form Schiff bases and then Amadori compounds (early glycation products), which are potentially reversible. Early glycation product formation may be followed by irreversible dehydration, condensation, and cross-linking reactions, resulting in a large, and a likely incompletely known heterogeneous family of derivatives termed Advanced Glycation End Products (AGEs). AGEs are also known as late glycation products, Maillard products, or glycoxidation products (as formation of many AGEs involves oxidative chemistry, see Fig. 11.2) [9].

Similar reactions can occur, by both enzymatic and non-enzymatic pathways, without glucose, providing the non-glucose materials containing an aldehyde group. Reactive metabolites such as the dicarbonyls (methylglyoxal (MG), glyoxal, and 3-deoxyglucosone (3DG)) from the glycolysis pathway, and from the metabolism of lipids and ketones can also interact with protein residues to form AGEs, including in lipoproteins [10]. Increased production of reactive dicarbonyls or their reduced detoxification by the glyoxalase system or by endogenous scavengers leads to increased carbonyl stress, which is a major driving force for AGE formation and accumulation [11]. AGE formation occurs in many extracellular and intracellular proteins, including lipoproteins, and AGEs are present in all people. AGE levels in long-lived tissues, such as skin and in the lens of the eye, usually increase with chronologic age [12]. AGE formation is accelerated by hyperglycemia as in diabetes [10–13] and also by impairment of kidney function, even in the non-diabetic milieu [12–14].

AGEs are chemically heterogeneous groups of both fluorescent and non-fluorescent compounds with over 25 fully characterized AGE structures [15]. The (type and concentration) of glycation products formed depend on both the range and concentration of substrates available and the duration of their interaction. Nε-carboxymethyl-lysine (CML) is the simplest and to date best characterized AGE and the main epitope for many commercially available antibodies used for AGE detection and quantification. Many of these products such as CML (thought to be the most abundant AGE in vivo), pentosidine, and erythronic acid are formed oxidatively [10]. Non-oxidatively derived AGEs such as the imidazolones and pyrraline have also been identified and characterized [16, 17]. Pyrraline is formed by the reaction of 3-deoxyglucasone with lysine, and imidazolone-type AGEs are formed by the reaction of 3-deoxyglucasone with arginine. The value of each specific AGE, or group of AGEs, as a marker or mediator of diabetic microvascular and macrovascular damage is not fully elucidated.

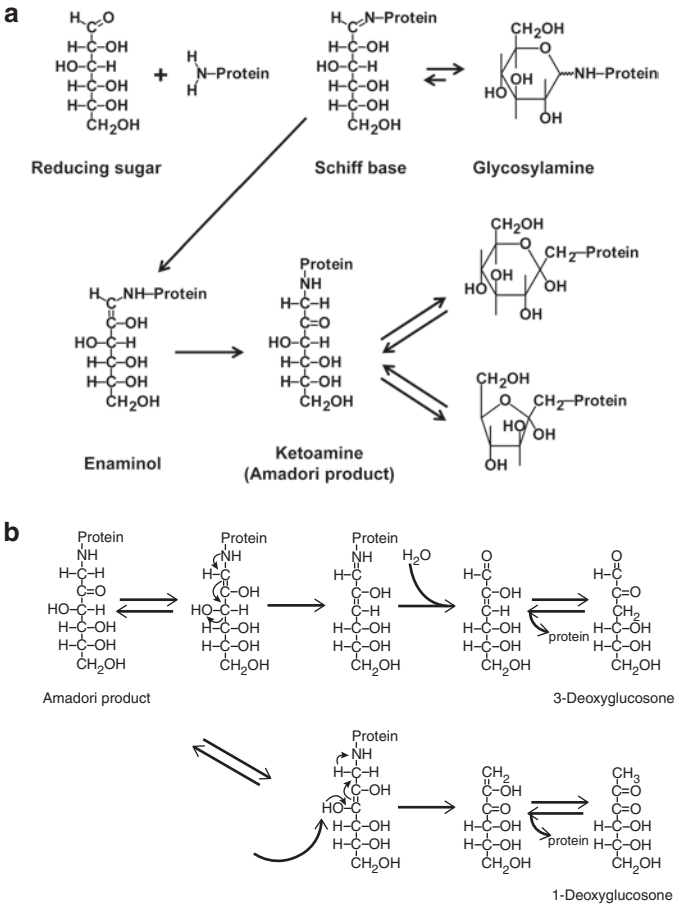

Fig. 11.1 Biochemistry of early and late glycation. (**a**) Early steps of the Maillard reaction. The reducing sugars in open chain form reacting an amino group on proteins to form a reversible Schiff base. The Schiff base then forms a cyclic glycosylamine or can rearrange to an enaminol and then to a ketoamine (Amadori compound). The Amadori compound is also stabilized by its cyclization to a furanose or pyranose ring. (**b**) The Amadori compound fructoselysine can undergo decomposition to form both 1- and 3-deoxyglucosone (1-DG and 3-DG). 3-DG is more reactive than glucose in the formation of AGEs. (**c**) Various pathways leading to the formation of AGEs. The Maillard pathway involves the reaction of a reducing sugar with an amine on a protein to form a ketoamine, which can break down to form AGEs. Alternatively, the autoxidation of glucose forms reactive compounds like arabinose and glyoxal that can further react with amino groups and form AGEs (Wolff pathway). The Schiff base intermediate can also form reactive carbonyl compounds under oxidizing conditions and can also react with an amine leading to AGE formation (Namiki pathway). Lastly, the ketoamine, under both oxidative and non-oxidative conditions, can fragment to form reactive deoxyosones that can form AGEs (Hodge pathway). (Reproduced (modified) with permission from: J.W. Baynes, "The role of AGEs in aging: causation or correlation", Exp. Gerontol. (2001) 36(9), 1527–1537)

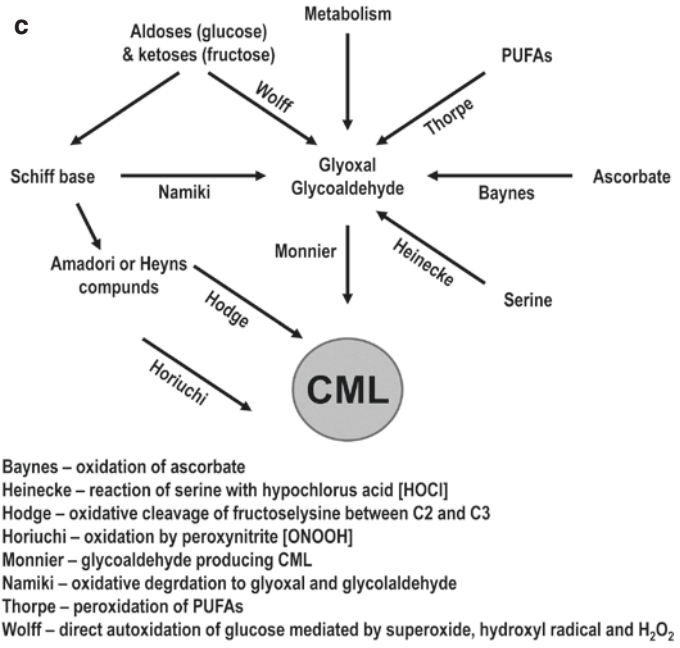

Fig. 11.1 (continued)

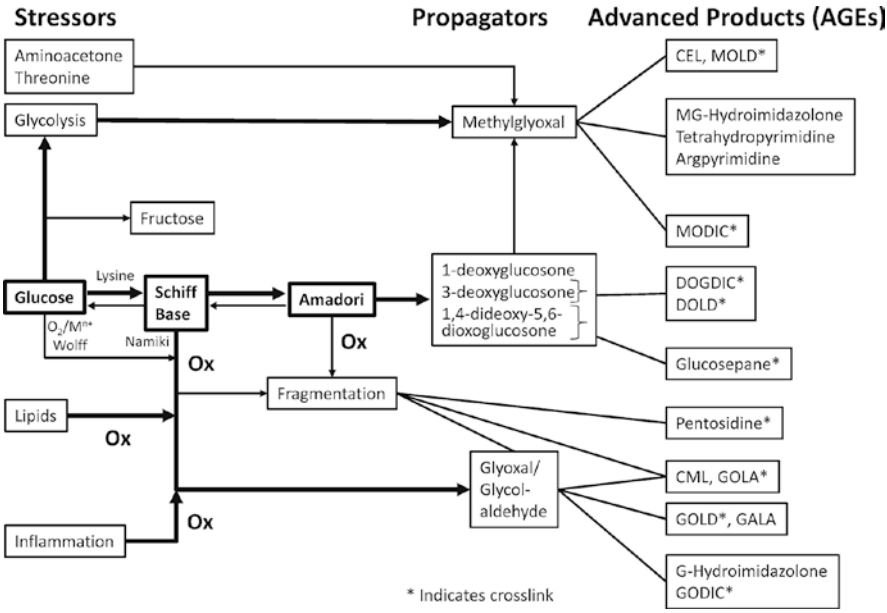

Fig. 11.2 Factors affecting AGEs formation and accumulation. (Reproduced with permission from: V.M. Monnier and X. Wu, "Enzymatic deglycation with amadoriase enzymes from Aspergillus sp. as a potential strategy against the complications of diabetes and aging" Biochem. Soc. Trans. (2003) 31, 1349–1353)

AGEs can also be derived exogenously, such as from the diet and smoking [18–20]. Dietary AGEs are abundant in foods such as (all as per 100 g of product) fried pork bacon, roast chicken skin, sesame oil, parmesan cheese, sweet butter cream, pan fried beef, or pizza [21]. AGEs in food are partially absorbed from the gastrointestinal tract, and approximately two-thirds are thought to remain in contact with tissues for several days, whereas the rest is rapidly excreted by the kidneys [22]. AGE restriction in mice, without energy or nutrient change, alleviates inflammation, prevents vascular complications, and extends their normal life span [23]. Human studies have showed that a low-AGE diet reduces inflammatory markers (C-reactive protein (CRP), Tumor Necrosis Factor alpha (TNF-α)) and vascular cell adhesion molecule (VCAM-1) levels [24]. In Type 2 diabetes, high-AGE meals have been shown to acutely impair vascular reactivity as measured by flow mediated dilation (FMD) [25]. HDL does suppress TNF-α induced VCAM-1 suppression in vitro, but it is not known how much of the low-AGE diet benefit, in animals or in humans, relates to effects on AGE-modified lipoproteins.

Differences Between Glycation and Glycosylation

The term "glycation" refers to non-enzymatic reactions between amino acid residues of proteins and reducing sugars. Glycosylation is a different set of usually enzymatic chemical reactions. Glycosylation is a major post-translational modification of both intracellular and extracellular proteins. Most intracellular proteins in humans contain sugars and are also known as glycoconjugates. Depending on the nature of the covalent attachment, glycosylated proteins can be divided into glycoproteins (in which the major component is a protein) and proteoglycans (in which typically >95% mass is a carbohydrate). Glycoproteins are an integral part of plasma membranes and serve important functions such as hormones, receptors, and mediators in intercellular interactions. Proteoglycans are major components of the extracellular matrix (ECM) [26]. These ECM proteins can also become modified by (early and late) glycation, which is discussed in another chapter in this book.

Glycation of Apolipoproteins in Lipoproteins

Within lipoproteins, apolipoproteins are major sites of glycation. Theoretically any amino compound with at least one hydrogen atom on its nitrogen can participate in the Maillard reaction. Chemically, within a protein moiety, only amino acids with one or more nucleophilic residues (lysine (Lys), arginine (Arg), cysteine (Cys), methionine (Met), and histidine (His)) are likely to become glycated. Although the amino acid cysteine is the strongest nucleophile, Lys residues are particularly abundant in apolipoproteins [27] and thus are the preferred site of glycation. For example,

apoA-I, found in HDL, contains 243 amino acids residues, including three Met, 21 Lys, five His, and 16 Arg residues, but no Cys residues. ApoB-100, found in VLDL, LDL, and Lp(a), contains 4563 amino acids residues: 79 Met, 356 Lys, 114 His, 150 Arg, and only 25 Cys (0.5%). The extent of lipoprotein glycation will depend on (1) the time of lipoprotein exposure to the glycating agent, which may in turn be influenced by the location of the lipoprotein being glycated (e.g., intra- or extravascular); (2) the concentration of the glycating agent; (3) the potency of the glycating agent; and (4) the efficacy of any deglycating or anti-glycating factors. The nature of the glycating agent determines the type of glycation products formed. For example, protein glycation with glucose leads to the formation of the late glycation product CML, whereas protein glycation with methylglyoxal results in formation of CEL [28]. In humans, the major circulating glycating agent is glucose in an open chain form [26]. Circulating levels of glucose in non-diabetic subjects average about 5 mmol/L while that of methylglyoxal is about 147 nmol/L [29]. In addition, glycating agents may also act on amino acids in both the extracellular and intracellular milieu.

Extent of Lipoprotein Glycation

The extent of lipoprotein glycation usually correlates with other measures of glycemia such as HbA1c and fructosamine [4, 30, 31], which are widely available assays in clinical laboratories. Any inconsistencies in the level of correlation may relate to differences in half-lives of the glycated protein moieties, methodologies for the quantification of lipoprotein glycation (discussed below), the range of glycemia related values in the study group, and the actions of any deglycating factors. The half-life of lipoproteins is days, while HbA1c from within red blood cells reflects glycemia over the previous 2–3 months, hence it is probable that the extent of lipoprotein glycation is more strongly correlated with shorter term measures of glycemia over days, such as mean glucose levels (perhaps measured by Continuous (Interstitial Fluid) Glucose Monitoring (CGM) or frequent finger-prick blood glucose monitoring), or by 1,5 anhydroglucitol levels [32]. We are not aware of any such comparative studies.

The Measurement of Lipoprotein Glycation

The quantification of glycated lipoproteins is currently a research laboratory tool. Various techniques have been used and predominantly applied to LDL and HDL. The most specific measure is the direct quantification of *fructoselysine* (an early glycation product) in lipoproteins by High Pressure Liquid Chromatography (HPLC) [33], which requires the physical separation of lipoproteins by ultracentrifugation. We have utilized this technique to study lipoproteins from diabetes patients [34, 35].

Glycated proteins, such as albumin, and glycated lipoproteins bind to boronate, so *boronate affinity chromatography* has been used in both a preparative manner [36] and in a rapid relatively simple HPLC and gel permeation column-based assay, developed by Tanaka et al. [37] which has been used to quantify glycated LDL and HDL from small volumes (5 μL) of serum.

Antibodies to glycated apoB have also been developed and used in in-house ELISA assays [38] and in a commercially available indirect competitive ELISA (Glyacor, Exocell, Philadelphia, PA). In this assay, a monoclonal antibody (ES12) is directed against a specific epitope in apoB in glycated LDL and does not cross-react with other human plasma proteins, including non-glycated LDL. The assay range is 3–40 μg/mL (corresponding to 0.3–4 mg/dL) in serum. Other antibodies have also been used to quantify glycated HDL and glycated Lp(a) [39]. Unlike purely glycated unoxidized lipoproteins, AGE-modified lipoproteins have increased electrophoretic mobility [40], a technique usually used for the characterization of physically separated isolated lipoproteins. AGEs can also be quantified by Gas Chromatography/Mass Spectroscopy (GC/MS) [33, 34, 41] in separated lipoproteins or in long-lived proteins such as skin and ocular lens tissue. An AGE-LDL antibody-based capture assay has also been developed [42] and used to quantify AGE-LDL in Type 1 diabetes. A less specific biochemical tool to measure the extent of lipoprotein glycation is the TNBS (trinitrobenzene sulfonic acid) assay [43]. The TNBS assay measures the amount of free Lys in a protein. As mentioned earlier, Lys is the most abundant amino group in human lipoproteins and is a strong nucleophile (Lys is the only one amino acid with two amino groups: alpha and epsilon). Unfortunately, due to the secondary and tertiary structure of proteins not all Lys residues (regardless of whether free or modified) are always available for reaction and therefore detection by the TNBS assay.

There is great interest in precision medicine, including the use of proteomics, lipidomics, and metabolomics in medicine, including in insulin resistance, prediabetes, and diabetes and its complications [44–48]. Such research tools usually detect small molecules using mass spectroscopy techniques and can detect glycating agents, such as methylglyoxal, but not intact glycated lipoproteins. Lipidomics detects small lipid species such as phospholipids, ceramides, and sphingolipids. We are not aware of any "omics" studies quantifying glycated lipoproteins or their breakdown products and correlating them with other measures of glycated lipoproteins. The development and validation of low-cost high throughput assays relevant to lipoprotein glycation would expedite this area of clinical research.

General Consequences of Lipoprotein Glycation

The potential consequences of increased lipoprotein glycation are summarized in Table 11.1. These include effects on lipoprotein metabolism (such as on their half-life in the circulation) and on cell interactions and responses, including effects related to important processes (e.g., systemic and vascular inflammation,

Table 11.1 Adverse effects of lipoprotein glycation

Effects on circulating half-life of lipoproteins
Foam cell formation
Increased matrix binding
Pro-oxidant and reduction of antioxidant effects
Pro-inflammatory or reduced anti-inflammatory effects
Pro-apoptotic effects
Effects on lipoprotein related enzyme activities
Altered lipoprotein receptor interactions
Cell signaling effects
Effects on gene expression
Promotion of antibody and immune complex formation
Altered reactivity in assays

thrombosis, vasoreactivity) relevant to the neurovascular complications of diabetes. Lipoproteins modified by glycation and by oxidation and extravasated are more likely to bind to vascular matrix, such as proteoglycans, than unmodified lipoproteins [49]. Tsmikas et al. demonstrated that the concentration of oxidized LDL in the arterial wall is 70-fold that in the circulation [50], but we are not aware of similar studies related to glycated lipoproteins. Matrix binding of lipoproteins is discussed in more detail in another book chapter herein.

It is important to recognize that even normoglycemic people have some lipoproteins that undergo non-enzymatic glycation, and that more extensively modified (late glycation or AGE modified) lipoproteins, may not remain in the circulation very long. Indeed AGE-modified lipoproteins are likely to exist predominantly outside the potent antioxidant milieu of blood in the extravascular spaces (of arteries, the retina, and renal beds), being rapidly removed from the circulation by pathways such as scavenger receptors in liver and in white blood cells. Antioxidants in blood include albumin, urate, bilirubin, and vitamin C [51], all of which are water soluble. Some fat-soluble vitamins, which can be carried within the lipoproteins (e.g., Vitamin E) are also antioxidants [52]. The low concentrations of modified lipoproteins in the circulation (relative to unmodified lipoproteins) may reflect both that formed within blood and that has effluxed from the extravascular bed.

Another challenge in this area of research is that in vitro modified lipoproteins studied in the laboratory setting may be differentially or more extensively modified than that occurring in vivo. Often the glucose or reactive intermediate (e.g., methylglyoxal) concentrations and incubation times used in the laboratory are well beyond that present in people. Later in this chapter, we will point out some studies in which both in vivo and in vitro glycated lipoproteins were studied, with divergent responses.

In the literature related to in vitro modified lipoproteins, the term glycation is often used loosely, not specifying if it is early or late glycation and there is often insufficient characterization to confidently discern which type of glycation is

present. Both may coexist. The effects of early glycation and late glycation often differ. For example, in in vitro studies of modified LDL on cultured retinal or renal cells by Lyons et al. both LDL modified by early glycation (glycated LDL) and LDL modified by late glycation (Heavily oxidized glycated LDL (HOG-LDL)) have been studied. HOG-LDL effects were generally significantly greater than that of less extensively modified glycated LDL [53–56]. Ideally researchers should present data related to the preparation and characterization of the modified lipoproteins they have studied. The in vitro modification of lipoproteins by early glycation alone requires the presence of metal chelating antioxidants, such as EDTA and DTPA in adequate concentrations and reduced exposure to oxygen such as may be achieved by incubation under nitrogen or argon and dialysis against nitrogen purged buffers [7]. In general, if there is increased electrophoretic mobility of lipoproteins on agarose gels, or increased AGEs or lack of recognition of modified LDL by the classical LDL receptor, then the glycation is more advanced (late glycation).

While in vivo studies, including longitudinal human or animal studies, can also be informative as to the effects of lipoprotein glycation, we must evaluate their findings while also recognizing that improved glycemic control may use lifestyle changes and drugs which may have direct effects on lipoprotein related gene or protein expression or other pleiotropic effects, and that more than just glycemia (and lipoprotein glycation) may change. Factors such as oxidative stress, inflammation, and cell signaling may also change. Furthermore, many of the particularly relevant sites of change induced by lipoproteins or modified lipoproteins, such as within the vascular wall, in the retina or within glomeruli or renal tubules, may not be accessible for sampling, particularly in living humans.

Human Studies of Glycated Lipoproteins

Glycated lipoproteins, particularly those modified by early glycation, are present in the circulation of both non-diabetic and diabetic people at relatively high concentrations [57, 58]. Durrington et al. have demonstrated that circulating levels of glycated apoB (which may reflect glycated apolipoprotein B within LDL, VLDL, VLDL remnants, Lp(a), chylomicrons, and chylomicron remnants) are increased in conditions in which LDL is raised, such as heterozygous familial hypercholesterolemia [57]. As with hyperglycemia itself, which is the hallmark of diabetes mellitus, enhanced lipoprotein glycation occurs from diabetes onset, and likely during the pre-diabetes phases the precede both Type 2 and Type 1 diabetes diagnosis. This likely reflects both an increase in the number of glycated amino acids per lipoprotein particle and also a greater proportion of lipoprotein particles with glycated residues. Based on our studies of in vivo glycated LDL as assessed by boronate affinity chromatography, in non-diabetic subjects approximately 5% of LDL particles are sufficiently glycated to bind to these columns (and have increased fructoselysine levels), whereas in people with diabetes (depending on their level of glycemic control) up to 25% of LDL may bind to the boronate affinity columns [34, 36]. Even within an individual,

the extent of glycation of lipoproteins will likely vary, in the same way that not all LDL, HDL, or VLDL particles are the same size [59, 60]. At any given time, circulating lipoproteins will include some that are newly secreted, hence are likely to be less glycated, and lipoproteins that are several days older, hence more likely to be more glycated. Ambient glucose levels which can fluctuate substantially over days, even hours, particularly in Type 1 diabetes, and lipoprotein size, apolipoprotein content, and chemical composition are also likely to affect the extent of lipoprotein glycation. For example, Younis et al. demonstrated that small (protein rich, lipid poor) LDL is more likely to undergo in vitro glycation than larger LDL [58].

Glycation of Major Lipoprotein Classes

The adverse effects of the early and late glycation of LDL and HDL on vascular endothelial cells were well-reviewed by Toma et al. in 2021 [61]. Implicated mechanisms related to promotion of oxidative stress, inhibition of antioxidant defences, reduced nitic oxide (NO) bioavailability, enhanced monocyte adhesion, impaired fibrinolysis, increased endothelial cell apoptosis and endoplasmic reticulum (ER) stress [61]. Other aspects discussed below include increased matrix binding and immune complex formation.

VLDL Glycation

While hypertriglyceridemia is common in people with Type 2 diabetes and in those with Type 1 diabetes and poor glycemic control, obesity, or kidney damage, there are few studies of VLDL glycation.

Levels of Glycated VLDL

Using a simple and non-specific agarose gel electrophoresis assay for glycated lipoproteins in sera from diabetic and non-diabetic subjects, levels of glycated VLDL were estimated to be four-fold higher in diabetes subjects and higher in diabetic patients with vs. without clinically evident atherosclerosis [62].

Effects on VLDL Metabolism

Hypertriglyceridemia may relate to both increased hepatic VLDL production and delayed VLDL clearance. In keeping, in in vivo VLDL kinetic studies in rodents, the clearance of triglycerides and apoB of in vitro glycated VLDL was slower than that from normal VLDL. Also in in vitro studies, the glycated VLDL was a poorer substrate for lipoprotein lipase [63].

There are several studies comparing VLDL from diabetic subjects and non-diabetic subjects which demonstrate that VLDL from people with Type 1 and Type 2 diabetes has a different lipid and apolipoprotein content from that of non-diabetic subjects, and within the same Type 1 or Type 2 diabetic patient can differ when their glycemic control is poor vs. improved, and is associated with increased rates of cholesteryl ester synthesis by human monocyte-derived macrophages [64–66] and endothelial cells [64–67]. Levels of or the extent of VLDL glycation were not quantified in these studies of modified VLDL.

LDL Glycation

Studies of LDL glycation are more numerous than those of other lipoprotein fractions, likely because LDL is highly atherogenic, especially when modified, and is usually the most abundant lipoprotein in blood and in atherosclerotic plaque.

Levels of Glycation

Relative to non-diabetic people, the levels of glycated LDL are increased (by approximately 50% to several fold) in Type 1 and Type 2 diabetes subjects and usually correlate with other measures of glycemia or with LDL-C levels, the two major required substrates for LDL glycation [38, 57, 68–70]. In people with Type 2 diabetes, levels of AGE-LDL were also elevated (about three-fold) relative to non-diabetic subjects and were lower in diabetic patients taking metformin than in those not on metformin [70]. Levels of circulating glycated LDL have been shown to be higher in people with Type 2 diabetes fed a high-AGE diet than in low-AGE diet fed diabetic and non-diabetic subjects [71].

LDL Size and Glycation

Small dense LDL is more atherogenic than larger more buoyant cholesterol-rich LDL particles [4]. There are divergent results from studies relating LDL size and LDL glycation. Glycated LDL (in the absence of LDL antibodies) has a longer residence time in the circulation than non-glycated LDL [72], thus may be smaller due to further lipolysis and lipid exchange. By evaluating in vivo modified and in vitro glycated LDL particles, some studies suggest that small dense LDL is more susceptible to glycation [58, 73]. Isolated LDL modified in vitro with methylglyoxal to form AGE-LDL was also significantly smaller than unmodified LDL [74]. However, in adults with Type 1 diabetes, using NMR spectroscopy we found no significant difference in the size of their in vivo glycated and relatively non-glycated LDL separated by boronate affinity chromatography [34].

Susceptibility to Oxidation

Oxidized LDL is more pathogenic than unmodified (native) LDL. Results of studies of the effects of LDL glycation on LDL's susceptibility to oxidation are divergent, perhaps related to differences between in vivo and in vitro modification, the type, concentration, and exposure time to the pro-oxidant, and the assays used to quantify oxidation. Tsai et al. demonstrated increased susceptibility of LDL from Type 1 diabetic patients with poor glycemic control to in vitro (copper-induced) oxidation [75]. This was not so in our study of complication-free Type 1 diabetic subjects with relatively good glycemic control, in which the lag time of LDL from Type 1 diabetic and non-diabetic subjects was similar [76]. We also determined the in vitro susceptibility to copper-induced oxidation of glycated LDL (G-LDL) and relatively non-glycated LDL (NG-LDL) prepared by boronate affinity chromatography from 13 subjects with Type 1 diabetes. Lipid soluble antioxidant levels did not differ between the two subfractions, in keeping with a lack of increased oxidative stress to G-LDL in plasma. The lag time to in vitro oxidation of the G-LDL was significantly less than that of the non-glycated LDL subfraction. There were no significant differences in the rate of or extent of lipid oxidation during the reaction, nor did the lag time, rate, or extent of protein oxidation of the two LDL subfractions differ [34]. In cross-sectional analyses of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort, we did not observe any statistically significant relationship between LDL susceptibility to lipid or protein oxidation and HbA1c and severity of diabetic nephropathy or retinopathy [77].

Glycated LDL and Immune Complex Formation

Antibodies to, and immune complexes with, modified lipoproteins such as glycated and AGE-modified LDL are implicated in human vascular damage. Modified lipoproteins themselves are pro-inflammatory, but when in immune complexes, they are even more pathogenic. Such immune complexes can increase foam cell formation and have pro-inflammatory effects, features of microvascular complications as well as atherosclerosis [78]. In Type 1 diabetes subjects of the DCCT/EDIC, cohort levels of AGE-LDL in circulating immune complexes are associated with and predict progression of carotid intima-media thickness [79] and also predict progression of diabetic retinopathy [80].

Matrix Binding by LDL

Lipoprotein matrix interactions, also discussed in another book chapter herein, may promote atherosclerosis and may also accelerate diabetic nephropathy by binding to glomerular matrix and affecting renal cell signaling [76]. Similar changes may also occur in the retina, where leaky retinal vessels and lipoprotein extravasation are a

feature. Matrix binding and retention of LDL and of glycated and/or oxidized LDL are thought to increase LDL's likelihood of further modification by glycation, oxidation, and AGE formation in vivo.

In vitro generated AGE-LDL has been found to be smaller and to bind more avidly to proteoglycans than unmodified LDL [74]. Using an in vitro model system of binding to arterial wall proteoglycans, Edwards et al. demonstrated that improved glycemic control in Type 2 diabetes patients reduced LDL proteoglycan binding, even in the absence of significant improvements in lipid levels. LDL glycation (fructosamine) was the only LDL compositional variable that correlated significantly ($r = 0.95$) with the proteoglycan binding [77].

Effects on Receptor Interactions and Cell Signaling

Lipoprotein glycation can change LDL's cell-based receptors and responsive cell signaling pathways in cells relevant to the vascular complications of diabetes. In general, LDL modified by early glycation can still interact with the classical LDL receptor on cells, as does unmodified (native) LDL, but with increasing degrees of glycation major pathways of cellular uptake are via scavenger receptors, the Receptor for AGEs (RAGE), and by endocytosis [53, 78–80]. Glycated LDL was isolated from diabetic and non-diabetic subjects. In cultured human fibroblasts, which express only the classical LDL receptor, the rates of receptor-mediated accumulation of relatively non-glycated LDL from both subject groups were greater than those of glycated LDL. In contrast, when incubated with human monocyte-derived macrophages, the rates of receptor-mediated accumulation of glycated LDL from both groups were significantly greater than those of non-glycated LDL [36].

We exposed cultured rat mesangial cells to native LDL or to LDL modified (in vitro) by early glycation or by extensive oxidation and glycation (AGE-LDL). Glycated LDL was taken up via the classical LDL receptor, induced a transient intracellular calcium spike and marked extracellular signal-regulated protein kinase (ERK) activation. AGE-LDL, recognized by the scavenger receptor, induced a sustained rise in intracellular calcium and less marked ERK activation [53]. In cultured human vascular smooth muscle cells relative to native LDL, AGE-LDL significantly increased protein and/or gene expression of receptors for modified LDL and AGE proteins (LRP1, CD36, and RAGE), which was associated with adverse cellular responses related to oxidative stress and cell proliferation [79].

Adverse Cellular Effects of Glycated LDL

Early and late glycation of LDL has been demonstrated to have many adverse cellular effects which may promote macro- and microvascular damage in diabetes. Most studies involve cultured monocytes, or arterial, retinal, and glomerular cells exposed to in vivo or in vitro glycated LDL. Adverse cellular responses include foam cell formation, cell proliferation or death (commonly by apoptosis), matrix overproduction (of particular relevance to glomerulosclerosis), pro-inflammatory

effects, and (discussed in subsequent sections in this chapter) impaired vasorelaxation and pro-thrombotic effects.

Macrophages are implicated in atherosclerosis and also in diabetic microvascular damage. Lopes-Virella et al. demonstrated that human monocyte-derived macrophage had increased cholesteryl ester accumulation when exposed to in vivo modified LDL from diabetic subjects, or to in vitro glycated LDL [30, 35]. Several groups demonstrated increased cholesterol uptake and cholesteryl ester accumulation by macrophages in response to glycated LDL, with greater effects of more extensively modified LDL, such as AGE-LDL generated by glycolaldehyde [80–82].

In cultured human vascular endothelial cells (HUVEC) in vivo and in vitro, glycated LDL can induce apoptosis [83] and in vitro generated AGE-LDL can increase expression of monocyte chemoattractant protein (MCP) [84], which may also promote atheroma. AGE-LDL induced MCP-1 expression in cultured human endothelial cells has been shown to be ameliorated by the PPARα agonist lipid drug fenofibrate, and by the anti-platelet agent dilazep, both of which suppressed the AGE-LDL induction of NFκB [85].

With regard to cultured microvascular cells, Lyons et al. demonstrated reduced cell viability of retinal capillary cells after exposure to in vitro glycated vs. native LDL [86] and reduction in this cytotoxicity by the in vitro glycation of LDL in the presence of the AGE inhibitor aminoguanidine [87].

While we found that glycated LDL did not reduce mesangial cell viability, it increased mesangial cell TGFβ mRNA expression and induced hemeoxygenase-1 (HO-1) expression, an intracellular marker of oxidative stress (personal communication A Jenkins). Others have demonstrated altered mesangial cell modified LDL binding and increased matrix (e.g., fibronectin and laminin) production by cultured mesangial cells exposed to glycated LDL than to native LDL [88–92]. These changes may promote glomerulosclerosis, a major feature of diabetic nephropathy.

Glycated LDL Effects on Modulators of Fibrinolysis

Exposure of cultured human vascular endothelial cells to in vitro glycated LDL increases PAI-1 production [93, 94]. This process is via activation of the PAI promotor [95] and involves the Golgi apparatus [96] and RAGE [97] and decreases generation of tissue plasminogen activator (tPA) [94]. In contrast, using in vivo modified LDL from people with Type 1 diabetes separated by boronate affinity chromatography into glycated and relatively non-glycated LDL subfractions, the production of PAI-1 and tPA by cultured human aortic endothelial cells did not differ significantly [34]. The different responses may relate to different extents of LDL glycation and cell types.

Glycated LDL Effects on Platelet Reactivity

Platelet hyperactivation is a common feature of diabetes and may promote thromboses in both large and small vessels. LDL that was AGE modified in vitro and LDL from Type 2 diabetic patients with poor glycemic control stimulated platelet

p38MAPK phosphorylation and thromboxane B2 production [98]. Another group demonstrated that relative to native LDL in vitro glycated LDL increased platelet TBARS levels (a measure of oxidative damage), NO production, intracellular calcium levels, and ADP-induced aggregation [99].

Glycated LDL Effects on Vasoreactivity

Glycated LDL can also impair vascular reactivity. While early glycation of LDL (without oxidation) had no effect on aortic ring acetylcholine-induced endothelium-dependent relaxation, AGE-modified LDL attenuated their vasorelaxation to an even greater extent than Ox-LDL [100]. In keeping with these results, AGE-LDL impaired acetylcholine-induced endothelium-dependent vasorelaxation of isolated mouse aortas, which was prevented by pharmacological inhibition of calpain. Exposure of bovine aortic endothelial cells to this same type of AGE-LDL reduced eNOS protein levels in a dose and time-dependent manner, without altering eNOS mRNA levels, increased intracellular calcium and reactive oxygen species (ROS) production [101].

In cultured porcine aortic endothelial cells exposed to in vivo glycated LDL and relatively non-glycated LDL from diabetic and non-diabetic subjects (separated by boronate affinity chromatography), the glycated LDL increased superoxide release by five-fold relative to the non-glycated LDL [102].

Both in vivo modified LDL from diabetic patients and in vitro glycated LDL caused vasoconstriction of arterioles in skeletal muscle of living mice [103], in keeping with similar adverse effects on vascular tone in the microvasculature.

HDL Glycation

Glycation of HDL in diabetes may ameliorate the efficacy of some of HDL's vasoprotective functions, which include reverse cholesterol transport, antioxidant, anti-inflammatory, anti-thrombotic, and vasodilatory effects. As with other lipoprotein subclasses, there is an admixture of studies using in vivo and in vitro modified HDL, including some studies of in vitro modified HDL use glycating agent concentrations or incubation times which may not occur in vivo.

Levels of Glycated HDL

Relative to that in non-diabetic subjects, the level of glycation of HDL is increased about four-fold in people with Type 1 or Type 2 diabetes and correlates with other measures of glycemic control. While all HDL associated apolipoproteins are glycated, about 80% of HDL glycation is located on apoA1. In in vitro studies for any given glucose concentration, the extent of apoA1 glycation was significantly greater in the presence of phospholipids [104].

Antioxidant Effects of HDL

The antioxidant effects of HDL can be assessed by measuring the susceptibility to efficacy of HDL in breaking down preformed lipid peroxides. Oxidation is implicated in the formation of late glycation (AGE) products, which also occur in HDL. The literature is divergent as to the effects of HDL glycation of its susceptibility to oxidation, which may relate to different oxidation techniques and measures of oxidation. Using 50 mM D-glucose, aluminum, and iron, one group demonstrated increased oxidative damage in glycated HDL [105], while another group found that glycated HDL was less, not more susceptible to in vitro oxidation by copper based on a xylenol orange assay, with no difference in levels of induced conjugated dienes or thiobarbituric acid reactive substances (TBARS) [106].

In people with Type 2 diabetes and diabetic nephropathy, serum AGE levels were increased and isolated (in vivo modified) HDL was less effective than that from non-diabetic subjects in protecting against ex vivo LDL oxidation (induced by DCFH), however the extent of HDL glycation was not reported [107].

Using in vivo and in vitro modified HDL and oxidized red blood cell (RBC) membranes, we found that the efficacy of HDL to remove preformed lipid peroxides (LPO) from RBC membranes was significantly impaired with HDL from adults with complication-free Type 1 diabetes relative to healthy subjects [108]. We did not quantify HDL glycation, but relative to unmodified HDL in vitro glycated HDL from non-diabetic subjects did not have impaired LPO removal efficacy, while AGE-modified HDL did, suggesting that late but not early glycation may be deleterious [108]. In a similar model system, HDL from Type 2 diabetes patients with in vivo glycated paraxonase-1 (PON-1) was less able to break down preformed LPO, with in vitro AGE modification having greater function effects on this HDL function than in vitro HDL glycation [109].

HDL Effects on Modulators of Fibrinolysis

In people with diabetes, circulating levels of PAI-1 are often increased, and in cultured vascular endothelial cells, Shen et al. demonstrated that glycated HDL increased HUVEC PAI-1 production, while unmodified HDL had no effect. Neither native nor glycated HDL altered endothelial cell tPA production [94, 95]; however, in HUVEC cell culture, the effects of HDL from non-diabetic and diabetic patients on tPA or PAI-1 production were similar. If HDL glycation has such an effect in vivo, this could promote thrombosis.

HDL Effects on Vasoreactivity

HDL can have vasodilatory effects. In a rabbit aortic ring model HDL from Type 1 diabetic patients could not attenuate the inhibitory effects of oxidized LDL on endothelial dependent vasodilatation as well as HDL from non-diabetic subjects.

However, this effect was not correlated with HDL-fructosamine levels (reflecting HDL glycation) or other systemic measures of glycemia [110].

Reverse Cholesterol Transport

The transport of cholesterol from cells to HDL and then to the liver is one of the more well-known functions of HDL. Results of studies related to the effects of HDL glycation on this process are divergent, which again may reflect the extent of HDL glycation and the model systems used. In general, reverse cholesterol transport is thought to be impaired in people with Type 2 diabetes and in mouse models of diabetes, but some investigators have reported greater cholesterol efflux with HDL from Type 2 diabetic subjects than from non-diabetic subjects, but no measures of HDL glycation were reported [111]. In a model of cholesterol efflux from mouse, peritoneal macrophages HDL from Type 1 diabetes subjects had impaired cholesterol efflux, but this did not correlate with measures of HDL glycation, nor was the function of in vitro glycated HDL impaired [112]. In another study of in vitro glycated HDL, its ability to promote cholesterol efflux was not significantly altered [106].

In an in vivo model of macrophage-to-feces, RCT HDL-mediated cholesterol efflux was reduced (about 20%) in Type 1 diabetic rodents vs. non-diabetic rodents, with unchanged cholesterol efflux to diabetic HDL but lower SR-BI mediated uptake from Type 1 diabetic HDL. Both in vitro and in vivo experiments supported effects due to HDL glycation [113].

Anti-inflammatory Effects of HDL

Another role of HDL is inhibition of vascular inflammation, such as reflected by expression of endothelial cell adhesion molecules (CAMs), such as VCAM-1 and ICAM [114, 115]. CAMs promote the attachment, rolling, and ingress of monocytes into the vascular wall, and levels of circulating forms, such as soluble(s) VCAM-1, sICAM, and sE-selectin, are increased in people with 1 and Type 2 diabetes [116], and circulating CAM levels have been correlated with circulating HDL-C levels, but correlations with glycated HDL levels have not been reported. CAM expression is also implicated in diabetic nephropathy [117] and diabetic retinopathy [118], and serum levels can be acutely lowered by intensive insulin treatment [119], but levels of glycated HDL were not reported. In our rabbit studies of collared carotid arteries, the favorable suppression of vascular CAMs was attenuated by methylglyoxal glycated apoA1 and by apoA1 from diabetic patients relative to unmodified apoA1[120]. The collars caused intima/media neutrophil infiltration and increased endothelial expression of VCAM-1 and ICAM. Unmodified apoA1 infusions decreased neutrophil infiltration and CAM expression substantially, while in vitro glycated apoA1 was less effective at suppressing neutrophil infiltration and did not significantly lower CAM expression. The in vivo glycated apoA-I from

diabetic patients did not inhibit neutrophil infiltration or CAM expression. These reduced anti-inflammatory properties of glycated apoA1 were associated with reduced inhibition of NFκB and reactive oxygen species (ROS) formation [120].

In keeping, another group demonstrated that in vitro glycated and AGE-modified HDL, with increased levels of both fructoselysine and CML, had reduced PON activity and did not suppress oxidized LDL-induced monocyte adhesion to human aortic endothelial cells, as did unmodified apoA1 [121]. In contrast, in vitro glycation of HDL did not impair its ability to inhibit monocyte adhesion to cultured aortic endothelial cells [121]. Perhaps also related to CAM expression glycated HDL increased breast cancer cell adhesion to HUVEC and to extracellular matrix, implicating HDL glycation in cancer metastasis [122].

In another model of inflammation, using high glucose-induced redox signaling in human monocyte-derived macrophages, apoA1 inhibited glucose-induced oxidative stress (ROS generation, NADPH expression, Nox2, SOD 1, and superoxide production), while in vitro glycated apoA1 and that from Type 2 diabetic subjects was less effective and inhibiting oxidative stress [123]. In THP1 cells, human monocyte-derived macrophages and mouse RAW2647 cells native HDL can suppress lipopolysaccharide (LPS) induced cytokine (TNF-α and interleukin-1β(IL-1β) release, while in vitro (28-fold) and in vivo (four-fold) glycated HDL were significantly less effective than native HDL [124].

Lipoprotein(a) Glycation

The pro-atherogenic and pro-thrombotic lipoprotein lipoprotein(a) (Lp(a)), which is discussed in another book chapter, also undergoes non-enzymatic glycation in diabetes, and this may enhance its adverse vascular effects.

Levels of Glycated Lp(a)

In a small cross-sectional study using boronate affinity chromatography and immunonephelometry, serum levels of glycated Lp(a) were found to be increased (more than double) in Type 2 diabetes patients relative to non-diabetic subjects, and higher in those with vs. without diabetes complications, but the extent of apoB glycation within Lp(a) was relatively higher [125]. In keeping, Doucet et al. demonstrated (using boronate affinity chromatography and ELISA) that levels of glycated Lp(a) were about 50% higher in diabetic than non-diabetic patients, with apo(a) being less glycated than the apoB within Lp(a) [39]. Glycated Lp(a) levels correlated positively with HbA1c levels, in spite of the major difference in half-lives: days for Lp(a) and months for HbA1c. Their in vitro glycation studies demonstrated that Lp(a) was less susceptible to non-enzymatic glycation by glucose than LDL [39].

Susceptibility to Oxidation of Lp(a)

As often found with LDL, glycation of Lp(a) increases its susceptibility to in vitro copper-induced oxidation [126], but as yet there are no human studies with substantially different levels of glycemic control.

Effects on Lp(a) Glycation on Modulators of Fibrinolysis

Relative to native Lp(a), glycation (including late glycation) of Lp(a) increases the production of PAI-1 and PAI-1 mRNA expression in cultured HUVEC and human coronary artery endothelial cells and suppresses tPA synthesis and secretion (but not mRNA expression). These changes are attenuated by the AGE inhibitor aminoguanidine and by the lipid soluble antioxidant butylated hydroxytoluene (BHT) [127], implicating the importance of combined glycation and oxidation (AGE modification) in lipoprotein function. If these types and extent of Lp(a) modifications occurred in vivo, they may impair fibrinolysis and promote vascular thrombosis and clinically evident vascular events.

Effects of Glycated Lp(a) on Vascular Reactivity

In people with diabetes, vascular reactivity is usually impaired, contributed to by reduced nitric oxide (NO) bioavailability (which is also discussed elsewhere in this book). In a model system of isolated rat aortic rings, glycated Lp(a) without concomitant oxidation did not impair acetylcholine (Ach)-induced endothelium-dependent vasodilation, while oxidized Lp(a) and AGE-modified Lp(a) did, with AGE-Lp(a) having the most deleterious effects. The likely mechanism is by increased superoxide formation, which may inactivate NO [126].

Glycation of Lipoprotein Related Enzymes

Lipoprotein related enzymes, found on the lipoproteins themselves and on cells with which they interact, mediate exchange of constituents between lipoproteins, alter lipoprotein composition (e.g., by cholesterol esterification), and have antioxidant effects. Glycation may affect these enzymes directly by modification of their amino acid components, by altering their reactivity with their glycation modified lipoprotein substrates or receptors, or by a combination thereof. The role of altered activity of these enzymes due to glycation and their potential as a therapeutic target for amelioration of diabetes vascular complications has not been fully delineated. We now review studies of the effects of glycation on some important lipoprotein related enzymes, including Platelet Activating Factor Acetylhydrolase (PAFAH), located mainly on LDL, and of paraoxonase (PON), Lecithin-Cholesterol Acyl

Transferase (LCAT), and Cholesteryl Ester Transfer Protein (CETP), which are predominantly located on HDL.

Platelet Activating Factor Acetylhydrolase (PAFAH)

The enzyme PAFAH, which is also known as lipoprotein-associated phospholipase A(2), hydrolyzes and inactivates the lipid mediator Platelet Activating Factor (PAF) and/or oxidized phospholipids. PAF is a phospholipid that activates neutrophils, macrophages, platelets, and smooth muscle cells and increases vascular cell adhesion molecule (CAM) expression and vascular permeability. Increased PAF and/or decreased PAFAH levels or activity have been associated with atherosclerosis and inflammation [128]. PAFAH circulates on LDL and to a lesser extent on HDL and can inhibit lipoprotein oxidation [128, 129], but there are few studies of the effects of lipoprotein glycation on PAFAH. Serum PAFAH activity levels have been found to be increased in people with Type 1 diabetes [130–132] and with Type 2 diabetes [133] relative to non-diabetic subjects, and to be increased in people with kidney failure [134], perhaps as a compensatory protective response. PAFAH activity in diabetes correlated with LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) levels in both common forms of diabetes [130–132, 135] and correlated inversely with HbA1c levels in Type 1 diabetes [130]. While serum PAFAH activity in Type 1 diabetes correlates with LDL susceptibility to oxidation and with oxidized LDL levels [132, 135], the relationships between lipoprotein glycation and PAFAH are not yet reported.

Paraoxonase (PON)

There are three PON genes and related proteins [136]. PON1 and PON3 proteins are located on HDL and have protective effects against LDL oxidation. PON2 is also implicated in vascular damage in diabetes [137], but is not known to be associated with lipoproteins. The glycoprotein PON1 is predominantly synthesized in the liver, is located in tissues, in particular the kidney [138, 139] and in serum is located exclusively on HDL [140], with a preference for certain apoJ containing and smaller HDL subclasses [141, 142]. PON protects against exogenous organophosphate poisons and in vivo is thought to hydrolyze phospholipid oxidation products [138], homocysteine, thiolactone [143], “statins” [144] and to protect against modifications of lipoproteins and cell membranes. Acute-phase HDL is less protective against LDL oxidation: this type of HDL has greatly reduced PON1 activity [145]. PON1 activity is usually assessed in vitro by hydrolysis of the artificial substrates of paraoxon and phenylacetate [138] and lactones [146].

A major determinant of PON activity are PON genotypes, which have also been associated with cardiovascular disease in the general [138, 147] and diabetic [148,

149] populations, and with diabetic retinopathy and nephropathy [150–152]. PON genotype may also modulate glycemia in both non-diabetic [153] and diabetic subjects [154, 155], which in turn may affect glycation of all lipoprotein classes.

PON protein levels are usually normal in diabetes [156, 157], but there is reduced serum PON activity in people with Type 1 and Type 2 diabetes [151, 156, 157]. In some cross-sectional studies, serum PON activity is lower in diabetic subjects with neuropathy [158], retinopathy [159], and nephropathy [160], but not in others [154]. PON activity in humans can be increased by statins and fibrates [136, 161].

Mackness et al. postulated that the low PON1 activity observed in diabetes is due to non-enzymatic glycation [154], which is in keeping with in vitro studies [157] or a circulating inhibitor of PON [156]. HbA1c and serum PON activity were not well-correlated in our cross-sectional studies [155], but this may relate to major differences in their half-lives. Shorter term measures of glycemia (e.g., glucose records over a few days, such as by CGM) are preferable because they correspond more closely to the (several days) half-life of PON. Longitudinal studies of improved glycemic control and PON activity and lipoprotein glycation are also desirable.

Lecithin: Cholesterol Acyl Transferase (LCAT)

LCAT, a glycoprotein produced by the liver, is preferentially bound to circulating HDL and is also found on VLDL and LDL [114]. LCAT which catalyzes esterification of free cholesterol to cholesteryl ester and may also hydrolyze oxidized lipids, is the rate-limiting enzyme in reverse cholesterol transport [162]. LCAT activity is inhibited by HDL_2, lipid peroxidation products [163–165], and activated by apoA-I and apoA-IV, both of which may become glycated. LCAT activity is decreased in both Type 1 and Type 2 diabetes subjects [166, 167] and in uremia [168]. While some have found that LCAT activity and glycemia do not correlate in diabetes [169]. Nakhjavani et al. found that LCAT activity and HbA1c were negatively correlated (rho = 0.951) in Type 2 diabetes subjects, and on multivariate analysis, HbA1c was a strong independent predictor of LCAT activity [170]. LCAT activity and oxidized LDL levels in serum also correlated, but relationships between LCAT and glycated lipoproteins were not reported [170]. In longitudinal studies, LCAT activity decreases with glycemia improved by insulin [171, 172], but not by diet or sulfonylureas [171].

In 1995, Fournier et al. reported both in vivo and in vitro modified LCAT and its reactivity to non-diabetic and diabetic (in vivo glycated) HDL [173]. The kinetics of isolated non-diabetic LCAT activity varied according to the extent of in vitro LCAT glycation. Moderate glycation (<30% residues on the TNBS reactivity assay) increased Km and Vmax, while greater glycation reduced both Km and Vmax. At all levels of LCAT glycation, the LCAT reactivity was lower in the presence of in vitro glycated HDL, related to the extent of lysine glycation in (the potent LCAT activator) apoA1. With in vivo modified HDL (from people with diabetes) as LCAT substrate Km values were not altered, but Vmax and LCAT reactivity were reduced by about 30% [173]. These differences between in vitro and in vivo glycated HDL

may relate to physiochemical changes other than glycation. More recently in in vitro studies Nobecourt et al. demonstrated that methylglyoxal-induced late glycation of apoA1 impaired its ability to activate LCAT, which was ameliorated by the late glycation inhibitors aminoguanidine and pyridoxamine, the AGE breaker alagebrium, and the insulin sensitizer metformin [115].

Cholesteryl Ester Transfer Protein (CETP)

CETP, a glycoprotein, stimulates transfer of cholesteryl ester, triglycerides, and phospholipids between circulating lipoproteins, such that triglyceride-rich lipoproteins lose triglyceride and gain cholesteryl esters [114], and is a key enzyme in reverse cholesterol transport [174]. Synthesized by hepatocytes, adipose tissue, and arterial smooth muscle cells [175], CETP binds to VLDL, LDL, and HDL. CETP gene polymorphisms influence HDL levels and vascular disease [176]. The effects of glycemia and lipoprotein glycation on CETP activity have been studied. CETP activity is increased in people with Type 1 [177] and Type 2 diabetes [178] relative to non-diabetic subjects. In diabetes patients, subcutaneous insulin delivery activates, while intraperitoneal insulin delivery reduces, CETP activity [177]. Glycemia may influence CETP activity via non-enzymatic glycation of the enzyme [178] and via conformational changes which affect enzyme binding and lipid exchange. Passarelli et al. showed that in vitro glycated and in vivo glycated lipoproteins are associated with increased cholesteryl ester transfer rates from HDL to VLDL and LDL. While in vitro glycation of partially purified CETP markedly impaired its activity [178], greater lipid transfer rates were observed when in vivo glycated lipoproteins from diabetic subjects were used, which was attributed to glycation of HDL protein. Lemkadem et al. demonstrated that in vitro glycation of HDL3 (with glucose concentrations up to 200 mM) increased cholesteryl ester transfer, but kinetic studies showed a paradoxical increase in CETP activity associated with a decrease of CETP affinity. HDL lipid and protein composition was unchanged but its fluidity was decreased and its electronegativity increased, which may affect CETP reactivity [179].

CETP inhibitors substantially increase HDL-C levels in people with and without diabetes, but the first major clinical trial on the cardiovascular effects of CETP inhibitors was stopped early due to adverse off-label effects (hypertension) [180, 181]. The development of other CETP inhibitors is ongoing.

Treatment of Lipoprotein Glycation in Diabetes

General approaches that may reduce lipoprotein glycation are listed in Table 11.2. These include reduction in “substrate stress” by lowering levels of glucose (and other glycating agents, such as methylglyoxal) and of lipids, the inhibition of early

Table 11.2 Potential approaches to reduce lipoprotein glycation

Lower glucose levels
Lifestyle, e.g., diets such as low-AGE diets
Glucose control drugs, e.g., metformin, insulin, sulfonylureas, incretins, SGLT2 inhibitors
Lower lipid levels
Lifestyle
Drugs such as statins, fibrates, ezetimibe, resins, PCSK9 inhibitors
LDL apheresis
Combined glucose and lipid lowering drugs, e.g., colestimide
Inhibit glycation reactions
Early glycation, e.g., saponins, some nutrients
Late glycation, e.g., amadorins, antioxidants
Removal of preformed AGEs
AGE breakers
Increase deglycation
Deglycating drugs
Increase activity of deglycating enzymes

and late glycation reactions, the use of deglycating agents, and the removal of existent AGEs. Another strategy would be to modulate adverse cellular and extracellular matrix responses to glycated lipoproteins.

Improving glucose control in people with diabetes also reduces diabetic neurovascular complications [182–184]. As higher glucose variability is now known to be a risk factor for chronic diabetes complications and mortality [185–188], improving this aspect of glucose control is also likely important. The evaluation of glucose variability with glucose therapies in relationship to chronic complications should be evaluated in randomized controlled trials and observational studies. This is discussed in another book chapter herein (by Dr. Jenkins).

Improving glycemic control will also improve the traditional lipid profile and also reduce post-translational lipoprotein glycation, reducing substrate stress. Unfortunately in clinical practice achieving normoglycemia is often challenging related to availability, affordability, and efficacy of current glucose control drugs, insulin pumps, CGM devices, and patient and clinician fears of hypoglycemia. Hypoglycemia has also been associated with adverse cardiovascular effects via similar mechanisms as hyperglycemia, including increased oxidative stress, inflammation, and endothelial dysfunction [187, 189, 190].

Strategies that can reduce lipoprotein glycation, or the adverse cellular and enzymatic responses to lipoprotein glycation, even in the setting of hyperglycemia, are desirable. Apart from glucose lowering drugs and perhaps HMG CoA reductase inhibitors (statins) [191], there are currently no regulatory body approved therapies in routine clinical practice known to reduce lipoprotein glycation. Some studies suggest benefit of "nutraceuticals," such as Vitamin B group derivatives, carnosine, and caffeic acid (discussed below) which may reduce some forms of lipoprotein glycation or the adverse cellular responses to the glycated lipoproteins.

Glucose Control Agents

Prospective longitudinal studies such as the DCCT trial and UKPDS [182–184] have demonstrated that drugs, such as insulin and metformin which improve glucose control, are associated with reduction in chronic complications, and although not reported likely with lower levels of glycated lipoproteins [182–184]. Some, such as metformin, may also have pleiotropic effects such as antioxidant or anti-AGE effects [192]. This is most likely related to effects on lowering glucose levels and related improvements in the lipid profile and other pleiotropic effects.

More recently clinically available glucose lowering agents, sodium glucose transporter 2 inhibitors, which induce glycosuria via inhibition of glucose reabsorption by the renal tubules, have shown great benefit for reducing cardiovascular events, renal damage and mortality in people with diabetes, predominantly Type 2 diabetes [193–196]. Other benefits include weight loss, improved lipids, decreased insulin resistance, and improvement in non-alcoholic fatty liver disease [197, 198]. Several of this drug class are now approved in some countries for clinical use in Type 2 diabetes and in some countries for subgroups of adults with Type 1 diabetes. Major concerns relate to the risk of euglycemic diabetic ketoacidosis [199, 200]. As yet there are no published studies related to their effects on lipoprotein glycation, though reduction in glucose levels would likely translate to reductions in at least the levels of lipoproteins modified by early glycation.

Lipid Control

As discussed elsewhere in this book, improving the lipid profile is an important aspect of preventing the macro- and microvascular complications of diabetes, but other risk factor management is also important. Improved glycemia, weight control, a healthy diet, exercise, and non-smoking are important goals which will also improve the lipid profile, but often, lipid drugs are required to reach the low LDL targets proven to reduce cardiovascular risk. The benefits of statins and fibrates for cardiovascular and as secondary or tertiary outcomes for retinal and renal protection have been shown in prospective placebo-controlled randomized clinical trials, predominantly in Type 2 diabetes [201–209] and a meta-analysis by the Cholesterol Treatment Trialist Collaboration shows similar statin benefit for CVD and mortality reduction in adults with Type 2 and Type 1 diabetes [209]. More recently, as reviewed in other chapters in this book, other classes of lipid lowering drugs such as PCSK9 inhibitors [210], bempedoic acid [211] and ezetimibe [212] are also vasoprotective, but there are no reports of their effects on lipoprotein glycation.

While meta-analyses support that statins may increase glycaemia and risk of new onset Type 2 diabetes [213], there are few studies of statins on lipoprotein glycation. In a cross-sectional study, Younis et al. demonstrated lower levels of plasma glycated apoB in statin-treated type 2 diabetes patients compared with those not on

statins [214]. This may relate to changes in LDL levels rather than a direct effect on lipoprotein glycation. Longitudinal studies are preferable.

The anion exchange resin colestimide improves both glycemia and lipid levels in people with Type 2 diabetes, hence could be expected to reduce lipoprotein glycation, but as yet there are no related publications [215]. Conversely, nicotinic acid, particularly the rapid release preparations, while improving the lipid profile (in particular lowering VLDL and increasing HDL levels), can slightly worsen glycemia [216], so may increase lipoprotein glycation, but as yet there are no published data of glycated lipoprotein levels. Due to its side effects (worsening of glycemia and flushing) and availability of other potent lipid drugs, this drug class is infrequently used in clinical practice.

LDL Apheresis

LDL apheresis, originally used for the treatment of familial hyperlipidemia (FH), and more recently for refractory LDL-C elevations and cardiovascular disease, often in the setting of statin intolerance. Apheresis effectively lowers LDL and Lp(a) levels, including in people with diabetes, and has been shown to lower circulating levels of malondialdehyde (MDA) modified (oxidized) LDL [217–220], but again, there are no studies of the effects on glycated lipoproteins. The costs, need for specialized facilities, and availability of liver transplantation for homozygous FH, and other potent LDL and Lp(a) lowering drugs such as PCSK9 inhibitors have reduced the need for LDL apheresis.

Anti-glycation Agents, AGE Preventers, Decoys, and Breakers

Drugs which inhibit glycation reactions directly rather than by lowering glucose levels could also reduce lipoprotein glycation. Saponins and some other compounds identified in traditional Chinese medicines used for diabetes have demonstrated in vitro anti-glycation effects against model proteins such as albumin [221, 222], but we have not identified any studies related to lipoprotein glycation. There are more studies of the inhibition of late glycation than of early glycation of lipoproteins.

Effective glycation inhibitory compounds include those primarily with anti-AGE effects, such as aminoguanidine and pyridoxamine, and various drugs classes, some already in common clinical usage with pleiotropic antioxidant/anti-AGE effects. Progression to AGEs from the “early glycation” Amadori product requires chemical rearrangements to create reactive intermediates before the formation of AGEs, and drugs such as aminoguanidine can inhibit this process [87, 223–225]. Aminoguanidine has demonstrated favorable effects in cultured cell systems relevant to diabetes complications, including our work with LDL and retinal cells [87] and has prevented vascular complications in diabetic animal models [87]. In human studies, aminoguanidine achieved some success with lowering AGE-LDL [226, 227] and AGE-modified hemoglobin, decreasing albuminuria and slowing progression of

nephropathy and retinopathy [228, 229], but was poorly tolerated [230, 231]. Aminoguanidine inhibits AGE formation in a range of short and long-lived proteins, including lipoproteins [232, 233], and also inhibits a range of other important pathways, most notably nitric oxide production via eNOS [234–236], hence it is difficult to proportion benefit to its anti-AGE effects.

Another approach to AGE inhibition is to scavenge post-Amadori dicarbonyls and so inhibit conversion of the Amadori intermediates to AGEs [237]. Such agents are classed as "Amadorins." Examples include the vitamin B12 derivative pyridoxamine [238, 239] and benfotiamine, a lipophilic vitamin B1 (thiamine) derivative [240–246], which are usually well-tolerated oral medications. *Pyridoxamine* (Pyridorin™) inhibits formation of both AGEs and Advanced Lipoxidation End Products (ALES), including in lipoproteins. We demonstrated in in vitro studies of LDL oxidation that pyridoxamine decreased late, but not early glycation products [238]. In animal studies, pyridoxamine prevented renal dysfunction [247, 248] and retinopathy [249] in diabetic rats and also had favorable effects on lipid levels [250]. While effective for reducing AGEs, in a 4-week human trials benfotiamine lowered levels of CML in adults with diabetic nephropathy, but did not benefit renal function or renal biomarkers [240]. Levels of glycated lipoproteins were not reported in these studies.

Another means of reducing AGE formation is by lowering levels of the reactive dicarbonyl metabolite, methylglyoxal (MG), which is usually increased in diabetes and in obesity, and is implicated in the development of insulin resistance, Type 2 diabetes, and the vascular complications of diabetes. MG is an arginine-directed glycating agent and precursor of AGE, arginine-derived hydroimidazolone MG-H1. MG can be reduced by increasing expression of the deglycating enzyme glyoxalase-1 (Glo1), which can be induced by a combination of trans-resveratrol and hesperetin, which lowered MG, insulin resistance, and inflammation in overweight and obese subjects [251]. Levels of glycated lipoproteins were not assessed and such measures in future human studies particularly in diabetes are of interest.

Deglycating Agents

Deglycating enzymes and drugs could also reduce lipoprotein glycation. Comparisons of human and in vitro studies suggest that for a given ambient glucose level, people vary in their propensity to form glycation products [251, 252]. This may be tissue specific [253] and also relate to genes and/or activity of deglycating enzymes [254, 255]. We are not aware of any studies of glycated lipoprotein levels in relationship to enzyme activities or genotypes. While at least two categories of deglycation enzymes have been identified, fructosyl amine oxidases and fructosyl amine kinases, there are no papers related to their effects on lipoprotein glycation.

The prevention of AGEs, including those on toxic AGE-modified lipoproteins, interacting with other proteins or with AGE receptors may also prevent diabetic complications. There are several potential approaches, but relatively little existent research specific to lipoprotein glycation. Antibodies to glycated albumin have

prevented basement membrane thickening in db/db mice [256], but there are no studies of the effects of therapeutic antibodies to glycated lipoproteins. Lysozyme has demonstrated in vitro ability to bind in vivo generated AGEs in uremic sera and dialysate [257–259], and highly efficient lysozyme removing dialysis membranes may potentially reduce AGE levels, which may also include AGE-modified lipoproteins, and vascular disease in dialysis patients.

Soluble RAGE (sRAGE) can act as a decoy for AGE binding and has shown benefit for reducing vascular damage in animal models, including vascular hyperpermeability [260], atherosclerotic lesion area and complexity [261], periodontal disease, impaired wound healing, renal dysfunction [262], and pro-inflammatory effects [263] such as CAM expression and neutrophil infiltration [264], but effects on glycated lipoproteins have not been evaluated. As yet there are no sRAGE drugs in clinical practice.

AGE or cross-link breakers are a novel class of anti-AGE drugs, which have shown some benefit for improving vascular and renal damage and erectile dysfunction in diabetic animal models and in patients. The most well-studied is alagebrium, which has demonstrated some benefits related to peripheral arterial function [265], cardiac contractility [266], and erectile dysfunction [267], but in other studies of heart failure [268] and glaucoma [269], both of which are more common in diabetes, was ineffective. AGE breakers may also act by inhibition of AGE formation [270], effects on NO [267, 271] and on thiamine metabolism [272]. None of the studies has reported effects on AGEs in lipoproteins. None is yet approved for use in clinical practice.

Compounds Altering Responses to Glycated Lipoproteins

Caffeic acid is a phenolic acid with antioxidant effects present in normal diets. Toma et al. evaluated caffeic acid effects on inflammation and its mechanism of action in cultured human endothelial cells incubated with glycated LDL in the presence and absence of caffeic acid [273]. Caffeic acid reduced levels of RAGE, inflammation (CRP, VCAM-1; MCP-1), oxidative stress, and endoplasmic reticulum stress (ERS) markers. RAGE and ERS specific blockers were used to elucidate mechanisms. Glycated LDL increased CRP via NADPH oxidase-dependent oxidative stress and ERS. Glycated LDL interaction with RAGE, oxidative stress, and ERS stimulated VCAM-1 and MCP-1 secretion. Caffeic acid reduced the secretion of CRP, VCAM-1, and MCP-1 by inhibiting RAGE expression, oxidative stress, and ERS [273]. Pre-clinical and if merited clinical studies are of interest.

More recently carnosine has been shown to reduce glycation and oxidation of LDL in rodents, prompting lipidomic studies in humans. In 24 overweight and obese adults, 2 g daily carnosine supplementation was given to 13 adults and placebo to 11 adults for 12 weeks [48]. Carnosine supplementation had favorable effects on lipid species, such as trihexosylceramide, phosphatidylcholine, and free cholesterol, some of which correlated with insulin levels and insulin secretion and resistance [48], but relationship with glycated lipoproteins was not reported.

Conclusions and Future Directions

Diabetes is already a major cause of morbidity and premature mortality globally. The onset and progression of diabetes-related micro- and macrovascular complications are likely to involve a wide range of pathogenic mechanisms, including lipoprotein glycation (of both early and late stages). Glycated lipoproteins can directly cause damage such as related to toxic effects on vascular cells, foam cell formation, and pro-thrombotic and pro-inflammatory effects. Glycated lipoproteins, while present in all types of diabetes from its onset, and to relatively higher levels than oxidized lipoproteins, are not as well-studied as other forms of lipoproteins. Additional assays to quantify a range of glycated lipoprotein classes in the circulation and in tissues are of interest. Further clinical and basic science studies are merited as lipoprotein glycation is likely a therapeutic target that may reduce residual vascular risk. The long-term management of the ever-growing number of diabetic patients will likely involve lifestyle measures, tight glycemic, lipid and blood pressure control, in combination with additional therapies that may reduce the (early and late) glycation of lipoproteins, even in the setting of ongoing hyperglycemia and dyslipidemia.

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Chapter 12
Lipid: Extracellular Matrix Interactions as Therapeutic Targets in the Atherosclerosis of Diabetes

Danielle Kamato and Peter J. Little

Introduction

The major focus of the role of lipoproteins in cardiovascular disease in both the absence and presence of diabetes is their role in the initiation and progression of atherosclerosis. Atherosclerosis is a chronic inflammatory disease considered to develop due to the binding and retention of atherogenic lipoproteins in the blood vessel wall as an early step in an inflammatory process. The "response to retention hypothesis" first defined by Williams and Tabas [1, 2] highlights that the extracellular matrix response of modification of the glycosaminoglycan chains on proteoglycans is one of the earliest signs of inflammation [1–3]. Atherosclerosis manifests as the focal development of atherosclerotic plaques. The rupture of a plaque leads to vessel occlusion and downstream tissue ischemia, advancing to heart attacks and strokes. The plasma milieu of diabetes is known to accelerate the development and progression of atherosclerosis and double the rate of cardiovascular disease [4], but the exact mechanisms have been difficult to define.

Establishing the role of elevated and modified lipoproteins in the development of atherosclerosis is accompanied by strong evidence that targeting dyslipidaemia

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reduces cardiovascular events [5]. Accordingly, most of the research and clinical activity have focused on ways to reduce or modify the lipoprotein profile of individuals to reduce the global cardiovascular disease burden [6]. This subject is covered extensively in this treatise and elsewhere. However, there is strong evidence that the initiating step in atherosclerosis is the trapping of lipoproteins in the vessel wall by binding to proteoglycans. For several decades, it has been acknowledged that the role of the extracellular matrix (ECM), of which proteoglycans are a major functional and structural component, is underappreciated; however, this situation has persisted for this considerable period.

Appreciating the role of the ECM, specifically proteoglycans in the development and progression of atherosclerosis, this chapter is focused on the various ways through which the interaction between lipoproteins and proteoglycans can be regulated to prevent atherosclerosis. There are cellular, animal, and human data which support the role of proteoglycans in atherosclerosis [7–10] and animal data indicating that interfering with the lipoprotein: proteoglycan interaction can reduce atherosclerosis [11, 12].

It is implicit that an independent mechanism such as the lipoprotein: proteoglycan interaction could be addressed therapeutically concurrently with strategies to lower the plasma concentrations of atherogenic lipoproteins. Such combined therapeutic strategies have become widely used in clinical medicine in the last decade or two [13–16]. However, there have emerged multiple instances where lipid lowering strategies are either not suitable, not tolerated, or not preferred by patients, and this cohort is tending to increase over time [17, 18]. Accordingly, it may be necessary to have approaches to the prevention of atherosclerosis that are not solely based on reducing plasma lipoprotein levels and a strategy such as reducing the impact of the lipoprotein: proteoglycan interaction might emerge as a desired therapeutic option.

This chapter briefly presents the evidence supporting the role of proteoglycans in the initiation and development of atherosclerosis and presents in detail the multiple strategies which have emerged to block this interaction and reduce lipid deposition in the vessel wall. These strategies are at various stages of development, but there is sufficient evidence and clear pathways to the development of therapeutic agents that can be presented as future therapies for lipoprotein-based disease in people with diabetes.

Biochemical and Cellular Mechanisms of Atherosclerosis

Atherosclerosis is defined by the hardening of a blood vessel wall due to the build-up of complex biochemical entities known as plaques. Atherosclerosis is slow and progressive and can be segmented into three phases, initiation, progression, and plaque rupture [19]. Over many years, smooth muscle cells of the media migrate into the intima to form the neointima [20]. The migration of smooth muscle cells is driven by growth factors, chemokines and cytokines leading to ECM production. Proteoglycans fill most of the extracellular interstitial space in the neointima and are

synthesized primarily by smooth muscle cells [21]. Proteoglycans are composed of a polypeptide backbone (core protein) with one or more glycosaminoglycan chains covalently anchored [22–24]. The glycosaminoglycan chains are negatively charged entities with a strong anionic attraction to positively charged amino acids on apolipoproteins on low-density lipoprotein (LDL) [25–27]. The binding of LDL to modified proteoglycans results in the retention of LDL in the neointima and increased susceptibility of the apolipoproteins to oxidation [28–30]. The oxidized LDLs act on endothelial cells to stimulate the expression of cell adhesion molecules (vascular cell adhesion molecules-1, VCAM-1, P and E-selectins) which recruit monocytes and T-cells into the subendothelial space. Chemoattractant proteins such as monocyte chemoattractant proteins (MCP)-1 and interferon (IFN)-γ interact with the inflammatory cells which migrate into the intima. The modified LDLs are recognized by scavenger receptors on the surface of macrophages that promote the internalization of the oxidized LDLs, leading to the formation of foam cells. The foam cells in the intima release inflammatory cytokines, growth factors and stimulate reactive oxygen species (ROS) generation, which further promotes the migration of smooth muscle cells from the media layer to the subendothelial space [31]. Simultaneously smooth muscle cells secrete collagen fibres that transform a fatty atherosclerotic plaque to a stabilized fibrous cap.

Therapeutic Advances for Treatment of Atherosclerosis

Vascular diseases are responsible for 30% of all deaths globally, with atherosclerosis responsible for 80–85% of mortality [32–34]. Atherosclerosis was a problem that initially concentrated in industrialized countries; however, the clinical consequences and impact of atherosclerosis now span the globe. Approaches that are available to treat atherosclerosis include lifestyle strategies to reduce modifiable risk factors and therapies to reduce risk factors. The current therapeutic approach for the prevention or treatment of atherosclerosis is to target associated risk factors. The therapies for the treatment of atherosclerosis include LDL cholesterol-lowering drugs such as statins, cholesterol absorption inhibitors, and proprotein convertase subtilising/kexin type 9 (PCSK9) inhibitors [35]. Therapeutically statins have led as the gold standard treatment for both primary prevention in high-risk patients and secondary regression in patients with established atherosclerosis. Statin therapy over a 5-year period can prevent 10% of recurring major vascular events (secondary) and prevent 5% of major vascular events in patients at high risk with no previous vascular events (primary) [36]. A meta-analysis of 27 clinical trials reveals that statins only reduce the risk of major adverse cardiovascular events by ~20–25% [37] which leaves approximately 70% unmet clinical need.

The use of statins has been complemented by the introduction of PCSK9 inhibitors that target cholesterol levels via a completely different mechanism. The use of PCSK9 inhibitors on top of a statin reduces cardiovascular-related risks by 15%

more than statins alone, and a combined therapy reduced cardiovascular-related events by 53% which shows that there was over 40% with unmet clinical need. More recent interventions have investigated anti-inflammatory therapies in reducing recurrence of cardiovascular events. The CANTOS trial revealed that targeting interleukin-1β reduced recurrent cardiovascular events, however, had no benefit on mortality rates [15]. In addition to the very high cost of this treatment, patients had a twice as likely increased risk of fatal infections as compared to the placebo group [15]. This highlights that there is still an important unmet clinical need for therapies that can treat atherosclerosis for which a vessel wall directed therapy would be a warranted approach.

Proteoglycans: Structure and Function in the Vessel Wall

Atherosclerosis develops in different stages and usually involves intimal hyperplasia, lipid accumulation and formation of fatty streaks, early and late-stage fibrous plaques, and eventually plaque rupture with thrombosis and vessel occlusion leading to myocardial infarction or stroke. At each stage, ECM composition is enriched by different types of proteoglycans. Proteoglycans are large complex macromolecules made up of a core protein with one or more covalently attached glycosaminoglycan chains. In a normal vessel wall, proteoglycans make up 4% of the total ECM and this increases to 50% in early atherosclerosis and proteoglycans make up approximately 20% of the ECM in advanced lesions [38].

The building blocks of glycosaminoglycan chains are made up of non-branching repeating disaccharide units consisting of either *N*-acetyl-D-glucosamine (GlcNAc) or *N*-acetyl-D-galactosamine (GalNAc) and uronic acid, either glucuronic (GlcA) or iduronic acid (IdoA) acid which, depending on the alternating glycan unit, determine the class of proteoglycans. There are three main proteoglycan subclasses: chondroitin/dermatan sulphate proteoglycans (CS/DSPG), heparan sulphate proteoglycans (HSPG), and keratan sulphate proteoglycans (KSPG). The subclasses are determined based on the building blocks of the glycosaminoglycan chains. CSPG consists of alternating GlcNAc and GalNAc while DS chains are alternating IdoA and GalNAc residues [39]. KSPG does not contain a uronic acid and is composed of repeating units of galactose and GlcNAc. HSPG are made up of repeating units of GlcNAc or N-sulphated glucosamine (GlcNS) and a combination of either GlcA or IdoA. The proteoglycans present in the vessel wall include versican (CSPG), biglycan and decorin (CS/DSPG), perlecan (HSPG), and mimecan (KSPG) [40].

The core protein of a proteoglycan has a defined molecular weight, however, the size of the glycosaminoglycan chains attached to the core protein varies due to the synthetic process for the formation of glycosaminoglycan chains. Glycosaminoglycan chain formation is a sequential event from the endoplasmic reticulum (ER) to the Golgi apparatus due to the combined actions of glycosyltransferases and sulfotransferases [41]. The glycosaminoglycan chains on CS/DSPG are closely associated with binding LDL which contributes to the trapping and accumulation of lipid in the

vessel wall. Retention of lipid and the body's response to this phenomenon forms the basis of the Williams and Tabas "response to retention hypothesis" [1]. The hypothesis proposes that LDL which normally diffuses through the blood vessel wall is trapped by intimal proteoglycans produced by vascular smooth muscle cells (VSMCs) and is consequently retained in the vessel wall [1, 2, 42]. The apolipoprotein B (apoB) moiety of an LDL particle contains a proteoglycan binding region [43, 44]. The positively charged LDL interacts with the negatively charged constituents of the vessel wall ECM [45] of which the majority are sulphated proteoglycans. The proteoglycans have a high degree of negative charge owing to the sulphate and carboxyl groups on the glycosaminoglycan chains. Mice expressing defective apolipoprotein B binding proteoglycans failed to develop atherosclerosis although cholesterol levels were elevated, demonstrating that retention of LDL by proteoglycans is critical in the early stages of atherosclerosis [43, 46, 47]. In the human coronary artery, proteoglycans secreted by VSMCs include biglycan and decorin, and to a much lesser extent, versican and perlecan [48].

Lipoproteins are transported from the plasma to the intimal space via endothelial transcytosis. The movement of lipoproteins into the subendothelial space does not correlate to the amount of LDL retention in the intimal space [49]. The retention of ApoB containing lipoproteins, rather than the rate of transcytosis, is the rate-limiting factor in the deposition of lipoproteins in the artery wall and the development of atherosclerosis [50]. VSMCs synthesize proteoglycans with modified glycosaminoglycan chains that bind lipoproteins [51–53]. The glycosaminoglycan chains have several structural features that can be modified, which will affect their ability to interact with lipids, this includes the sulfation pattern, the length of the glycosaminoglycan chain, and the isoform of the uronic acid moiety on the glycosaminoglycan chain. Glycosaminoglycan chain synthesis requires the combined actions of glycosyltransferases and sulfotransferases that are involved in the synthesis of the tetrasaccharide linkage region on the core protein and the addition of GlcA and GalNaC residues, sulfation, and polymerisation to create the glycosaminoglycan chain. The widespread expression and multi-functionality of proteoglycans suggest that the enzymes that lead to the synthesis of glycosaminoglycan chains would not represent a therapeutic target; however, the signalling pathways that regulate the expression of the enzymes may be cell/tissue specific and as such represent potential therapeutic targets.

Role of Proteoglycans in Atherosclerosis

The role of glycosaminoglycan chain synthesizing enzymes has been demonstrated in vivo [9, 54, 55]. Mice susceptible to atherosclerosis fed a western diet, had an increase in lipid deposition in the aortic root, an increase in the mRNA expression of glycosaminoglycan synthesizing enzymes chondroitin 4-*O*-sulfotransferase (CHST11) and chondroitin *N*-acetylgalactosamine transferase (ChGn-2), and an increase in CS proteoglycan size [9]. This demonstrates that the expression of

glycosaminoglycan chain synthesizing enzymes and glycosaminoglycan chain length were concurrent with atherosclerotic plaque formation in vivo. The impact of targeting glycosaminoglycan synthesizing enzymes for the treatment of atherosclerosis has been evaluated in genetically modified mice [54, 55]. Mice lacking ChGn-2 and LDL receptors (ChGn-$2^{-/-}$/$LDLR^{-/-}$) were fed a western diet and compared to ChGn-$2^{+/+}LDLR^{-/-}$ mice, there was a significant reduction in lipid retention and a reduction of smooth muscle cell migration associated with the deletion of ChGn-2 [54]. This study demonstrates that glycosaminoglycan chain synthesizing enzymes correlate with atherosclerotic plaque formation in vivo. Importantly reduction or inactivation of glycosaminoglycan synthesizing enzyme(s) expression may be a therapeutic avenue to lessen plaque progression by reducing LDL retention and smooth muscle cell migration.

The signalling intermediates involved in the modification and elongation of glycosaminoglycan chains on proteoglycans are potential targets of therapeutic agents because of the association of glycosaminoglycan chains and lipid binding. Vascular growth factors and hormones stimulate cellular pathways in VSMCs to synthesize proteoglycans with modified glycosaminoglycan chains. Vasoactive factors that stimulate glycosaminoglycan chain elongation include seven-transmembrane G protein-coupled receptor agonists, such as lysophosphatidic acid (LPA) [56], endothelin-1 (ET-1) [57], and thrombin [58–62]; tyrosine kinase receptor agonists such as PDGF and epidermal growth factor (EGF); serine/threonine kinase agonist such as TGF-β [63–66] and toll-like receptor agonist lipopolysaccharide (LPS) (Fig. 12.1) [67]. There has been considerable progress in characterizing the pathways through

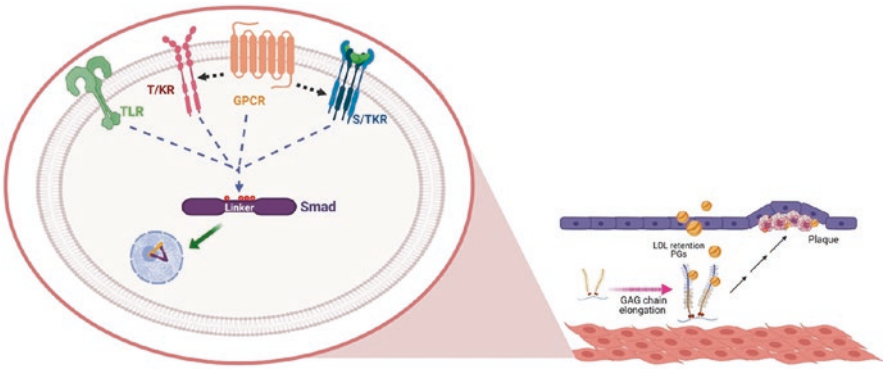

Fig. 12.1 Receptor pathways leading to the production of proteoglycans with elongated glycosaminoglycan chains. Vasoactive growth factors, cytokines, hormones, and endotoxins transmit their signal via G protein-coupled receptors (GPCRs), tyrosine kinase receptors (T/KR), serine threonine kinase receptors (S/TKR), or toll-like receptors (TLRs) to stimulate the phosphorylation of the Smad2 linker region. The phosphorylation of the Smad2 linker region activates downstream signalling cascades that lead to an increase in the expression of the rate-limiting enzymes associated with glycosaminoglycan chain elongation. This leads to the synthesis of proteoglycans with elongated glycosaminoglycan chains that have an increased susceptibility to bind LDL. (Figure created with BioRender.com)

which receptor pathways can elongate glycosaminoglycan chains on proteoglycans.

Cellular Signalling Pathways That Drive Glycosaminoglycan Chain Elongation

There have been major advances in defining the pathways that regulate glycosaminoglycan chain lengths [60, 63, 64, 68]. TGF-β is involved in atherosclerosis [69] and is a prototypical activator of glycosaminoglycan chain elongation [70]. The cognate TGF-β receptor triggers the rapid and direct activation of Smad2 transcription factor in the carboxyl terminal [71]. The Smad transcription factor has several amino acid residues in the linker regions that can become phosphorylated. The phosphorylation of the Smad2 linker region occurs via activation of serine/threonine kinases, thus not a direct response of the TGF-β receptor. In human VSMCs, TGF-β mediated Smad2 linker region phosphorylation is regulated by Erk and p38 dependent pathways which correlate with TGF-β mediated glycosaminoglycan chain elongation [68] and the expression of rate-limiting glycosaminoglycan elongation genes [63]. Mechanistic studies reveal that glycosaminoglycan synthesizing genes CHSY1 and CHST11 have a Smad2 binding domain [64]. These findings define an important role for the Samad2 transcription factor in the regulation of glycosaminoglycan chains on proteoglycans.

Vasoactive agonists thrombin, lysophosphatidic acid (LPA), and endothelin-1 (ET-1) are elevated in patients with atherosclerosis [72, 73]. In human VSMCs, thrombin, LPA, and ET-1 acting via respective GPCRs stimulate glycosaminoglycan chain synthesizing enzymes and glycosaminoglycan chain elongation (Fig. 12.1) [56, 58, 67, 74, 75]. GPCRs via receptor-to-receptor communication or transactivation dependent signalling can activate serine/threonine kinase receptors or tyrosine kinase receptors and their downstream signalling pathways [76, 77]. GPCR activation of tyrosine kinase receptors was first described by Daub et al. in 1996 [78]. This was followed by Little et al. [61] in the early 2000s, who described that GPCRs can transactivate serine/threonine kinase receptors leading to Smad2 phosphorylation. As such the Smad2 transcription factor was evaluated in the GPCR-mediated signalling pathways. Cell surface receptors can communicate with each other to activate a second class of cell surface receptors and activate downstream signalling pathways [76, 77]. Taking the GPCR agonist, thrombin, as an example [58–60], there has been a tremendous amount of work characterizing the signalling pathways leading to glycosaminoglycan chain elongation. Thrombin acting via its cognate GPCR, proteinase activated receptor (PAR)-1, transactivates tyrosine kinase receptors and serine/threonine kinase receptors, namely EGF and TGF-β receptors, respectively, to stimulate glycosaminoglycan chain elongation [62] through increased expression of glycosaminoglycan chain synthesizing

enzymes [58]. Detailed signalling studies reveal that thrombin via transactivation dependent pathways stimulates the phosphorylation of the Smad2 linker region. Thrombin mediated Smad2 linker region phosphorylation was regulated by different serine/threonine kinases which correlates to the expression of glycosaminoglycan synthesizing enzymes. A similar signalling pathway was described with GPCR agonists LPA and ET-1 [56, 75, 79].

Bacterial infections produce endotoxins such as LPS. LPS mediates its biological responses through pathogen sensing toll-like receptors (TLRs). Gut derived LPS and TLR4 were present in human atherosclerotic plaque demonstrating that pathogens can travel the distance to propagate their effects [80]. Exposure of human VSMCs to LPS stimulated the expression of glycosaminoglycan chain synthesizing enzymes via the linker region of the Smad2 transcription factor [67]. The Smad2 linker region has several sites that can be phosphorylated which are a threonine residue and three serine residues [81]. Using specific antibodies to each of these residues has highlighted a highly specific signalling pathway. The phosphorylation of the Smad2 threonine residue correlates with the expression of glycosaminoglycan chain initiation enzyme XT-1, and the phosphorylation of the serine residues of the Smad2 linker region correlates with the expression of glycosaminoglycan elongation genes CHSY1 and CHSt11 [60]. This highly specific signalling pathway has been identified downstream of three vasoactive receptors: thrombin [60], TGF-β [63], and LPS [67]. The findings demonstrate that it is feasible to target glycosaminoglycan chain elongation without affecting the initiation of new glycosaminoglycan chains. The high specificity of the signalling pathway suggests that perhaps there could be a single integrating target to prevent glycosaminoglycan chain elongation in vessels exposed to the various vasoactive agonists.

Proteoglycans as a Therapeutic Target in Atherosclerosis

Traditional risk factors such as hypercholesterolemia and hypertension are contributing less towards atherosclerosis, with a decline in the prevalence of heart disease between 2014 and 2019 [32]. Despite effective intervention for the traditional risk factors, a considerable risk remains and novel approaches to treat cardiovascular disease are warranted. Lipid retention to modified proteoglycans is one of the earliest steps in the development of atherosclerosis, however, no treatments currently target these pathological events in the vessel wall. Therefore, targeting proteoglycan components for the prevention or treatment of a vascular disease may be of merit due to their impact on the development of atherosclerosis. Approaches that target the lipoprotein:proteoglycan interaction include vessel wall directed small chemical entities, glycosaminoglycan targeting monoclonal antibodies and competing peptide mimetics (Fig. 12.2).

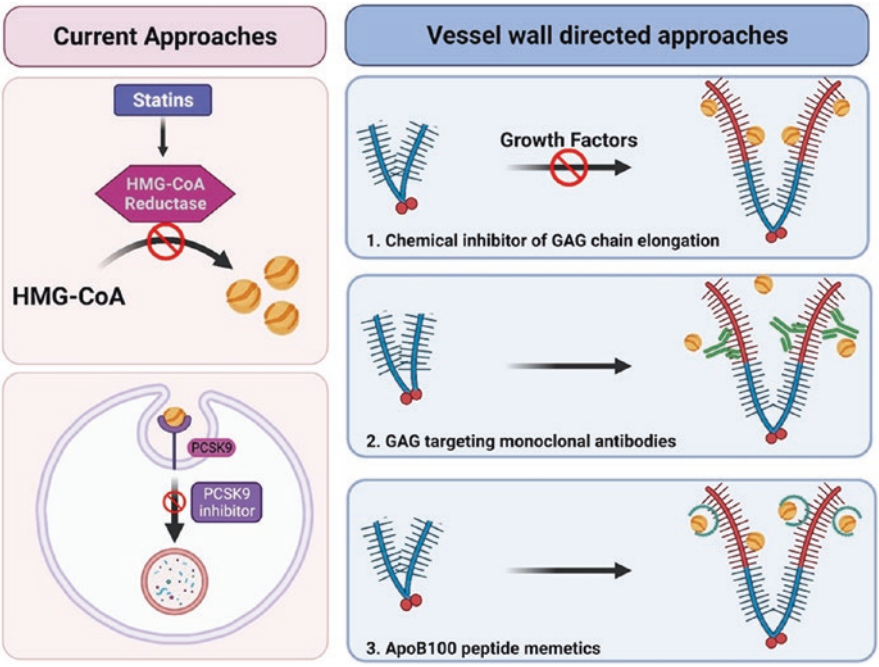

Fig. 12.2 Current and future vessel wall directed therapies to treat atherosclerosis. *Current approaches*: Statins inhibit HMG-CoA reductase involved in the biosynthesis of cholesterol leading to a reduction in hepatic cholesterol production. PCSK9 leads to the degradation of LDL receptors in hepatocytes, therefore PCSK9 inhibitors prevent LDL receptor degradation and recycling. *Vessel wall directed therapies*: Vasoactive growth factors act on the cells in the vessel wall to produce proteoglycans with longer glycosaminoglycan chains that have an increased binding capacity to LDLs. **1.** Targeting the growth factor mediated cellular signalling pathway to prevent glycosaminoglycan chain elongation. **2.** The use of chimeric monoclonal antibodies that target sulfation on the glycosaminoglycan chains to interfere with their ability to bind LDL. **3.** The use of ApoB100 peptide that disrupts the interactions between proteoglycans binding of LDL. (Figure created with BioRender.com)

Glycosaminoglycan Targeting Monoclonal Antibodies

The use of chimeric mouse/human monoclonal antibodies that recognize the sulphated glycosaminoglycans to interfere with LDL interactions has been effective at preventing the progression of atherosclerosis (Fig. 12.2) [11, 82–85]. The chimeric mouse/human IgG1 P3 monoclonal antibody (chP3) that recognizes sulfatides was modified with the addition of an arginine residue in position 99 (chP3R99) to display higher reactivity, was assessed for its anti-atherosclerotic properties [85]. ChP3R99 recognized heparin, heparan sulphate, dermatan sulphate and had the highest reactivity with chondroitin sulphate. In vitro, chP3R99 interfered with the ability of LDL to bind to CS chains with a 70%

reduction in the LDL binding [85]. The role of chP3R99 in preventing lipid accumulation and lesion progression was studied in vivo in New Zealand White rabbits administered lipid emulsion Lipofundin [85, 86]. Immunization with chP3R99 accumulates in atherosclerotic lesions to prevent atherosclerosis progression in rabbits. In a late-stage model of atherosclerosis, mice that had developed atherosclerosis were administered ChP3R99, which reduced lesion progression by 88%, with no change in total plasma cholesterol levels [11]. Age and sex influence the development of atherosclerosis and therapeutic interventions. Male and female mice immunized with ChP3R99 showed a reduction in lesion area by 31% and 38%, respectively. Adolescent, young adult and middle-aged mice were immunized with ChP3R99 showed that there were no differences in the anti-CS responses irrespective of age. These results identify that interference with LDL retention to proteoglycans with an anti-glycosaminoglycan antibody is a potential therapeutic target for preventing and treating atherosclerosis. For relevance, we note the increasing use of biologicals, including antibodies, in human therapeutics.

ApoB100 Peptide Mimetics

The electrostatic interaction between the amino acids on the ApoB100 and the sulphate groups on elongated glycosaminoglycan chains is the driving factor for lipid retention. Therefore, a potential therapeutic mechanism would be to inhibit this interaction using a small peptide mimetic [47, 87]. Two major approaches which have adopted this process include mutations at siteB of ApoB100 and immunization with malondialdehyde (MDA) modified polypeptides covering the ApoB100 sequence [43, 87]. Immunization with an ApoB100 peptide with MDA modifications provides athero-protective actions in $ApoE^{-/-}$ mice [87]. Clinically the use of MDA ApoB100 peptide could predict the development of atherosclerosis. In patients with coronary heart disease, the IgM antibodies levels against MDA ApoB100 correlated with carotid intima-media thickness, oxidized LDL and clinical outcomes, suggesting clinical significance for the use of MDA-ApoB100 peptides for protection against atherosclerosis. Screening of 302 peptides comprised of the ApoB100 sequence with human plasma identified native MDA-ApoB100 peptide 210 (p210) as an important epitope recognized by autoantibodies [88]. This native epitope was studied in vivo for functionality. An $ApoE^{-/-}$ model of atherosclerosis immunized with p210-PADRE peptide resulted in a reduction of atherosclerotic plaque formation [88]. These studies demonstrate that interference with the proteoglycan and ApoB100 binding domain with peptides represents a potential therapeutic strategy for lowering cardiovascular risk.

Small Chemical Entity Targeting Changes in the Vessel Wall

Targeting the modification of the glycosaminoglycan chains on proteoglycans is a potential therapeutic strategy to prevent atherosclerosis. In diseased vessels, glycosaminoglycan chains on proteoglycans are modified (elongation and sulfation), leading to an increase in the "stickiness" of the vessel wall and an increased capacity to attract and retain LDL. The actual enzymes associated with the synthesis of the glycosaminoglycan chains are involved in physiological processes, therefore directly targeting the enzymes with classical inhibitors would most likely produce adverse reactions. However, targeting the pathways that lead to an increased expression of the enzymes would be tissue specific and an optimal target. Inhibiting the pathways that lead to glycosaminoglycan chain modification with a chemical inhibitor would be an effective approach that can be used together with cholesterol-lowering therapies to ameliorate atherosclerosis. A proof-of-concept study demonstrates feasibility for this therapeutic approach [12, 89]. Imatinib, developed as a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukaemia, was used as a platelet derived growth factor (PDGF) receptor inhibitor [89]. Treatment of VSMCs with imatinib dose-dependently inhibited PDGF mediated glycosaminoglycan chain elongation [12, 89]. In vivo in an ApoE$^{-/-}$ model of atherosclerosis treatment with imatinib reduced lipid deposition in the vessel wall by approximately 30% [12]. Treatment with imatinib had no effect on the circulating lipid levels in mice, demonstrating that inhibition of glycosaminoglycan chain elongation by imatinib reduced atherosclerosis.

The modification and elongation of glycosaminoglycan chains in vitro have been regulated by various pharmacological agents, including fenofibrate used for the treatment of hypertriglyceridemia, which has pleotropic actions in the vessel wall. Treatment of VSMCs with fenofibrate reduces TGF-β and PDGF mediated proteoglycan:LDL binding and glycosaminoglycan chain elongation [90]. Another fibrate, gemfibrozil, also used to treat hypertriglyceridemia, has a similar action on growth factor stimulated proteoglycans synthesis and glycosaminoglycan chain elongation [90]. Thiazolidinediones sometimes used to improve insulin sensitivity in type 2 diabetes reduce lipid deposition and atherosclerosis in a mouse model [91, 92]. In vitro treatment of aortic smooth muscle cells with thiazolidinediones, troglitazone, and rosiglitazone produced proteoglycans with shorter glycosaminoglycan chains [93]. The pleotropic actions of the agents including their ability in preventing LDL:proteoglycan interaction advance the "response to retention" hypothesis for developing vessel wall directed therapies.

The role of anti-parasitic and anti-malarial drugs, suramin and artemisinin, as potential vessel wall directed therapies has been investigated. Suramin dose dependently inhibits PDGF mediated glycosaminoglycan chain elongation [74, 94]. Mechanistic studies with artemisinin revealed that TGF-β mediated glycosaminoglycan chain elongation measured as the expression of the glycosaminoglycan

chain synthesizing genes was regulated by artemisinin. Specifically, artemisinin inhibited TGF-β mediated Smad2 linker region phosphorylation [74]. In a high fat-fed diet model of atherosclerosis artemisinin attenuates atherosclerotic lesions [95]. The cellular studies of receptor mediated pathways to proteoglycan synthesis and glycosaminoglycan chain elongation reveal that the Smad2 transcription factor is a common integrating point. Vasoactive growth factors and cytokines mediate their biological responses via different classes of cell surface receptors. Cellular studies demonstrate that activation of different receptors results in the activation of serine/threonine kinases which activate the Smad2 linker region. Intricate studies revealed that the serine residues of the Smad2 linker region regulated the genes associated with glycosaminoglycan chain elongation and the threonine residue is associated with the initiation of glycosaminoglycan chains onto the core protein [60, 67]. These studies show feasibility for targeting the glycosaminoglycan chain elongation process to prevent LDL retention and the development of atherosclerotic plaque.

Proteoglycans as Biomarkers of Atherosclerosis

The earlier the identification that patients may have a future risk for cardiovascular events due to atherosclerosis may enable the establishment of earlier risk factor control of reducing the severity and impact of the disease. Biomarkers are valuable tools for this purpose. Currently measuring classical risk factors associated with atherosclerosis including inflammatory C-reactive protein, LDL-cholesterol, and HDL-cholesterol serve as predictive biomarkers for cardiovascular disease. Biomarkers thus far have been limited to the assessment of the modifiable risk factors of atherosclerosis. However, there is a rise in patients that develop atherosclerosis who lack the traditional risk factors. This group of patients have similar rates of plaque progression to patients who exhibit traditional risk factors [96]. Thus, the identification of predictive biomarkers represents an unmet clinical need. The binding of LDL particles to proteoglycans in the vessel wall is an early key event in the development of atherosclerotic lesions, as such several groups have investigated LDL: proteoglycan binding as a potential predictive biomarker approach. Using purified solutions of proteoglycans, a standard procedure was developed to measure LDL: proteoglycan interaction from complex serum derived from patients. This ex vivo method was used to compare the binding affinity from LDL derived from patients. In 214 healthy and 77 probably ischemic subjects those who had no history of myocardial infarction showed ischemic subjects had a higher prevalence of LDL: proteoglycan complex formation than the healthy subjects [97]. In LDL from obese subjects with or without type 2 diabetes, a higher binding affinity to proteoglycans was observed as compared to controls [98]. The LDL: proteoglycan binding affinity was also measured in patients with moderate hypercholesterolemia pre- and post-treatment with lipid lowering therapies. In patients administered gemfibrozil, pravastatin, or combined therapy, there were substantial reductions in the amount of LDL binding to proteoglycan [99]. More recently in renal transplant recipients, the

LDL: proteoglycan binding susceptibility was associated with cardiovascular mortality and kidney graft failure. Cholesterol retention to proteoglycans showed a higher correlation at predicting graft failure than the quantity of LDL-cholesterol suggesting a higher relevance to proatherogenic properties of LDL in chronic graft failure [100]. These studies indicate that the LDL affinity to bind proteoglycans is associated with an increased risk of atherosclerosis, and as such this interaction has the potential to be explored as a potential predictive marker for atherosclerosis.

Conclusions

Since the original introduction of the response to retention hypothesis, there has been significant advances in the understanding of the pathways between proteoglycan interactions with lipids that leads to lipid retention in the vessel wall. Although several highly effective therapies that target modifiable risk factors associated with atherosclerosis and cardiovascular disease exist, the prevalence of cardiovascular disease has continued to rise. The global rise in prevalence can be attributable to the increase in patients who develop atherosclerosis in the absence of standard modifiable risk factors [96]. Although this cohort lacks traditional risk factors, they exhibit development of atherosclerotic plaque to a similar extent to patients with standard risk factors. This highlights that current therapeutic approaches that target traditional risk factors would not be feasible in this cohort. Hence, vessel wall directed approaches can be used in combination with lipid lowering therapies or alone in patients who lack traditional risk factors to prevent the development of atherosclerosis and cardiovascular disease.

Medical therapies in addition to lipid lowering therapies are required to address the global burden of atherosclerosis. Such areas include antioxidants and anti-inflammatory strategies which are relatively broad and have not produced outstanding novel therapeutic advances. One well-appreciated vessel wall mechanism of atherosclerosis is the interaction between glycosaminoglycan chains on proteoglycans and lipoproteins. In this chapter, we have outlined the pre-clinical and emerging clinical research underlying a vessel wall directed therapy as a novel therapeutic approach for the prevention and treatment of cardiovascular disease in patients with and without diabetes.

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Part II
Lipoproteins and the Complications of Diabetes

Chapter 13
The Role of Modified Forms of LDL and Corresponding Autoantibodies in the Development of Complications in Diabetes

Maria F. Lopes-Virella and Gabriel Virella

Introduction

Hyperglycemia and hyperlipidemia in diabetes lead to overproduction of reactive oxygen species (ROS). Oxidative stress contributes to modification of lipoproteins which is a critical factor to initiate endothelial dysfunction and activate pathogenic pathways that lead to the development and progression of complications in diabetes [1, 2]. Hyperglycemia plays a key role by inducing mitochondrial overproduction of reactive oxygen species (e.g., superoxide anion, hydrogen peroxide, and others), which, in turn, will lead to a variety of modifications of proteins, enzymes, and other substrates, including the formation of advanced glycation end-products (AGE) and oxidation [1, 3, 4].

Lipoproteins can be modified as a consequence of oxidation and glycation. Endothelial cells, monocytes/macrophages, lymphocytes, and smooth muscle cells (SMC) are all able to enhance the rate of oxidation of low-density lipoprotein (LDL). Reactive oxygen species and sulfur-centered radicals initiate metal ion-dependent lipid peroxidation resulting in the generation of aldehydes that interact with lysine residues in ApoB-100. Myeloperoxidase, a heme enzyme secreted by activated macrophages, is able to catalyze lipid peroxidation independently of free metal ions. Oxidation of arachidonic acid, usually secondary to oxidative stress, prostaglandin synthesis by endothelial cells (EC) and platelet activation, lead to the

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formation of aldehydes that interact with the lysine residues of ApoB100 causing its aggregation, and the resulting modification is generally referred to as malondialdehyde (MDA)-modified LDL [5].

Modified forms of LDL induce endothelial dysfunction and vascular inflammation. Inflammation derives from modified LDL-induced activation of the innate immune system and from the induction of antibodies against the different LDL modifications that lead to the formation of circulating immune complexes that exhibit strong immunomodulatory properties, leading to a robust atherogenic and pro-inflammatory response. LDL-containing IC serve as a predictive biomarker of macrovascular disease in diabetes.

The Pathogenic Role of Modified Forms of LDL

The pathogenic role of modified LDL in the development and progression of atherosclerosis is well established. It has been investigated from two different angles: the direct pro-atherogenic effect of modified forms of LDL [3, 6] and the consequences of the immune response directed against neo-epitopes resulting from lipoprotein modification [7]. Both types of effects have been extensively characterized in the case of oxidized LDL (oxLDL) and of advanced glycation end products-modified LDL (AGE-LDL).

Modified lipoproteins stimulate the release of pro-inflammatory mediators and can affect epigenetic mechanisms leading to the reprogramming of cells such as endothelial cells and monocytes. For instance, oxLDL induces the transformation of macrophages into foam cells, but that only occurs after an epigenetic reprogramming of monocytes. Exposure of reprogrammed monocytes to oxLDL leads to an enhanced response to TLR 2 and 4 as well as to upregulation of CD36 and SRA [8]. Another way to induce an epigenetic reprogramming of monocytes is 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′ untranslated regions of their target mRNAs. To better assess the role of miRNAs in the development of atherosclerosis and other complications in diabetes, miRNAs that regulate cholesterol homeostasis, endothelial cell homeostasis, and the inflammatory response are being carefully studied, but well-validated knowledge in this field is still not available, although many promising results are starting to emerge [9–12].

As well as inducing the transformation of macrophages into foam cells, a hallmark of the atherosclerotic process, due to its uptake by macrophages via receptor-mediated pathways [3, 13, 14], oxidized LDL can also present oligopeptides to the cell-mediated immune system, leading to activation of T helper 1 cells (Th1 cells) in the vascular wall. As a consequence of their activation, Th-1 cells release, among others, interferon-γ and TNF that activate macrophages and induce the release of chemokines that attract more T cells to the area. The process becomes

self-perpetuating, resulting in a chronic inflammatory reaction [15, 16]. Furthermore, oxidized phospholipids generated during LDL oxidation may also activate inflammatory cells through their interaction with TLR4 [17, 18], and oxLDL containing oxidized phospholipids can mediate the uptake of oxLDL by scavenger receptors and it can also be taken up by oxLDL-IC opsonized after interaction with Fc receptors. The differences observed when macrophages are incubated with copper-oxidized LDL versus highly oxidized MDA-LDL could result from differences in the content of oxidized phospholipids in those two forms of oxidized LDL [19, 20].

In addition, oxLDL has chemotactic effects on monocytes [21], enhancing monocyte adhesion to EC in culture [22, 23] as well as the expression of vascular cell adhesion molecule 1 (VCAM 1) and intercellular adhesion molecule 1 (ICAM 1) by human aortic endothelial cells induced by tumor necrosis factor (TNF) [24] and of ICAM-1 in resting human endothelial vein cells [25], thus contributing to the migration of monocytes into the vessel wall. Also high concentrations of oxLDL are cytotoxic and experimental data suggests that oxLDL can injure vascular cells, both endothelial cells and smooth muscle cells (SMC) [26, 27]. Multiple microRNAs and epigenetic modifications also have been described as influencing endothelial and SMC dysfunction [12, 28, 29]. OxLDL induces enhanced synthesis of growth factors including platelet-derived growth factor-AA (PDGF-AA) and PDGF receptors in SMC, as well as of granulocyte-monocyte colony-stimulating factor, macrophage colony-stimulating factor (M-CSF), and granulocyte colony-stimulating factor in aortic endothelial cells from humans and rabbits [30]. In addition, oxidized LDL may affect fibrinolysis by inhibiting the secretion of tissue plasminogen activator (tPA) by human endothelial cells [31] and stimulating the secretion of plasminogen activator inhibitor (PAI)-1 [31]. Thus, oxLDL is unable to stimulate the endothelium-dependent activation of fibrinolysis and may promote a chronic pro-thrombotic state.

The endothelial dysfunction and chronic inflammation induced by oxLDL are extremely relevant to the development of atherosclerosis and other complications in diabetes. Our group has found a positive association between the levels of inflammatory and endothelial dysfunction biomarkers and diabetic retinopathy [32], nephropathy [33], and subclinical atherosclerosis [34].

These pro-inflammatory effects are the result of the activation of a variety of functional pathways. Oxidized LDL has been shown to activate a variety of cell types expressing CD36 and other scavenger receptors and to contribute to the generation of reactive oxygen species (ROS) [35]. On macrophages, the interaction of oxLDL and CD36 (mediated by oxidized phospholipids) results in activation of the src family members Fyn/Lyn, and of several components of the MAP kinase pathway, including MKKK, MKK, FAK, and MAPK (JNK) [14]. The activation of these kinases and associated proteins, such as Vav, is associated with foam cell formation as well as with unregulated actin polymerization and loss of cell polarity causing a migration defect and the trapping of activated cells in the atheromatous lesions [14]. Recently, it was demonstrated that exposure of monocyte-derived macrophages to cytokines and oxLDL through binding to CD36, oxLDL significantly increases production of pro-thrombotic microparticles expressing tissue factor, via a caspase 3/7 dependent pathway [36]. In platelets, the same signaling events lead to enhanced

platelet reactivity and enhanced formation of thrombi [37]. It has also been reported that ligation of CD36 by oxLDL leads to the formation of a toll-like receptor heterodimer (TLR-4–TLR-6) that, in turn, activates MyD88 and nuclear factor kappa B (NFkB), a critical step in inducing the synthesis and release of pro-inflammatory cytokines [38]. The balance of pro- and anti-inflammatory mediators, together with resolvins [39], agents that promote the resolution of inflammation, is responsible for atherosclerotic lesion progression or regression

The advanced glycation end-product modified LDL, AGE-LDL, as well as other AGE-modified proteins have also been shown to have pro-inflammatory properties [40, 41]. AGE-modified proteins will impact endothelial cells eliciting increased permeability and pro-coagulant activity [42] as well as overexpression of VCAM-1 [43]. AGEs also contribute to fibroblast proliferation and T lymphocyte activation, which results in the release of increased amounts of interferon-γ that will activate monocytes and macrophages, inducing in turn the release of pro-inflammatory cytokines and chemokines [42], thus creating the conditions for a chronic inflammatory reaction in the arterial wall. The predominant impact of AGE/RAGE in the pathogenesis of oxidative stress in cardiovascular diseases and diabetes has been extensively discussed [44], and the impact of AGE in the atherosclerotic process associated with diabetes was confirmed in streptomycin-induced diabetic ApoE−/− mice [45]. Administration of soluble forms of AGE receptors (RAGE) resulted in reduction of vascular permeability and reduced the progression of atheromatous lesions [45].

The Adaptive Immune Response Elicited by Modified LDL

The pro-inflammatory properties of modified LDL appear to be considerably enhanced as a consequence of their immunogenicity. The immunogenicity of modified LDL was first reported by Steinbrecher et al. based on the immunization of laboratory animals with modified lipoproteins [46]. Of all the modified forms of LDL, oxLDL has been studied in greatest detail from the immunological point of view. Steinbrecher as well as Palinski et al. characterized its immunogenic epitopes [47, 48]. Furthermore, human autoantibodies to oxLDL were the first to be purified and characterized [49–51]. Immune complexes (IC) containing modified LDL have been isolated from the peripheral blood of patients with diabetes, cardiovascular disease, and healthy individuals [52, 53]. Both oxidized LDL and corresponding antibodies have been isolated from atheromatous human tissue [49, 54]. Thus, it seems reasonable to use circulating IC as an indicator of the IC that are deposited in the vessel wall. The formation of LDL-IC in circulation is likely to be inconsequential, but those IC formed in the vessel wall will result in enhanced phagocytosis and increased presentation of peptides derived from modified LDL to T helper cells, which is a critical step in the perpetuation vascular inflammation, as described above.

In several studies, we have consistently found that the predominant isotype of modified LDL antibodies is IgG [50, 51, 55–57]. This is a significant finding

because IgG antibodies are pro-inflammatory [50, 51, 55–57]. As reported by our group, predominance of circulating IgG antibodies with higher avidity over IgM antibodies in isolated oxLDL-IC is associated with parameters indicative of deteriorating renal function in the type 1 diabetes Diabetes Control and Complications Trial/Epidemiology of Interventions and Complications (DCCT/EDIC) cohort [57]. We observed significant positive associations of IgG oxLDL antibody concentration in isolated IC with serum creatinine and the urinary albumin excretion rate, as well as a negative correlation with the estimated glomerular filtration rate. IgM oxLDL antibody concentrations did not show any correlation with those parameters [57]. This study, however, was based on a small group of patients with type 1 diabetes. Later we studied a much larger population of 905 patients with type 2 diabetes [58], and this study shows the predominance of IgG over IgM oxLDL antibodies in isolated immune complexes and also shows that high levels of AGE-LDL as well as of IgG antibodies, but not IgM antibodies, reacting with MDA-LDL lysine epitopes in circulating IC, predict the development of macroalbuminuria in patients with type 2 diabetes. Several groups have reported data suggesting that IgM antibodies to oxidized phospholipids and oxidized LDL have protective effects in relation to the development of atherosclerosis [59–64], although whether this protective effect extends to antibodies recognizing that modified peptides seem questionable based on data published by Fredrickson and co-workers [65]. If a predominant IgM response has protective effects against the development of atherosclerosis, it is difficult to see how that information can be translated into the clinical setting.

The Composition of Circulating Modified LDL Immune Complexes and Diabetes Complications

Besides studying the pathogenic role of modified LDL antibodies [57, 58, 66–69], we developed methodology that allows the measurement of modified forms of LDL and the corresponding antibodies involved in IC formation through the isolation and fractionation of circulating IC [53, 57, 70]. This is an important methodological improvement over the direct assay of modified LDL or their corresponding antibodies in serum or plasma samples, as most modified LDL in the circulation is associated with the corresponding antibodies, and measurements of either component of the circulating complexes are inaccurate due to the mutual saturation of antigen and antibody binding sites [53, 56, 70].

In contrast with the conflicting data generated by studies of modified LDL or antibodies to modified LDL [56, 71], data generated in clinical studies carried out on the DCCT/EDIC cohort with our assay have shown that high levels of oxLDL and AGE-LDL in isolated and fractionated IC are associated with increased risk for developing diabetic nephropathy [72]. Also in the DCCT/EDIC cohort, using coronary artery calcification (CAC) indices and carotid intima-media thickness (IMT) as end-points indicative of cardiovascular disease progression, we also found that

increased levels of oxLDL and of AGE-LDL in circulating IC are associated with the development of coronary calcification [73] and with increased levels and progression of carotid IMT [74–76]. The levels of MDA-LDL in isolated IC showed a significant but weaker correlation with increased carotid IMT [74–77]. Recently, our group have demonstrated that the levels of AGE-LDL, oxLDL, and MDA-LDL in circulating IC isolated from plasma collected at entry into the DCCT/EDIC study predicted CVD outcomes in people with type 1 diabetes occurring over a 25-year period, even after adjustment for other risk factors including LDL-C levels [78]. When subsequent measurements of these IC were incorporated over time, adjustments by other risk factors mainly LDL-C attenuated the predictive value of the baseline levels and only oxLDL-IC remained independently associated with the risk of all major adverse cardiac and cerebrovascular events, myocardial infarction (MI), and coronary artery disease (CAD). Our data strongly points to a causal association of modified LDL-IC with the development and progression of atherosclerosis. Supporting this concept are our studies showing that F(ab′)2 fragments of anti-oxidized LDL IgG attenuate vascular inflammation and atherosclerosis in a diabetic LDL receptor deficient mice [79]. Our results in type 1 diabetes differ from those obtained in patients with type 2 diabetes (the Veterans Affairs Diabetes Trial (VADT) cohort), in whom the levels of oxLDL and AGE-LDL in circulating IC are not significantly associated with the occurrence of acute events, but high concentrations of MDA-LDL in IC are strong predictors of acute events, especially myocardial infarction (MI) [80]. In agreement with our data, Holvoet et al. reported in two separate studies a link between high levels of oxLDL and established CAD, and between elevated plasma MDA-LDL levels and plaque instability [81, 82].

The correlation between MDA-LDL levels and plaque instability is particularly significant because it has been well established that atherosclerotic plaque rupture is a critical event triggering thrombus formation, arterial luminal obstruction, and subsequent acute coronary syndromes [83]. Plaques that are prone to rupture consist of a larger intimal lesion with abundant macrophages and foam cells and a thinned fibrous cap [84]. Necropsy studies have demonstrated that atherosclerosis in people with diabetes is more extensive and accelerated than that in non-diabetic subjects [85]. Furthermore, studies have also shown that atherosclerotic lesions in diabetic patients were more vulnerable as they had larger intimal lesions and increased macrophage infiltration as compared to those in non-diabetic patients [86]. Analysis of gene expression in atherosclerotic plaques showed that when compared to stable plaques, vulnerable plaques have higher expression of matrix metalloproteinases (MMPs) with collagenase activity, which contribute to the thinning of the fibrous cap, causing plaque instability and rupture [87]. Among the metalloproteinases, MMP-9 has been the object of considerable interest in recent years and according to some studies is an independent risk factor for atherothrombotic events [88, 89]. MMP-9 synthesis and release can be induced through TLR-4 stimulation, usually involving bacterial endotoxins [17] but also by minimally modified LDL [90]. The association of circulating MDA-LDL and IC-associated MDA- LDL with plaque instability/acute CV events raises interesting questions such as whether IC containing different modified forms of LDL may lead to distinct gene regulation and cell reprogramming. MDA-LDL-IC

seems to lead to plaque instability by inducing macrophage apoptosis and/or increased synthesis of matrix metalloproteinases, such as MMP-9 [91]. OxLDL-IC, in contrast, induce the release of pro-inflammatory cytokines [66] and promote collagen synthesis by smooth muscle cells [92], and therefore are more likely to contribute to atheroma progression without a significant effect on plaque stability (Fig. 13.1).

Considerable interest has been raised by the accumulation of apoptotic macrophages around the necrotic core of vulnerable plaques [91]. A variety of pro-apoptotic insults has been proposed to play a significant role in the evolution of atheromas, including oxidative stress, endoplasmic reticulum (ER) stress, accumulation of non-esterified (free) cholesterol, and effects of pro-inflammatory cytokines released by activated macrophages [91]. Accumulation of free cholesterol in macrophages in combination with signals delivered through scavenger receptors or with interferon-γ, known to be released by activated T lymphocytes in atheromas [16, 93], leads to serine phosphorylation of STAT-1 which is a critical element in the induction of apoptosis secondary to ER stress [94]. The apoptotic macrophages in atheromas are ingested by functional macrophages (efferocytosis). Efferocytosis in early lesions seems to result in suppression of inflammation, while in advanced lesions is associated with enhanced inflammation [91]. This evolution appears to be a result of defective efferocytosis, allowing the apoptotic cells to undergo necrosis, resulting in the accumulation of cell fragments that promote inflammation and plaque instability [91].

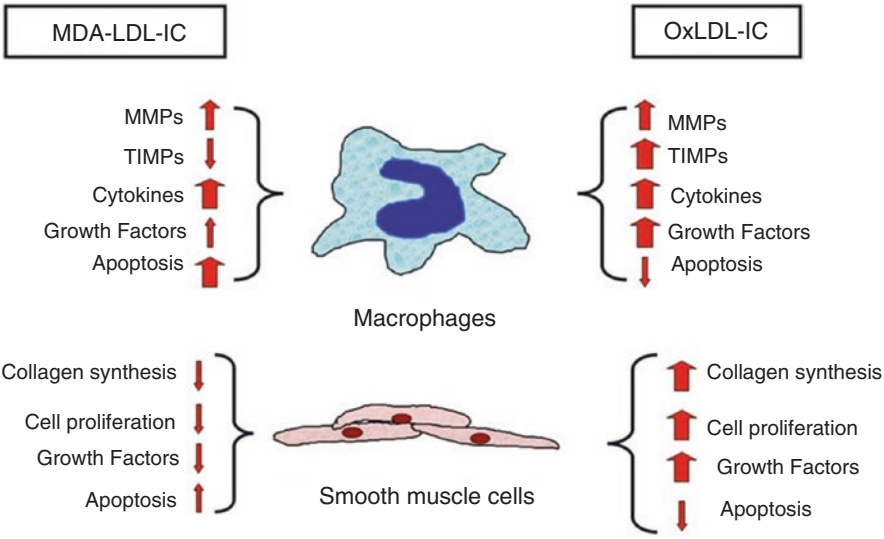

Fig. 13.1 Diagrammatic representation of the different effects of immune complexes prepared with human copper-oxidized malondialdehyde-modified LDL and the corresponding human antibodies reported by several groups (see text). While both types of immune complexes induce the release of pro-inflammatory cytokines, MDA-LDL-IC are pro-apoptotic while oxLDL-IC are anti-apoptotic and induce the release of proliferation and growth factors by macrophages and smooth muscle cells, and only oxLDL-IC induce collagen synthesis by smooth muscle cells

Pathogenic Mechanisms of Modified LDL-IC

We have published extensive data proving that oxLDL-IC are more potent activators of human macrophages than oxLDL [66, 67, 95, 96]. The uptake of IC prepared with native or copper-oxidized LDL by human monocyte-derived macrophages is primarily mediated by Fcγ receptors, primarily FcγRI [97–99], and it has been shown that the binding of oxLDL antibody blocks the interaction of oxLDL with CD36 [100], so scavenger receptors are not involved in the process. The dependency of the vascular inflammatory process on the activation of phagocytic cells via Fcγ receptors has been demonstrated in double-knockout (DKO) mice generated by crossing apolipoprotein E-deficient mice [apoE(−/−)] with FcγR γ-chain-deficient mice [gamma(−/−)] [101]. The progression of atherosclerosis in the DKO mice is significantly reduced in comparison with apoE(−/−) mice. For MDA-LDL-IC and AGE- LDL-IC, FcγRI is also involved, but possible involvement of scavenger receptors or receptors for AGE-modified proteins has not been excluded. One fundamental property of LDL-IC is their ability to deliver large concentrations of free and esterified cholesterol to macrophages [67, 97, 102]. The intracellular accumulation of free cholesterol is a known inducer of ER stress, which is believed to be the prime stimulus for the chain of events that results in modification of LDL and atheroma formation. However, experimental studies have shown that ER stress usually protects against apoptosis [91]. In fact, both oxLDL at concentrations not exceeding 75 μg/mL and oxLDL-IC prevent macrophage apoptosis [99, 103]. Whether the anti-apoptotic effect of oxLDL is a consequence of the induction of ER stress is not clear, because in addition to the enhanced generation of reactive oxygen and nitrogen species [104], several other mechanisms seem to be involved, including the release of M-CSF mediated by the activation of a PI3K-dependent pathway, upregulation of the anti-apoptotic Bcl-XL gene by NFkB activation, activation of sphingosine kinase, which causes the levels of anti-apoptotic sphingosine-1-phosphate to increase, and inhibition of acid sphingomyelinase, which prevents pro-apoptotic ceramide generation [103, 105]. The anti-apoptotic effect is more pronounced with oxLDL-IC [99, 106] and is not unique to oxLDL-IC, because it has also been reproduced with KLH-anti-KLH IC [99]. However, there are significant differences between oxLDL-IC and other IgG-containing IC. Only oxLDL-IC can induce foam cell formation, and the magnitude of the pro-inflammatory response induced in human macrophages is greater with oxLDL-IC than with KLH-IC, for example [66].

While oxLDL cell signaling is mediated by scavenger receptors, oxLDL-IC deliver activating signals via Fcγ receptors. The cross-linking of Fcγ receptors by IC induces phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) by kinases of the Src family, leading to activation of the Syk pathway [107, 108]. Activation of Syk triggers the mitogen-activated protein kinase (MAPK) signaling cascade, which includes ERK1/2, p38 MAPK, and c-Jun N-terminal kinase (JNK). MAPK activation is also essential for Fc-mediated activation of NFκB [109]. Following the general rule, oxLDL-IC primarily engage FcγRI and induce the activation of the MAPK pathway [110], which is responsible for the expression of

pro-inflammatory gene products. In addition, cross-linking of FcγRs by oxLDL-IC activates PI3K and c-Akt [99]. Activated c-Akt promotes cell survival by at least four different mechanisms: (1) phosphorylating the Bad component of the Bad/Bcl-X_L complex which results in its dissociation and cell survival, (2) caspase 9 inactivation, (3) regulation of the expression of transcription factors, and (4) activating IKK kinases which phosphorylate IκB and, as a consequence, release the active form of NFkB, which induces the expression of genes favoring cell survival [111] (Fig. 13.2). The repertoire of oxLDL-IC-induced pro-survival genes is much wider than that induced by oxLDL alone [96]. Also, oxLDL-IC induce HSP70B expression in macrophages. This protein binds to the internalized lipid moiety of oxLDL-IC and prevents its degradation, while at the same time inducing sphingokinase-1 [104, 112].

In contrast to oxLDL, there is no published information concerning pathways of cell activation triggered by MDA-LDL or MDA-LDL-IC. The association of MDA-LDL with acute coronary syndromes [5, 82] and the association of high levels of

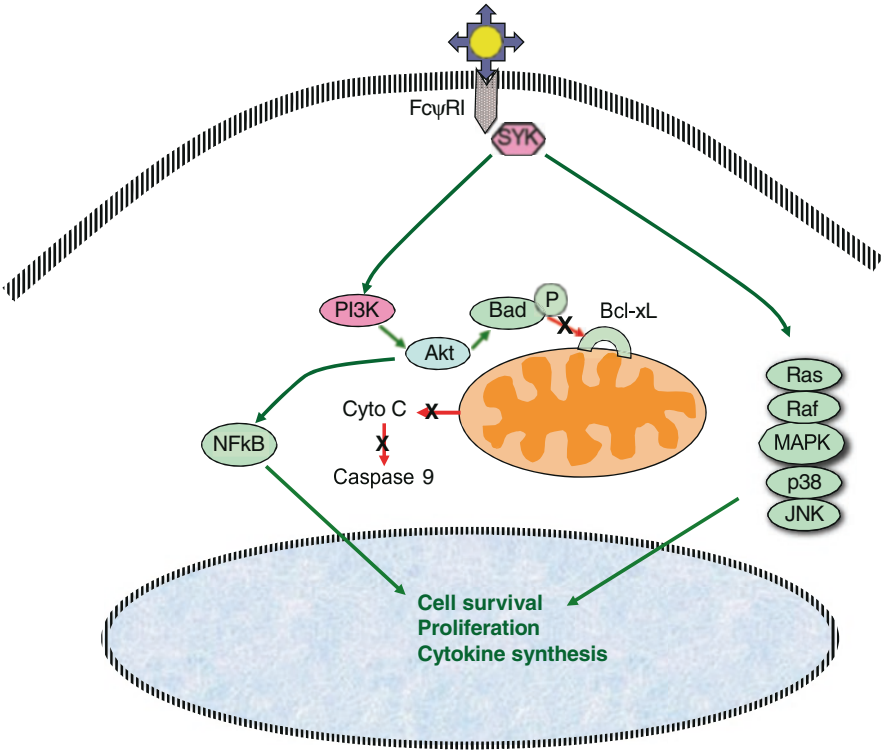

Fig. 13.2 Diagrammatic representation of the activation pathways triggered by oxLDL-IC through the engagement of FcγRI. Two main pathways are activated, the MAPK pathway which is important for the activation of cell proliferation and cytokine synthesis, and the Akt pathway, which also contributes to the induction of cell proliferation and cytokine synthesis through NFκB activation and also promotes cell survival through the dissociation of the Bad/Bcl-X_L complex, blocking the pathway that leads to the activation of caspase 9

MDA-LDL in the circulating IC isolated from patients with type 2 diabetes who had acute cardiovascular disease (CVD) events, mainly MI [80], strongly suggest that MDA-LDL and MDA-LDL-IC have pro-apoptotic activity. The different effects of cellular uptake of oxLDL-IC and MDA-LDL-IC (Fig. 13.1) could be a result of structural differences between MDA-LDL and oxLDL. The extent of MDA-lysine modification is much greater in laboratory produced MDA-LDL than in copper-oxidized LDL [70]. This difference results in the generation of epitopes unique to MDA- LDL, and the fact that MDA-LDL antibodies obtained by immunization of rabbits with laboratory-prepared MDA-LDL react with LDL isolated from IC proves that MDA-LDL with identical epitopes and, therefore, with similar structural characteristics, is generated in vivo. Also, while copper oxidation predominantly results in ApoB fragmentation, MDA modification is associated with ApoB aggregation [113]. Obviously, these differences in ApoB could determine different biological properties of the two forms of modified LDL. For example, it has been reported that the processing of heavily oxidized and aggregated LDL by macrophages is defective [114]. Thus, the uptake of MDA-LDL-IC could result in a variety of conditions that could promote apoptosis, including (1) the release of much higher concentrations of free cholesterol in the cell, (2) intracellular accumulation of aggregated LDL, (3) cytoplasmic release of lipoprotein degradation products and oxidized phosphatidylcholine, which could be transported to the extracellular compartment and then react with scavenger receptors and/or TLRs, delivering signals that would favor the activation of pro-apoptotic pathways.

There is considerable interest in identifying biomarkers indicative of plaque instability. A variety of proteins and enzymes have been proposed as candidates, as reviewed recently by Koenig [115]. Besides MMPs, reactive proteins (CRP), cytokines (IL-6, IL-18), enzymes (glutathione peroxidase, lipoprotein-associated phospholipase A-2 (Lp-PLA2)), myeloperoxidase, chemotactic proteins (monocyte chemotactic protein-1), and modified lipoproteins have been proposed as indicators of plaque instability [5, 81, 82, 89, 116, 117]. Our data suggest that modified forms of LDL can also be useful biomarkers for CVD [72, 73, 75] and plaque vulnerability risk [80].

In conclusion, modified forms of LDL play a key role as a persistent insult leading to chronic vascular inflammation and cell reprogramming. The pro-inflammatory effects of modified LDL are significantly enhanced as a consequence of the formation of immune complexes. In general, modified LDL-IC have pro-inflammatory properties, but both clinical and experimental data suggest that there are differences in the consequences of cellular uptake of IC depending on the predominant type of LDL modification. Furthermore, due to cell reprogramming, secondary to epigenetic factors and microRNAs, a major feature of diabetes since hyperglycemia is highly involved in the process, the expression of receptors involved in innate immunity responses and scavenger receptors, as well as expression of pro-thrombotic and pro-inflammatory mediators, may be considerably affected. These novel findings open a variety of basic and clinical research perspectives, ranging from the study of epigenetic and microRNAs cell reprogramming, the investigation of the molecular mechanisms that are responsible for the different cellular effects of different LDL

modifications, to the definition of specific LDL modifications as risk factors able to discriminate between people with different types or degrees of diabetes-associated complications.

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Chapter 14
Endothelial Dysfunction in Type 2 Diabetes with an Update on New Interventions

Natalie C. Ward, Wann Jia Loh, and Gerald F. Watts

Introduction

Type 2 diabetes mellitus (T2DM) markedly increases the risk of all forms of cardiovascular disease [1, 2]. Endothelial dysfunction is an early indicator of diabetic vascular disease and independently predicts cardiovascular risk [3]. Major factors that contribute to endothelial dysfunction include dyslipoproteinemia [4], oxidative stress, and inflammation [5–7]. Dysglycemia, hypertension, and insulin resistance

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are clearly important, but likely operate through oxidative stress and pro-inflammatory pathways [5, 8, 9]. Both invasive and non-invasive methods for assessing endothelial function have generated a wealth of knowledge concerning the pathogenesis and therapeutic regulation of endothelial dysfunction in T2DM [3, 10]. We provide a brief update on the previous chapter in the first edition of this book "Lipoproteins in Diabetes Mellitus."

Endothelial Function

Normal Endothelial Function and Nitric Oxide

The endothelium maintains vascular homeostasis through multiple regulatory functions, including the release of several vasoactive factors that maintain vessel wall tone and blood fluidity, while limiting smooth muscle cell proliferation and inflammation [10–12]. Arguably, the most important of the endothelium-derived molecules is nitric oxide (NO), although maintenance of endothelial function also involves endothelin-1 (ET-1), angiotensin II, prostacyclin, and endothelial-derived hyperpolarizing factor (EDHF) [3].

In response to shear stress or activation of muscarinic receptors by the G-protein signaling pathway, endothelial NO synthase (eNOS) is activated. This generates, in a tightly coupled process, NO and citrulline from L-arginine, molecular oxygen, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) [3, 11]. NO released by this process diffuses to the underlying smooth muscle layer where it stimulates soluble guanylate cyclase. The production of cyclic guanosine 3′,5′-monophosphate (cGMP) results in vasodilation and inhibits chemotaxis and platelet aggregation [13].

In Vivo Measurement of Endothelial Function

Endothelial function may be measured indirectly in the peripheral circulation by assessing the vasodilatory responses of conduit and resistance arteries to stimuli that increase NO release (Table 14.1) [3, 13, 14]. In the brachial artery, shear stress is generated by hyperemia following an induced period of local ischemia, and flow-mediated dilatation (FMD) is measured using high-resolution ultrasonography or even magnetic resonance imaging [15, 16]. Blood flow changes in the forearm microcirculation following hyperemia, or intra-arterial infusion of muscarinic receptor agonists such as acetylcholine, can be measured using venous occlusion strain-gauge plethysmography [17, 18]. A more recent non-invasive clinical tool to assess endothelial dysfunction is digital peripheral arterial tonometry (PAT) (Endo-PAT, Itamar Medical) [19, 20].

Table 14.1 Techniques and methods for assessing endothelial function in humans

Coronary circulation	Peripheral circulation	Circulating biomarkers	Vasodilatory stimuli
QC angiography Positron emission tomography	Ultrasonography: FMD Plethysmography: FABF Endo-PAT	ADMA, NO ET-1 hs-CRP vWF PAI-1 ICAM, VCAM Selectins EP cells EMP	Acetylcholine Shear stress Nitrates NOS inhibitors

Key: *QC angiography* quantitative coronary angiography, *PE tomography* positron emission tomography, *FMD* flow-mediated dilation, *FABF* forearm blood flow, *Endo-PAT* non-invasive peripheral artery tonometry, *NOS* nitric oxide synthase, *ADMA* asymmetric dimethylarginine, *NO* nitric oxide, *ET-1* endothelin-1, *hs-CRP* high sensitivity C-reactive protein, *vWF* von Willebrand factor, *PAI-1* plasminogen activator inhibitor-1, *ICAM* intercellular adhesion molecule, *VCAM* vascular cell adhesion molecule, *EP cells* endothelial progenitor cells, *EMP* endothelial-derived microparticles

Endothelial function in coronary arteries may also be assessed, in response to pharmacological agonist or shear stress stimuli, using quantitative angiography to measure vessel diameter changes [21]. Non-invasive methods, such as positron emission tomography (PET), may also be undertaken, but are costly [13]. In addition, as this procedure involves the infusion of ^{18}F-labeled deoxyglucose, which is similar in structure to natural glucose and is similarly taken up by GLUT receptors, it can lead to direct competition between plasma glucose and ^{18}F-labeled deoxyglucose, particularly in hyperglycemia. Hyperglycemia along with oral and insulin diabetes medications can affect PET scan accuracy and need to be considered in analysis of glucose uptake during the procedure [22].

Circulating biomarkers may be measured as indirect indices of endothelial cell damage, activation, and inflammation (Table 14.1) [23–29]. Impaired mobilization or depletion of endothelial progenitor cells derived from bone marrow is involved in the pathogenesis of endothelial dysfunction, and their circulating levels can also be used as a marker of endothelial dysfunction [30–33]. Subsequently, a relationship between progenitor cells and cell-derived microparticles has been demonstrated [32]. Microparticles (MP) are small membrane-shed vesicles derived from cell surfaces under conditions of cellular activation or injury/apoptosis [32, 34]. Thus, endothelial-derived microparticles (EMP) may be potential markers of endothelial dysfunction [32, 34]. Vascular extracellular superoxide dismutase (ecSOD) activity, the major antioxidant enzyme system of the vessel wall, was substantially reduced in patients with CAD and closely associated with NO-mediated vasodilation, suggesting that reduced ecSOD activity contributes to the reduced bioavailability of NO [35]. However, measurement of ecSOD requires the intravenous injection of heparin, therefore, its utility as a surrogate marker of endothelial dysfunction in the clinical setting is less practicable.

Endothelial Dysfunction

Endothelial Dysfunction: Uncoupling of eNOS

Endothelial dysfunction reflects an imbalance between release of vasodilator and vasoconstrictor endothelial-derived factors. A decrease in the bioavailability of NO involves either a decrease in NO synthesis or inactivation of NO due to increased endothelial production of reactive oxygen species (ROS) [36]. With increased oxidative stress, tetrahydrobiopterin (BH_4), a cofactor that tightly regulates NO production, is oxidized resulting in the uncoupling of eNOS and reduced NO production [37]. Elevated levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS through competition with L-arginine, may further reduce NO production [13]. This perpetuates a cycle of vascular oxidative stress through the transfer of electrons to molecular oxygen, forming oxidant species such as superoxide and peroxynitrite, which further consumes NO and increases oxidative stress [37–39].

Endothelial dysfunction may also involve altered levels of vasoconstrictors, such as endothelin-1 and angiotensin II, and other vasodilators such as endothelial-derived hyperpolarizing factor (EDHF) and prostacyclin [3, 36]. MicroRNAs have been implicated in the regulation of endothelial dysfunction and have been discussed in a recent review [40].

Predictive Value of Endothelial Dysfunction

Several studies in diverse groups of subjects have shown that endothelial dysfunction measured by the aforementioned techniques in different vascular beds is predictive of clinical events [41–56]. The principal studies are shown in Table 14.2. Some of these studies included type 2 diabetic patients. In type 2 diabetic patients with normal coronary arteries, coronary artery dysfunction, as assessed by cold-pressor test, was found to predict long-term cardiovascular outcomes, with a relative risk of 4.9 [51]. In a study of asymptomatic type 2 diabetic patients who underwent myocardial perfusion imaging, normal endothelial function had a 93% negative predictive value in excluding CAD [52]. In patients with newly diagnosed CAD and impaired brachial artery FMD (40% with diabetes), a persistently impaired FMD was an independent predictor of future cardiovascular events after 6-months of optimized lifestyle changes and pharmacotherapy [54]. A community based study in 1016 older adults (72% with diabetes) demonstrated that impaired forearm endothelial-dependent vasodilation was associated with a 5-year risk of major adverse cardiovascular events [56]. A meta-analysis of 14 observational studies and a review, both concluded that FMD is predictive of cardiovascular events and provides prognostic information that is at least equal to the information gained from conventional cardiovascular risk factors; however, future research is required to confirm FMD's efficacy in the assessment of CVD risk [57, 58].

Table 14.2 Selected studies evaluating endothelial dysfunction as a predictor of cardiovascular events

Study (year)	*n*	Patient population	Arterial bed	Technique(s)	Endpoints	Mean follow-up (months)	Endothelial dysfunction as an independent predictor	Estimated RR of CV events
Papaioannou et al. [52] (2006)	75	T2DM patients without CAD	Brachial	FMD, NMD MPI	CAD	60	No association	na
Nitenberg et al. [51] (2005)	124	Patients with HT or T2DM and normal coronary arteries	Coronary	CPT	CAD, CVD	112	Yes	4.9
Nitenberg et al. [50] (2004)	72	T2DM patients without CAD	Coronary	CPT	CAD, CVD	45	Yes	2.8
Chan et al. [48] (2003)	152	Patients with CAD	Brachial Carotid	FMD IMT	CAD, CVD	34	Yes	4.7[a]
Halcox et al. [46] (2002)	308	Patients with and without CAD	Coronary	Acetylcholine response	CAD, CVD	46	Yes	1.4
Perticone et al. [45] (2001)	225	Patients with untreated hypertension	Forearm	FABF	CAD, CVD, PVD	32	Yes	2.1
Schachinger et al. [43] (2000)	147	Patients with chest pain or SVD	Coronary	Acetylcholine response, CPT, FMD, NMD	CAD, CVD, PVD	80	Yes	na
Suwaidi et al. [41] (2000)	157	Patients with mild CAD	Coronary	Acetylcholine, adenosine, and nitroglycerin responses	CAD	28	na	na
Muiesan et al. [53] (2008)	172	Patients with uncomplicated hypertension (28% with diabetes)	Brachial	FMD	CV events	109	Yes	2.5[b]

(continued)

Table 14.2 (continued)

Study (year)	*n*	Patient population	Arterial bed	Technique(s)	Endpoints	Mean follow-up (months)	Endothelial dysfunction as an independent predictor	Estimated RR of CV events
Yeboah et al. [55] (2009)	3026	Population-based cohort of without known CVD	Brachial	FMD	CV event	60	Yes	na
Kitta et al. [54] (2009)	251	Patients with CAD and optimized therapy (40% with diabetes)	Brachial	FMD	CVD	36	Yes	1.8[c]
Lind et al. [56] (2011)	1016	Community based study of older adults (>70 years), 72% with diabetes	Forearm, brachial, radial, carotid	Acetylcholine and sodium nitroprusside, FMD, PWA, IMT	CV disease (MI or stroke	60	Yes, forearm EDV only	na

Key: *RR* relative risk, *CV* cardiovascular, *T2DM* type 2 diabetes mellitus, *CAD* coronary artery disease, *EDV* endothelial-dependent vasodilation, *FMD* flow-mediated dilation of brachial artery, *NMD* nitroglycerin-mediated dilation, *MPI* myocardial perfusion imaging, *na* not assessed, *HT* hypertension, *CPT* cold-pressor test, *CVD* cerebrovascular disease, *IMT* intima medial thickness, *FABF* forearm blood flow, *PVD* peripheral vascular disease, *PWA* pulse wave-based method, *SVD* single vessel disease

[a] Approximation for patients with high IMT plaque burden

[b] Estimated for patients with impaired FMD compared to those with preserved FMD

[c] Estimated for patients with persistently impaired FMD compared to those with improved FMD

Pathogenesis of Endothelial Dysfunction in Type 2 Diabetes Mellitus

Endothelial dysfunction has been demonstrated in T2DM in both the resistance and conduit vessels of the peripheral circulation [59–63], as well as in the coronary circulation [64, 65]. Plasma levels of the soluble adhesion molecules E-selectin, vascular cellular adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1 are elevated in subjects with T2DM [3, 28, 29, 66]. Similarly, increased plasma levels of von Willebrand factor (vWF), a measure of endothelial cell damage and activation, are found in diabetes [3, 29, 66]. Microalbuminuria is an independent predictor of endothelial dysfunction and may indicate widespread vascular dysfunction in diabetes [3, 67].

The precise pathogenetic mechanisms underlying the development of endothelial dysfunction in T2DM remain unclear, but at inception they probably involve uncoupling of both eNOS activity, and mitochondrial oxidative phosphorylation (Fig. 14.1), as well as the activation of vascular NAD(P)H oxidase. These three mechanisms essentially result in increased generation of superoxide ($O_2^{\cdot -}$) radicals, eNOS uncoupling, and the overproduction of peroxynitrite. The main factors that combine to cause these biochemical disturbances are dyslipoproteinaemia, oxidative stress [4], and inflammation [5–7]. Additional clinical factors that may contribute, either individually or synergistically, to endothelial dysfunction in T2DM

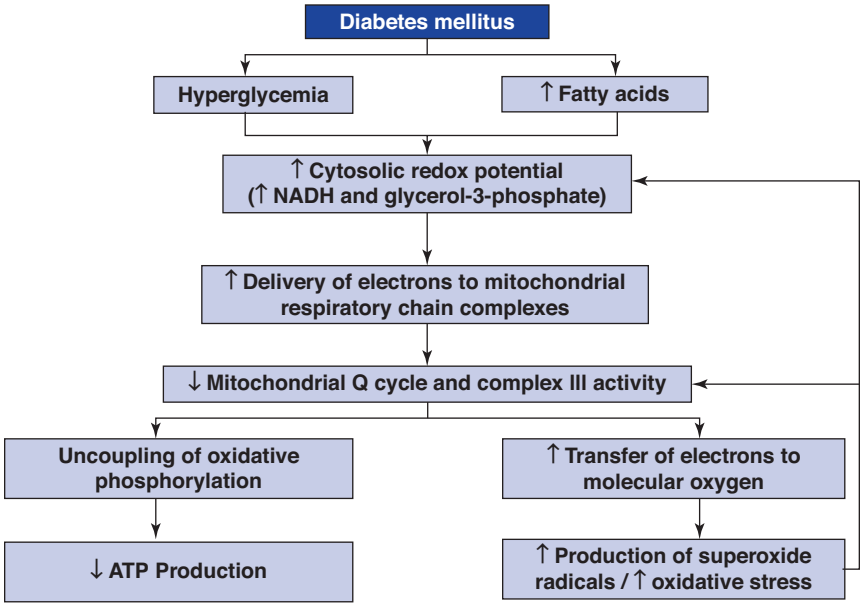

Fig. 14.1 Mechanism whereby hyperglycemia and elevated fatty acids induce uncoupling of mitochondrial oxidative phosphorylation and increased oxidative stress in diabetes. *ATP* adenosine triphosphate, *NADH* reduced nicotinamide adenine dinucleotide

include hypertension [68], visceral obesity [69], insulin resistance [5, 70, 71], postprandial hyperlipidaemia [72–74], fasting and postprandial hyperglycaemia [75–77], elevated levels of ADMA [36, 78], and glycated lipoproteins [79].

The impact of insulin resistance in T2DM operates at an insulin signaling level in endothelial cells and in adipose tissue and skeletal muscle [5]. Impaired insulin receptor substrate-1 (IRS-1) and phosphatidylinositol (PI) 3-kinase insulin signaling results in decreased production of NO and endothelial dysfunction on the one hand, and decreased glucose transporter (GLUT4) translocation and peripheral insulin resistance on the other. Insulin resistance also increases fatty acid availability which uncouples mitochondrial function in endothelial cells. This generates ROS by increasing advanced glycation end-products (AGES), protein kinase C (PKC), and *N*-acetylglucosamine (glcNAC), impairing eNOS activity and inducing endothelial dysfunction. Inflammation, lipotoxicity, and glucotoxicity are all increased in diabetes and collectively contribute to insulin resistance and endothelial dysfunction [5]. Figure 14.2 suggests that the pathogenesis of endothelial dysfunction in T2DM has oxidative stress as the central pathway for a wide spectrum of risk factors [3]. Inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), also play an important role in endothelial dysfunction in obesity and T2DM [80]. The pro-inflammatory cytokine tumor necrosis factor (TNF)-alpha may contribute to endothelial dysfunction by stimulating vascular NADPH oxidase and both superoxide production and oxidative stress.

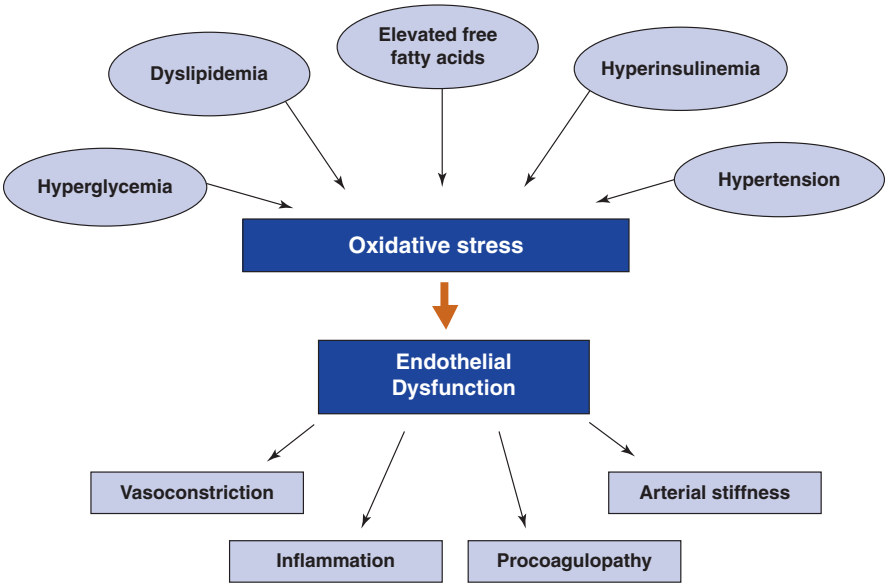

Fig. 14.2 Pathogenesis and consequences of endothelial dysfunction in type 2 diabetes mellitus. Oxidative stress also contributes to endothelial dysfunction by activating protein kinase C, polyol, hexosamine, and NFkappa B pathways, as well as by increasing asymmetric dimethylarginine and advanced glycation end-products

Treating Endothelial Dysfunction in Type 2 Diabetes

Strategies for treating endothelial dysfunction in T2DM will necessarily target the pathophysiological factors that underlie dysfunction, such as hyperglycemia, insulin resistance, dyslipidemia, increased oxidative stress, inflammation, and hypertension [81, 82]. Treatment options range from lifestyle interventions to nutritional supplements and specific pharmacological therapies. The results of selected intervention studies are summarized in Table 14.3.

Lifestyle Interventions

Diet and exercise programs aimed at achieving weight loss improve many of the metabolic abnormalities in T2DM that contribute to endothelial dysfunction, such as hyperglycemia, insulin resistance, visceral obesity, hypertension, and dyslipidemia. Weight loss and increased physical activity have been shown to improve endothelial dysfunction in T2DM patients. In an uncontrolled study, obese insulin resistant subjects (including subjects with T2DM), who underwent a 6-month lifestyle modification programme of caloric restriction and regular supervised exercise, achieved a 7% mean reduction in body weight, with improvement in brachial artery FMD and reduction in markers of endothelial activation and coagulation [83]. Insulin sensitivity, glycemic control, and HDL-cholesterol levels also improved. A study in healthy volunteers found that ingestion of a high fat meal resulted in delayed and lower peaks of both glucose and insulin compared to a high carbohydrate meal. This delay was associated with an increase in IL-6, which may impair vascular function [84]. The CORDIOPREV study investigated whether long-term consumption of a Mediterranean diet rich in olive oil or a low-fat diet was associated with improvements in endothelial function in pre-diabetic and T2DM patients. At 1.5 years, habitual consumption of the Mediterranean diet rich in olive oil was associated with increased FMD compared to both baseline and the low-fat control diet. These beneficial effects were seen in both pre-diabetic and T2DM participants [85].

A randomized, crossover study of combined aerobic and resistance exercise training for 8-weeks demonstrated an increase in brachial artery FMD and acetylcholine (ACh)-stimulated forearm blood flow (FABF) in T2DM subjects [86]. Although glycemic control also improved, reductions in HbA1c and fasting glucose were not correlated with changes in endothelial function. Indeed, it appears that the benefits of exercise in improving endothelial dysfunction are not necessarily dependent on improvement in traditional cardiovascular risk factors [87], suggesting that repeated exercise may also act directly on the vasculature via a shear stress-related mechanism, possibly involving endothelial nitric oxide synthase (eNOS) up-regulation or reduced nitric oxide (NO) degradation by free radicals [88]. A meta-analysis of databases evaluating the influence of exercise training programs >8-weeks found that exercise resulted in an overall improvement in FMD (1.8%) in

Table 14.3 Randomized controlled trials investigating the therapeutic regulation of endothelial function in patients with type 2 diabetes mellitus

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Lifestyle interventions					
Maiorana et al. [86] (2001)	16	Aerobic and resistance exercise	2	FMD, ACh, stimulated FABF	+
Torres-Pena et al. [85] (2018)	805	Mediterranean diet rich in olive oil	18	FMD	+
Antiglycemic agents and insulin sensitizers					
Bagg et al. [309] (2001)	43	Improved glycemic control/usual glycemic control	5	FMD	ns
Rask-Madsen et al. [193] (2001)	28	Insulin therapy/no hypoglycemic drug therapy	2	Insulin-stimulated FABF response to ACh	+
Vehkavaara et al. [191] (2000)	18	Insulin + metformin v metformin	6	FABF response to ACh, SNP	+
Sundaresan et al. [194] (1997)	14	Metformin/glibenclamide	1	FABF response to diazoxide, ACh, SNP, norepinephrine	ns
Williams et al. [195] (1998)	14	Glibenclamide/placebo	1	FABF response to ACh	ns
Spallarossa et al. [196] (2001)	20	Glibenclamide/glimepiride/diet	2	FMD	ns
Wascher et al. [197] (2005)	15	Gliclazide/glibenclamide	1	FABF response to hyperemia	
Abbink et al. [198] (2002)	24	Glibenclamide/glimepiride or metformin	2	FABF response to diazoxide, ACh, dipyridamole, forearm ischemia	ns
Manzella et al. [199] (2005)	16	Repaglinide/glibenclamide	2	FMD	+[a]
Schmoelzer et al. [200] (2006)	12	Repaglinide/control	2 h	FMD	+

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Mather et al. [201] (2001)	43	Metformin/placebo	3	FABF response to ACh	+
Natali et al. [202] (2004)	74	Rosiglitazone, metformin, or placebo	4	FABF response to ACh	+[b]
Caballero et al. [205] (2003)	87	Troglitazone/placebo	3	FMD	+[c]
Martens et al. [207] (2005)	20	Pioglitazone/placebo	1	FMD	+
Pistrosch et al. [208] (2004)	12	Rosiglitazone/nateglinide	3	FABF response to ACh	+[b]
Shimabukuro et al. [209] (2006)	14	Acarbose/placebo prior to a test meal	1 wk	FABF response to hyperemia	+
Nystrom et al. [211] (2004)	12	Recombinant glucagon-like peptide-1/saline	1 wk	FMD	+
Koska et al. [210] (2010)	28	Exenatide/placebo	Single dose	PAT	+
Sawada et al. [220] (2019)	50	Empagliflozin	6	FMD	+
Tanaka et al. [221] 2020	117	Empagliflozin/placebo	6	RH-PAT	–
Sezai et al. [223] (2019)	35	Canagliflozin	12	FMD	+
Zainordin et al. [224] (2020)	81	Dapagliflozin/placebo	3	FMD	–
Shigiyama et al. [227] (2017)	80	Dapagliflozin with metformin/ metformin	4	FMD	+
Sugiyama et al. [228] (2017)	54	Dapagliflozin	6	RH-PAT	+

(continued)

Table 14.3 (continued)

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Papadopoulou et al. [229] (2021)	85	Dapagliflozin/placebo	3	PWV	+
Tamura et al. [230] (2022)	63	Empagliflozin/glimepiride	3	FMD	–
Nomoto et al. (2015) [214]	31	Liraglutide/glargine	14 wk	FMD	–
Ikonomidis et al. [232] (2020)	160	Insulin/liraglutide/empagliflozin/ combination	12	PWV	+
Antihypertensive agents					
O'Driscoll et al. [239] (1999)	10	Enalapril/placebo	1	FABF response to ACh	+
Cheetham et al. [240] (2000)	9	Losartan/placebo	1	FABF response to ACh	+
Cheetham et al. [241] (2001)	12	Losartan/placebo	1	FMD	+
Ceriello et al. [103] (2005)	20	Atorvastatin/irbesartan/placebo	1 wk	FMD	+
Flammer et al. [242] (2007)	13	Losartan/atenolol	1	FMD	+[d]
Davies et al. [243] (2004)	42	Spironolactone/placebo	1	FABF response to ACh	—
Yilmaz et al. [248] (2010)	108	Valsartan/amlodipine/valsartan + amlodipine	3	FMD	+[e]
Antioxidants and nutritional supplements					
Watts et al. [251] (2002)	40	CoQ_{10}/placebo	3	FMD	+
Playford et al. [121] (2003)	20	CoQ_{10}/placebo	3	FABF response to ACh, BK, SNP	ns

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Lim et al. [252] (2008)	80	CoQ_{10}/placebo	3	Microcirculatory function	ns
Anderson et al. [255] (2006)	20	Vitamin C/placebo	2 wk	FMD	+
Paolisso et al. [257] (2000)	40	Vitamin E/placebo	2	FMD	+
Darko et al. [260] (2002)	35	Vitamin C/placebo	3 wk	FABF response to ACh	ns
Tousoulis et al. [116] (2007)	41	Vitamin C/atorvastatin/placebo	4 wk	FABF response to hyperemia	ns[f]
Chen et al. [261] (2006)	32	Vitamin C/placebo	1	FABF response to ACh, SNP or insulin	ns
Gazis et al. [262] (1999)	48	α-Tocopherol/placebo	2	FABF response to ACh, BK, SNP	ns
Beckman et al. [263] (2003)	23	Vitamin E and C/placebo	6	FMD	ns
Mangoni et al. [270] (2005)	26	Folic acid/placebo	1	FABF response to ACh	+
Title et al. [271] (2006)	19	Folic acid/placebo	2 wk	FMD	+
Miscellaneous therapies					
Desouza et al. [272] (2002)	14	Sildenafil/placebo	2 wk	FMD	+
Koh et al. [277] (2001)	20	Conjugated equine estrogen/placebo	2	FMD	ns
Silvestri et al. [278] (2005)	30	HRT/Tibolone/DHEAS	1	FMD	+
Howes et al. [279] (2003)	16	Red clover derived isoflavones/ placebo	1	FABF response to ACh	ns
Kang et al. [287] (2002)	35	Testosterone/placebo	3	FMD	+
Groti et al. [288] (2018)	55	Testosterone/placebo	12	FMD	+

(continued)

Table 14.3 (continued)

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Bilsborough et al. [307] (2002)	13	Pentoxifylline/placebo	2	FABF response to ACh, FMD	ns
Butler et al. [308] (2000)	11	Allopurinol	1	FABF response to ACh	+

Key: *wk* weeks, *FMD* flow-mediated dilation of brachial artery, *ACh* acetylcholine, *FABF* forearm blood flow, **+** indicates improved endothelial function, *ns* no significant effect, *SNP* sodium nitroprusside, *BK* bradykinin, **—** indicates a decreased response, *PAT* peripheral arterial tonometry, *HRT* hormone replacement therapy, *DHEAS* dehydroepiandrosterone-sulfate

[a] + in repaglinide only

[b] + in rosiglitazone only

[c] In recently diagnosed diabetics (<3 years) without microvascular disease

[d] + in losartan only

[e] + in all three treatment groups

[f] = in atorvastatin only

patients with T2DM. This benefit was seen with both aerobic (1.2%) and combined aerobic and resistance exercise (2.5%), with resistance only exercise having a non-significant effect. There was no added benefit with high intensity over moderate intensity, however improvements in FMD were smaller in T2DM patients compared to non-diabetic controls [89].

Epidemiological studies provide a large body of evidence supporting the association between cigarette smoking and cardiovascular events [90]. Cigarette smoking is also associated with the premature development of macrovascular and microvascular complications in patients with T2DM [91]. Cigarette smoke increases inflammation, thrombosis, and oxidation of LDL-cholesterol, with experimental and clinical evidence supporting the notion that increased oxidative stress results in vascular dysfunction [90]. Both active and passive cigarette smoking are associated with a dose-related impairment of endothelial function [92–94]. Brachial artery FMD was assessed in current and former healthy young adult smokers [92]. Former male smokers, but not former female smokers, had higher FMD than current smokers, suggesting that endothelial function may improve with smoking cessation [92]. A larger randomized, placebo-controlled study investigated the effects of five smoking cessation pharmacotherapies on brachial artery FMD in 1504 subjects [95]. Despite a greater weight gain, FMD significantly improved in subjects who quit and remained abstinent at 1 year, but did not change in those who continued to smoke [95]. Studies assessing the effects of smoking cessation in T2DM patients are warranted.

Lipid-Lowering Therapies

Hydroxymethylglutaryl (HMG)-CoA Reductase Inhibitors (Statins)

Statins, inhibitors of hydroxymethylglutaryl (HMG)-CoA reductase, have been proven in large clinical trials to reduce cardiovascular mortality in a wide range of population subgroups, including participants with diabetes [96]. Apart from their main effect in lowering LDL-cholesterol, statins may also have direct anti-inflammatory and antioxidant effects on the vasculature [81]. Statins have been shown to improve endothelial function in non-diabetic subjects with dyslipidemia [97, 98], but results in T2DM subjects have been inconsistent and contradictory. Uncontrolled studies have not shown any benefit of statin therapy on serotonin-stimulated forearm blood flow (FABF) or brachial artery FMD in T2DM subjects [99–102]. One trial suggested improvement in endothelial function in a subgroup who achieved greater LDL-lowering [102], but another showed no benefit despite intensive lipid-lowering [100].

A number of randomized, placebo-controlled studies have shown a beneficial effect of statins on brachial artery FMD in T2DM subjects [103–106]. On-treatment improvement in endothelial function occurred within days, prior to any plasma lipid changes and was correlated with a reduction in oxidative stress, inflammation, and endothelial cell activation [103, 104, 106]. Compared with placebo, atorvastatin

was associated with a reduction in vascular cell adhesion molecules (VCAM1) and E-selectin, suggesting an improvement in endothelial function in T2DM patients that was independent of the lipid-regulating effects of atorvastatin [107]. In male participants with stable atherosclerosis (30% with diabetes), treatment with rosuvastatin or atorvastatin inhibited Rho/Rho kinase pathway activity and this inhibition was associated with improvement in brachial artery FMD in the absence of a reduction in plasma LDL-cholesterol levels [108]. In statin-naïve, hypertriglyceridemic T2DM patients who had no history of CVD, atorvastatin, or rosuvastatin significantly improved FMD and plasma levels of CRP, but associations between FMD and CRP or triglycerides were not reported [109]. In a study in normocholesterolemic T2DM patients with no evidence of CAD, 4-weeks of low dose atorvastatin (10 mg/day) significantly improved brachial artery FMD compared with placebo. A third of the patients in this study were reported to have had dyslipidemia at baseline, but on-treatment lipids were not reported [110].

In contrast, there are randomized, double-blind, placebo-controlled studies that have shown no effect of statin therapy on brachial artery FMD in subjects with T2DM [111, 112], despite improvements in dyslipidemia [113–115]. Studies examining the effect of statin therapy on forearm vascular reactivity in T2DM subjects have shown improvement with atorvastatin [116], but not with cerivastatin [117]. However, in the latter study, ACh-stimulated FABF increased with co-infusion of L-NMMA (inhibitor of NOS), suggesting an effect of treatment on non-NO vasoactive mediators, such as EDHF.

Fibric Acid Derivatives

In addition to their lipid-regulating effects, fibrates may also reduce vascular inflammation and endothelial cell activation. In randomized controlled studies, fenofibrate and related drugs appear to have consistent beneficial effects on endothelial function in both non-diabetic and T2DM subjects. Fenofibrate has been shown to improve brachial artery FMD in non-diabetic subjects with mixed hyperlipidemia or primary hypertriglyceridemia [118, 119]. Fenofibrate improved brachial artery FMD in statin-naïve T2DM patients with dyslipidemia [120]. However, fenofibrate alone did not significantly improve forearm microcirculatory function in such patients [121]. Moreover, Chew et al. have demonstrated that fenofibrate and CoQ_{10} independently and interactively lowered 24-h ambulatory blood pressure [122] consistent with their beneficial effects on endothelial function in resistance arterioles. Ciprofibrate and gemfibrozil have been shown to improve brachial artery FMD in T2DM subjects in fasting and postprandial states [123, 124]. However, 12-weeks of fenofibrate therapy in T2DM patients did not improve microvascular endothelial-dependent function when assessed by skin blood flow response to the iontophoresis of acetylcholine [125]. In these studies, the effects of fibrates on markers of oxidative stress and insulin sensitivity were also inconsistent [120, 121, 123].

Although short-term fenofibrate therapy may improve endothelial function [118–120], a sub-study of the longer-term FIELD study showed no beneficial treatment effect on carotid intima-media thickness, augmentation index, or

biomarkers of endothelial function in T2DM participants [126]. However, the FIELD study subjects were mostly low risk (as evidenced by the low CVD event rate) and had not been selected for having endothelial dysfunction at baseline. The PROMINENT study [127] further investigated this by looking at the effect of the selective PPAR-α modulator, pemafibrate on residual cardiovascular risk in patients with T2DM and already on-treatment to manage their LDL-cholesterol. Although not yet published, following planned interim analysis, the data safety monitoring board concluded that the primary endpoint (composite of nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, and cardiovascular death) would not be met (https://www.lipid.org/nla/phase-3-prominent-cardiovascular-outcomes-study-discontinued-kowa).

Fenofibrate also has beneficial microvascular effects. In FIELD, monotherapy with fenofibrate, when compared with placebo, significantly reduced the need for laser therapy for diabetic retinopathy [128] and may delay albuminuria progression and impairment of renal function [129]. Reports from ACCORD show that both the addition of fenofibrate to simvastatin and intensive glycemic therapy reduced progression of diabetic retinopathy [130, 131]. In T2DM patients with hypertriglyceridemia and retinopathy, adding a fibrate to statin therapy and weight loss may be safe and effective treatment options for cardiovascular and retinopathy risk reduction compared with intensification of antihyperglycemic and/or statin therapy.

Nicotinic Acid (Niacin)

Niacin may also improve endothelial function and reduce CVD events through direct effects on the vasculature [132]. Two studies have reported on niacin monotherapy. In a controlled study in 22 healthy men with low HDL-cholesterol (<1.04 mmol/L), no-flush niacin 1.5 g/day for 12-weeks significantly improved FMD with no change in plasma lipids or chylomicron remnants suggesting a direct vascular effect by niacin [133]. In metabolic syndrome patients allocated to ER niacin (1000 mg/day) or placebo for 52-weeks, niacin improved FMD by 22% ($p < 0.001$), significantly regressed CIMT, decreased high sensitivity C-reactive protein (hs-CRP) by 20% ($p = 0.013$) and significantly improved plasma lipids (HDL-cholesterol, LDL-cholesterol, and triglycerides) [134]. No studies have reported on patients with T2DM. Collectively, these studies demonstrate that niacin is effective in improving endothelial function in subjects with low HDL-cholesterol. Further, improvements in both lipids and inflammatory markers suggest that both lipid-mediated and direct mechanisms are involved in the beneficial vascular effects of niacin.

Omega-3 Fatty Acids

Omega-3 fatty acids derived from marine fish oil predominantly lower triglycerides, but may also have beneficial effects on HDL subfractions and LDL particle size, as well as direct actions on the vasculature to reduce inflammation and endothelial cell activation [135]. Randomized, double-blind, controlled trials of omega-3 fatty acid

supplementation in T2DM subjects have shown improvement in ACh-stimulated FABF [136], but no change in brachial artery FMD [137]. In hypertriglyceridemic T2DM subjects, inclusion of omega-3 fatty acids in a meal containing predominantly unsaturated fatty acids reduced postprandial lipemia and improved brachial artery FMD [138], possibly by attenuating the postprandial rise in lipoprotein subclass containing apolipoproteins B and C (LpB:C) [139]. In subjects with metabolic syndrome, it is possible that improvement in forearm vasodilator response is attributable to docosahexaenoic acid (DHA) and not the eicosapentaenoic acid (EPA) component of fish oils [140]. The REDUCE-IT study in patients with established CVD or diabetes and other risk factors, currently taking statin therapy and with elevated triglycerides found pure icosapent ethyl reduced cardiovascular risk and ischemic events [141, 142]. In contrast, the STRENGTH trial in statin-treated patients with atherogenic dyslipidemia and high cardiovascular risk found no benefit of an omega-3 carboxylic acid formulation containing both EPA and DHA on the composite outcome of major cardiovascular events [143]. These findings suggest that the beneficial effects of omega-3 fatty acids may be specific to EPA [144, 145], and future studies investigating effects on endothelial function should consider use of pure EPA over omega-3 EPA/DHA combinations.

In summary, the lipid-lowering therapies discussed above (statins, fibrates, niacin, and omega-3 fatty acids) all correct diabetic dyslipidemia, improving lipid and lipoprotein composition and concentrations to varying degrees and by different mechanisms (Table 14.4). Collectively, these agents have been demonstrated to improve endothelial dysfunction, but not all the findings are consistent. PCSK9 inhibitors have also been investigated in patients with T2DM and shown to reduce LDL-cholesterol and cardiovascular risk and events, although no studies specifically looking at endothelial dysfunction have been carried out [146–148]. Endothelial

Table 14.4 Possible mechanisms of action of four lipid-regulating agents that improve endothelial function

Parameter	Statins	Fibrates	Niacins	Omega-3 fatty acids
↓ LDL-cholesterol	++	+/−	+	+/−
↑ LDL particle size	+	++	++	+
↓ Triglyceride	+	+++	++	++
↓ Chylomicron remnants	++	+/−	+/−	+/−
↑ HDL-cholesterol	+	++	+++	+/−
↑ PPAR activation/expression	+	+++	++	+
↓ Vascular inflammation	+	+	+	+
↑ NO production	+	+	+	+/−
↓ Endothelin-1 synthesis/expression	+	+	+/−	+/−
↓ Oxidative stress	+	+	+	+

Adapted from and reproduced with permission from Woodman et al. 2005 [3]
LDL low density lipoprotein, *NO* nitric oxide, *PPAR* peroxisome proliferator-activated receptor, *statin* HMG-CoA reductase inhibitor, ↓ indicates decreased, ↑ indicates increased, + indicates minor effect, ++ indicates moderate effect, +++ indicates major effect, +/− indicates equivocal effect.

dysfunction is a complex condition with multiple factors contributing to its pathogenesis. A multifactorial strategy that combines lipid-regulating drug therapy with other interventions, such as lifestyle changes, insulin sensitizers, and antioxidant and cofactor supplementation, is likely to achieve the best cardiovascular outcome.

Combination Therapies

In large prospective clinical outcome trials (HPS, CARDS, TNT, and CTT meta-analysis), the residual risk of CVD events in T2DM remains high, despite achievement of optimal or near optimal LDL-cholesterol levels with statin therapy [96, 149–151]. Studies examining the effects of statins on endothelial dysfunction have demonstrated inconsistent and contradictory results (Table 14.5) [103–117]. It is possible that in T2DM, treatment with a single therapeutic agent may not adequately improve endothelial function. Several complementary treatment options may be of interest.

Table 14.5 Randomized controlled trials investigating the therapeutic regulation of endothelial function in patients with type 2 diabetes mellitus: lipid-regulating therapies

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Lipid regulating agents					
Statins					
Tsunekawa et al. [104] (2001)	27	Cerivastatin/ placebo	3 days	FMD	+
Tan et al. [105] (2002)	80	Atorvastatin/ placebo	6	FMD	+
Ceriello et al. [106] (2002)	30	Simvastatin/ placebo	3–6 days +3	FMD	+
Ceriello et al. [103] (2005)	20	Atorvastatin/ irbesartan/ placebo	1 wk	FMD	+
Dalla et al. [107] (2003)	25	Atorvastatin/ placebo	12	VCAMI and E-selectin	+
Brunetti et al. [109] (2007)	22	Atorvastatin/ rosuvastatin	3	FMD	+
Adel et al. [110] (2010)	60	Atorvastatin/ placebo	4 wk	FMD	+
Economides et al. [111] (2004)	40	Atorvastatin/ placebo	3	FMD	ns
Beishuizen et al. [112] (2005)	250	Cerivastatin replaced by simvastatin	24	FMD	ns

(continued)

Table 14.5 (continued)

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Van Venrooij et al. [113] (2002)	133	Atorvastatin 10 mg/ atorvastatin 80 mg	7.5	FMD	ns
Tantikosoom et al. [115] (2005)	42	Atorvastatin/ placebo	7.5	FMD	ns
Tousoulis et al. [116] (2007)	41	Atorvastatin/ vitamin C/no treatment	1	FABF response to post-ischaemic hyperaemia	+[a]
Tran et al. [117] (2005)	11	Cerivastatin/ placebo	2	FABF response to ACh and L-NMMA	ns
Fibrates					
Playford et al. [120] (2002)	40	Fenofibrate/ placebo	3	FMD	+
Playford et al. [121] (2003)	20	Fenofibrate/ placebo	3	FABF response to ACh, BK, SNP	ns
Evans et al. [123] (2000)	20	Ciprofibrate/ placebo	3	FMD	+
Avogaro et al. [124] (2001)	10	Gemfibrozil/ placebo	3	FMD	+
Fegan et al. [125] (2005)	10	Fenofibrate/ placebo	3	Blood flow responses to iontophoresis of ACh	ns
Hiukka et al. [126] (2008)	170	Fenofibrate/ placebo	60	IMT, AIx, biomarkers of endothelial activation	ns
Niacin					
No studies identified in T2DM patients					
Omega-3 fatty acids					
McVeigh et al. [136] (1993)	23	Fish oil/placebo	1.5	FABF response to ACh	+
Woodman et al. [137] (2003)	51	EPA/DHA/ placebo	1.5	FMD	ns
West et al. [138] (2005)	18	MUFA ± omega-3 FA	3 test meals over 3 wk	FMD	+
Combination therapies					
Hamilton et al. [156] (2010)	15	Statin + fenofibrate/statin + placebo	3	FMD + forearm microcirculatory function	+
Fegan et al. [125] (2005)	11	Cerivastatin + fenofibrate	3	Skin blood flow response to iontophoresis and skin maximum hyperaemia	ns

(continued)

Table 14.5 (continued)

Study (year)	*n*	Treatment	Treatment duration (months)	Endpoint	Treatment effect
Lee et al. [166] (2009)	71[b]	Statin + niacin/ statin/placebo	12	Carotid MRI, aortic distensibility, MRI brachial artery FMD	+[c]
Hamilton et al. [167] (2010)	15	Statin + niacin/ statin alone	5	Small artery vasodilation and compliance	+
Hamilton et al. [173] (2009)	23	Statin+ CoQ_{10}/ statin + placebo	3	FMD	+
Koh et al. [175] (2005)	50	Simvastatin + ramipril	2	FMD	++
Ceriello et al. [103] (2005)	20	Atorvastatin + irbesartan	1 wk	FMD	++
Playford et al. [121] (2003)	20	Fenofibrate + CoQ_{10}	3	FABF response to ACh, BK, SNP	+
Luescher et al. [184] (2011)	476[d]	Dalcetrapib + statin/placebo + statin	9	FMD	ns

Key: *wk* weeks, *FMD* flow-mediated dilation of brachial artery, *ACh* acetylcholine, *FABF* forearm blood flow, **+** indicates improved endothelial function, *ns* no significant effect, *SNP* sodium nitroprusside, *BK* bradykinin, *L-NMMA* L-nitro-mono-methyl arginine, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *T2DM* type 2 diabetes mellitus, **++** indicates combination therapy improved FMD more than monotherapy alone, *AIx* augmentation index, *IMT* intima-media thickness, *MUFA* monounsaturated fatty acids, **±** with or without, *omega-3 FA* omega-3 fatty acids, *CoQ_{10}* coenzyme Q_{10}, *VCAM1* vascular cell adhesion molecules 1

[a] + in atorvastatin only

[b] Only 65% of the patients T2DM

[c] + in carotid MRI only

[d] Only 45% of patients T2DM

Statins and Fibrates

In T2DM, a combination of statin and fibrate therapy can significantly benefit dyslipidemia and cardiovascular risk status [152–155]. However, there is limited evidence investigating the effects of combined statin/fibrate therapy on endothelial dysfunction in T2DM patients. In a randomized, double-blind, crossover study, fenofibrate significantly improved brachial artery FMD and forearm microcirculatory function in statin-treated T2DM patients with LDL-cholesterol <2.6 mmol/L and endothelial dysfunction [156]. Improvement in FMD was inversely associated with on-treatment LDL-cholesterol and apoB concentrations, indicating that the improvement in endothelial function may in part relate to enhanced reduction in LDL-cholesterol and apoB concentrations [156]. In contrast, microvascular endothelial function, assessed by skin blood flow response to iontophoresis of acetylcholine and sodium nitroprusside and skin maximum hyperemia to local heating, was not improved in T2DM participants treated with combination cerivastatin and fenofibrate therapy [125].

Statins and Niacins

Nicotinic acid effectively raises HDL-cholesterol, lowers triglycerides, increases LDL particle size [157], and modestly lowers lipoprotein(a) levels [Lp(a)] [158]. In diabetic subjects, a combination of niacin and atorvastatin therapy improves the atherogenic lipid profile more effectively than monotherapy [159]. Combined statin and niacin therapy has been shown to reduce the progression of coronary and carotid artherosclerosis [160–163]. Two studies have reported on the effects of combined statin/niacin therapy on endothelial function in patients with CAD [164, 165]. In these studies, the addition of niacin significantly improved endothelial function in patients with low HDL-cholesterol levels [164, 165]. In the Oxford Niaspan Study, the effect of modified-release nicotinic acid (Niaspan) on atherosclerosis and endothelial function was assessed in statin-treated patients with low HDL-cholesterol, together with either T2DM and CAD, carotid atherosclerosis, or peripheral atherosclerosis; 65% of the patients had T2DM. Compared with placebo, 12-months of niacin treatment significantly reduced carotid atherosclerosis, but did not alter either aortic distensibility or brachial artery FMD [166]. In a parallel group study, 15 statin-treated T2DM participants with LDL-cholesterol <2.5 mmol/L and endothelial dysfunction were randomized to niacin (nicotinic acid prolonged release) or no additional therapy [167]. Niacin significantly improved small artery vasodilatory function and compliance and reduced serum triglycerides by 47%. An inverse association between maximal forearm post-ischemic blood flow and change in serum triglycerides suggests that a reduction in triglycerides may in part explain the improvement in endothelial function [167]. However, the AIM-HIGH study which randomized 3196 patients with metabolic syndrome already on statin therapy to placebo or extended-release niacin (1.5–2 g/day) for a year showed that niacin did not reduce cardiovascular risk [168]. Furthermore, HPS-THRIVE study which randomized 25,673 high risk patients, including those with diabetes, to extended-release niacin (2 g/day) and laropiprant (40 mg/day) or matching placebo, showed that the extended-release niacin and laropiprant did not significantly reduce the risk of major vascular events, but did impair glycemic control and induced diabetes [169].

Statins and Antioxidants

In patients with ischemic cardiomyopathy (40% with diabetes), atorvastatin (10 mg/day) significantly improved post-ischemic forearm blood flow. However, the co-administration of vitamin E (400 IU/day) with atorvastatin blunted the effect of atorvastatin on post-ischemic forearm blood flow although the effect remained significant [170].

Given the potential for statins to inhibit the cellular synthesis of plasma CoQ_{10}, a by-product of isoprenoid metabolism, their full benefit on improving endothelial function may be blunted [171, 172]. In a randomized, double-blind, crossover study, CoQ_{10} supplementation significantly improved FMD in statin-treated T2DM patients with LDL-cholesterol <2.5 mmol/L and endothelial dysfunction [173].

CoQ_{10} supplementation has been shown to improve ecSOD levels and endothelial relaxation of the brachial artery in patients with CAD (20% with diabetes and 80% statin-treated) [174], indicating that the beneficial effects of CoQ_{10} on endothelial function are in part related to improvements in local vascular oxidative stress.

Statins and Antihypertensive Agents

Statins and antihypertensive agents such as ACE inhibitors, angiotensin II receptor antagonist, or calcium channel blockers have differing mechanisms of action on the arterial wall. Therefore it is conceivable that in combination they will have additive and synergistic effects on endothelial function [103, 175–177]. In hypercholesterolemic T2DM patients, ramipril combined with simvastatin significantly improved FMD and reduced malondialdehyde (MDA) and hs-CRP levels compared to ramipril or simvastatin alone [175]. Both ramipril alone and combination therapy improved adiponectin levels and insulin sensitivity, but there was no additive effect with combination therapy [175]. In T2DM, postprandial hyperglycemia and hypertriglyceridemia independently and cumulatively decreased FMD and increased biomarkers of inflammation. Short-term treatment (1-week) with atorvastatin and irbesartan, alone or in combination counterbalanced these detrimental effects, combination therapy being more effective than either monotherapy [103]. Longer-term studies utilizing combined statin and ARB therapy in T2DM are required. In patients with hypercholesterolemia and hypertension, evidence supports the anti-atherosclerotic effects of combined statin and calcium channel blocker therapy, particularly the combination of amlodipine and atorvastatin [176, 178–180].

Fibrates and Antioxidants

In dyslipidemic T2DM patients with endothelial dysfunction, combination of fenofibrate and CoQ_{10} significantly improved endothelium-dependent and -independent forearm blood flow response to intra-arterial vasodilator infusions [121]. Moreover, it has been demonstrated that fenofibrate and CoQ_{10} independently and interactively lowered 24-h ambulatory blood pressure [122], consistent with their beneficial effects on endothelial function in resistance arterioles. This synergistic effect of fenofibrate and CoQ_{10} in improving endothelial function may involve co-activation of PPAR-α in endothelial and smooth muscle cells, improving the production and action of NO and decreasing the synthesis of endothelin-1.

Other Combinations: Ezetimibe, Omega-3 Fatty Acids, CETP Inhibitors

In patients with T2DM, co-administration of ezetimibe on background statin therapy significantly lowered CRP to a greater extent than that of statin alone [181]. In the Stop Atherosclerosis in Native Diabetics Study (SANDS), aggressive

LDL-cholesterol lowering with statins alone or statins plus ezetimibe resulted in similar regression of CIMT in those patients who achieved equivalent LDL-cholesterol reductions [182], but the comparative therapeutic effects on endothelial function were not studied. Omega-3 fatty acid supplementation has been consistently shown to improve endothelial function in T2DM [136, 138, 140], but whether it enhances the effect of statins and other agents reviewed above remain to be demonstrated. The REDUCE-IT study supports the use of high-dose pure EPA in addition to statin therapy to reduce risk of ischemic events; however, direct in vivo effects on endothelial function remain to be demonstrated [141].

Dalcetrapib, a CETP inhibitor, was investigated in the dal-VESSEL study for its efficacy and safety on endothelial function, blood pressure, lipids, and clinical outcomes in patients with CHD or CHD risk equivalent and below average HDL-cholesterol; 45% of patients had T2DM. Patients were also treated with a statin and/or other cholesterol lowering agents to a LDL-cholesterol <2.6 mmol/L [183]. In this randomized, double-blinded, placebo-controlled study, 36-weeks of dalcetrapib reduced CETP activity by almost 50% and HDL-cholesterol by 30%, but brachial artery FMD, ambulatory blood pressure and biomarkers of inflammation, oxidative stress, and coagulation did not alter with either dalcetrapib or placebo [184].

Lipid-regulating therapies improve diabetic dyslipidemia; however, the various agents work via differing mechanisms, targeting to a greater or lesser degree the various aspects of the dyslipoproteinemia. These therapies, through both lipid-lowering effects and direct effects on the vasculature, may improve endothelial dysfunction.

Antiglycemic Agents and Insulin Sensitizers

Hyperglycemia contributes to endothelial dysfunction by multiple mechanisms, many of which result in increased oxidative stress [185, 186]. The effect of short-term blood glucose control on endothelial function was examined in poorly controlled T2DM subjects, who were randomized to improved glycemic control (multi-agent therapy, including insulin, to achieve and maintain glycemic targets) or usual treatment for 20-weeks: no difference in brachial artery FMD was found between the treatment groups [9]. Poor glycemic control has also been shown to mediate the association between endothelial dysfunction, as assessed by FMD, and incidence of coronary artery disease in patients with T2DM [187].

Insulin

Insulin treatment not only reduces glycemia, but may also directly increase endothelial NO production through 1-phosphotidylinositol 3-kinase signaling [188]. In an uncontrolled study in T2DM subjects on oral hypoglycemic therapy, switching to pre-meal insulin lispro at a dose to maintain equivalent glycemic control improved fasting and postprandial brachial artery FMD, an effect that was further augmented

by concomitant vitamin C therapy [93, 189]. In uncontrolled studies in T2DM patients treated with oral hypoglycemic therapy, the addition of insulin treatment improved glycemic control and brachial artery FMD [190] or forearm vascular reactivity [191, 192]. A randomized, controlled trial in T2DM subjects with ischemic heart disease showed that insulin therapy reduced HbA1c levels and improved insulin-stimulated, but not unstimulated, FABF response to ACh [193]. A small study of 18 T2DM patients and 27 matched controls investigated the effect of insulin therapy in addition to standard metformin treatment, on endothelial function. After 6-months treatment, the combination of insulin and metform resulted in an increased response to acetylcholine and sodium nitroprusside [191].

Sulfonylureas and Insulin Secretagogues

Sulfonylureas reduce glycemia by binding to specific (SUR1) receptors, resulting in closure of pancreatic beta-cell potassium-dependent ATP channels and stimulation of endogenous insulin secretion. However, controlled, crossover studies of glibenclamide therapy in T2DM subjects did not show any change in acetylcholine-stimulated forearm blood flow response compared with metformin or placebo [194, 195], and treatment with either glibenclamide or glimepiride did not alter brachial artery FMD compared with diet treatment alone [196]. One double-blind, randomized, crossover trial in T2DM subjects suggested that gliclazide reduced forearm blood flow responses to hyperemia compared with glibenclamide, possibly due to differential binding of these agents to sulfonylurea receptors [197]. However, another study did not show any difference between these two agents on ACh-stimulated forearm blood flow [198]. In a randomized crossover study, treatment with repaglinide (a short-acting insulin secretagogue), but not glibenclamide, increased brachial artery FMD in diet-treated T2DM subjects; improvement in endothelial function was correlated with changes in postprandial glycemia [199]. In subjects with impaired glucose tolerance, endothelial dysfunction following a glucose challenge was related to the level of hyperglycemia. Reduction in the glycemic response following a single dose of repaglinide ameliorated endothelial dysfunction in a glucose-dependent manner [200].

Metformin

Although its main antihyperglycemic action is to suppress hepatic gluconeogenesis, possibly by stimulation of AMP-activated kinase pathways, metformin may also increase insulin sensitivity in peripheral tissues. In a placebo-controlled trial, metformin treatment increased ACh-stimulated forearm blood flow and insulin sensitivity in diet-treated T2DM patients [201]. However, another randomized, double-blind, placebo-controlled trial in T2DM patients failed to show improvement in insulin sensitivity or ACh-stimulated forearm blood flow with metformin therapy, despite improved glycemic control [202]. Despite this, a recent review has suggested that

metformin may have protective effects on coronary arteries above its hypoglycemic effects, with several pre-clinical and clinical trials demonstrating reductions in cardiovascular events in T2DM patients treated with metformin [203].

Thiazolidinediones

Thiazolidinediones improve insulin sensitivity and reduce glycemia via PPAR-gamma receptor-mediated effects on adipocytes resulting in decreased hepatic glucose output and increased peripheral glucose uptake by skeletal muscle [204]. In addition, as PPAR-gamma receptors are also present in the endothelium, vascular smooth muscle cells, and macrophages, these agents may also have direct anti-inflammatory and anti-atherogenic effects on the vasculature.

In a randomized, double-blind, placebo-controlled trial, troglitazone increased brachial artery FMD in recently diagnosed T2DM patients without macrovascular disease, but not in subjects with more long-standing disease or macrovascular complications [205]. In a small uncontrolled trial, pioglitazone-treated T2DM participants showed improvement in brachial artery FMD, with a significant association between changes in FMD and insulin sensitivity [206]. In a randomized, double-blind, placebo-controlled, crossover study in T2DM patients, pioglitazone was also shown to increase brachial artery FMD, but improvement in endothelial function was not correlated with favorable changes in plasma insulin, free fatty acids, adiponectin, or C-reactive protein (CRP) [207]. In double-blind, crossover trials, rosiglitazone was shown to increase ACh-stimulated forearm blood flow in T2DM patients [202, 208].

Alpha-Glucosidase Inhibitors

Administration of a single dose of acarbose, an alpha-glucosidase inhibitor that targets postprandial hyperglycemia, has been shown to attenuate postprandial impairment of hyperemic forearm blood flow response in diet-treated T2DM patients [209].

Incretins

Glucagon-like peptide (GLP)-1 is an incretin that reduces glycemia by stimulating insulin secretion, suppressing glucagon secretion, and slowing gastrointestinal motility. Gliptins inhibit dipeptidyl peptidase-4, thereby increasing incretin levels which in turn increases insulin secretion and decreases glycemia, predominantly through postprandial mechanisms [210]. In a randomized crossover study, infusion of recombinant GLP-1 was shown to increase brachial artery FMD in T2DM patients, without any change in insulin resistance [211]. In a randomized crossover

study, improved postprandial endothelial function following a single subcutaneous injection of exenatide (a DPP4 inhibitor) in T2DM patients was associated with decreased triglyceride but not glucose concentrations [210]. A recent meta-analysis of seven trials with 56,004 patients found that treatments with GLP-1 receptor agonists had beneficial effects on cardiovascular events and mortality, as well as renal outcomes in patients with T2DM [212]. A double-blind trial of 163 participants with T2DM, however, found that weekly exenatide for 18-months improved fasting and postprandial glycemic control but had no effect on carotid plaque volume or composition [213]. In a small study of 31 patients with T2DM on metformin and/or sulfonylurea treatment, the addition of either liraglutide or glargine therapy did not improve FMD [214]. Post-hoc analysis of a phase 2 trial of weekly tirzepatide, dulaglutide, or placebo found that tirzepatide (a dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist, or "twincretin") decreased ICAM-1, hs-CRP in addition to beneficial effects on HbA1c and body weight [215]. Although these latter studies did not use direct measures of endothelial function, the beneficial effect on surrogate markers suggests improvements and warrants further investigation.

Amylin Agonists

Pramlintide, a synthetic amylin agonist, is associated with modest improvements in HbA1c levels and weight loss in insulin requiring T2DM patients [216, 217]. Pramlintide has also been shown to improve cardiovascular risk factors in T2DM patients: modest reductions in triglyceride levels [218] and improvement in markers of inflammation and oxidation have been reported [217, 218].

Sodium Glucose Co-transporter (SGLT2) Inhibitors

SGLT2 inhibitors are a relatively new class of anti-diabetic drug that work by inhibiting glucose reabsorption in the kidney and have also been shown to have cardioprotective effects [219]. In an uncontrolled study, 50 T2DM patients with established CAD were treated with 10 mg/day of empagliflozin for 6-months. At the end of treatment, body weight and body fat percentage had significantly decreased, along with HbA1c, postprandial glucose levels, insulin secretion levels, CRP levels, and fasting and postprandial triglycerides. Accompanying these changes was increased in plasma concentrations of ketones and a significant improvement in FMD. This improvement in FMD was most strongly predicted by the reduction in triglycerides [220]. In contrast, a multi-center, randomized, double-blind placebo-controlled study investigated the effect of empagliflozin on endothelial function in T2DM patients at high risk of cardiovascular events. Treatment for 24-weeks with 10 mg/day empagliflozin improved glycemic control and resulted in significant reductions in BMI. Despite these improvements, there were only borderline differences in BP

between treatment and placebo groups and no significant difference in reactive hyperemia peripheral tonometry index [221]. Secondary analysis of this trial revealed that this was not related to changes in clinical variables, including glycemic parameters [222].

In another uncontrolled study of T2DM patients with chronic heart failure who were treated with 100 mg a daily of canagliflozin, there were significant improvements in FMD, arterial stiffness as measured by carotid-femoral pulse wave velocity, and blood pressure, with FMD changes seen immediately and at 1-, 3-, 6-, and 12-months follow-up. These changes were accompanied by significant reductions in subcutaneous, visceral, and total fat area [223].

In contrast, the EDIFIED trial, which employed dapagliflozin (10 mg/day), did not find any significant changes in FMD after 12-weeks of therapy, compared with placebo. This was despite a reduction in ICAM-1 expression [224]. While an acute study of dapagliflozin has shown improvements in FMD and renal artery vasodilation [225], these effects were not sustained over a longer period in a follow-up study in T2DM patients with hypertension [226].. Interestingly, the DEFENCE trial reported an improvement in FMD with 5 mg/day of dapagliflozin over 16-weeks in early uncontrolled T2DM patients on metformin [227]. Dapagliflozin also improved reactive hyperemia peripheral arterial tonometry in T2DM, which was also accompanied by reductions in BP and abdominal fat mass [228]. Twelve weeks of 10 mg/day dapagliflozin also improved pulse wave velocity, augmentation index, and central and brachial BP in T2DM patients [229]. In an open-label randomized parallel study, 63 patients with T2DM received metformin and insulin glargine for 12-weeks, followed by additional treatment of empagliflozin or glimepiride for 12-weeks. The empagliflozin and glimepiride groups showed no differences in FMD response of HbA1c at 24-weeks. However, there were significant improvements in body weight with glimepiride and body fluid volume with empagliflozin [230]. A recent review of nine randomized controlled trials and two cohort studies involving 868 patients found that SGLT inhibitors could significantly improve FMD in T2DM patients compared with the control group, with no significant effect on pulse wave velocity [231].

Finally, a study of GLP-1, SGLT2, and insulin on vascular and cardiac function in patients with T2DM treated with metformin found that treatment with the GLP-1 agonist, SGLT2 inhibitor and their combination resulted in improved vascular makers and cardiac work compared to treatment with insulin. Combined treatment was superior to the separate treatments [232]. While SGLT-2 inhibitors show clear benefits on weight reduction and glycemic control in patients with T2DM, their effect on endothelial function appears to be dependent on the inhibitor used and background medication. Empagliflozin and canagliflozin appear to improve endothelial function, but the findings with dapagliflozin are inconsistent. While the mechanisms remain unclear, improvement in endothelial function has been postulated to be due in part to their differing specificity toward SGLT-2 and that their beneficial effects in larger cardiovascular outcomes trials are only modestly mediated by direct changes in endothelial function [219].

Antihypertensive Agents

In hypertension, increased oxidative stress and release of endothelial-derived constricting factors result in ED [8]. The coexistence of diabetes and hypertension has been shown to have an additive deleterious effect on endothelial function in the forearm resistance arteries [233]. Hyperglycemia increases the production of angiotensin II (Ang II) in the vessel wall [234]. Ang II stimulates vascular NAPH oxidase, increasing oxidative stress [235], and NF-kappaB activity, thereby activating inflammatory cytokines and vascular expression of cell adhesion molecules [9]. Hence, RAS inhibition may improve endothelial function by reducing vascular oxidative stress and inflammation. Ang II may also promote release and production of vasoconstrictors such as endothelin-1 and prostaglandin-H2, which contribute to endothelial dysfunction and hypertension.

Angiotensin-Converting Enzyme (ACE) Inhibitors

In a small uncontrolled study in hypertensive T2DM subjects, treatment with perindopril reduced blood pressure but did not improve methacholine-stimulated FABF [236]. However, a randomized, open parallel group study showed that quinapril treatment increased serotonin-stimulated FABF in T2DM subjects, perhaps by increasing vascular adiponectin expression [237]. In T2DM patients with proteinuria, improvement in brachial artery FMD following short-term ramipril treatment was associated with a reduction in serum hs-CRP and plasma long pentraxin 3 (PTX3) [238]. Furthermore, a double-blind, placebo-controlled crossover study showed that enalapril lowered blood pressure and improved ACh-stimulated FABF in T2DM subjects without vascular disease [239].

Angiotensin Receptor Antagonists

In randomized, controlled crossover trials, angiotensin type 1 receptor antagonists were shown to improve both FABF response to ACh and brachial artery FMD in subjects with T2DM [103, 240–242]. Improvement in endothelial function occurred despite no significant change in blood pressure and may relate to other treatment effects on oxidative stress, inflammation, and endothelial cell activation.

Aldosterone Antagonists

On a cautious note, a randomized, double-blind, placebo-controlled trial showed that treatment with spironolactone worsened ACh-stimulated FABF in T2DM subjects, possibly due to worsening of glycemic control and increase in plasma Ang II [243].

Calcium Channel Blockers

Evidence for the effects of calcium channel blockade (CCB) on endothelial dysfunction is inconsistent. In a comparative study of antihypertensive agents, CCB (amlodipine) did not improve brachial artery FMD in patients with CAD [244]. In contrast, amlodipine improved endothelial function in hypertensive patients [245]. A study examining the effects CCB on endothelial function in hypertensive patients suggests a divergent effect for different types of these agents: efonidipine, a T- and L-type CCB, but not nifedipine, an L-type CCB, improved endothelial function and markers of oxidative stress [246]. Further, in patients with stable angina pectoris, combination of CCB and ACE inhibition improved endothelial function, arterial stiffness, and urinary albumin excretion more effectively than CCB alone [247]. However, in hypertensive T2DM patients with proteinuria, treatment with amlodipine, valsartan (an angiotensin II receptor blocker), or a combination of both improved brachial artery FMD and proteinuria. Improvement in endothelial function was associated with reductions in PTX3 and soluble TNF-like weak inducer of apoptosis (sTWEAK) [248].

Antioxidants and Nutritional Supplements

Supplementation with antioxidants and/or factors essential to NO production may potentially improve endothelial dysfunction in T2DM by recoupling eNOS and mitochondrial function, as well as decreasing vascular NAD(P)H oxidase activity.

Increased oxidative stress in T2DM may disrupt coenzyme Q_{10} (CoQ_{10}) composition and levels, resulting in defective antioxidant defences and further exacerbating oxidative stress and increasing membrane fluidity [37, 249, 250]. In endothelial cells, this may lead to uncoupling of eNOS and a reduction in the release and subsequent activity of NO. CoQ_{10} as a potent antioxidant may decrease oxidative stress by not only quenching reactive oxidant species, but also by "recoupling" mitochondrial oxidative phosphorylation, thereby reducing superoxide production [37]. CoQ_{10} supplementation improved brachial artery FMD in treatment naive diabetic patients with dyslipidemia, but there was no change in glyceryl-trinitrate mediated endothelial-independent response, forearm vascular reactivity, or plasma F_2-isoprostanes [121, 251]. However, CoQ_{10} supplementation did not improve microcirculatory endothelial function in type 2 diabetic patients, despite repleted plasma CoQ_{10} concentrations [252].

Vascular responses to several other antioxidants and nutritional supplements have been examined, with inconsistent results being reported. Vitamin C (ascorbic acid) and vitamin E (tocopherol) have well-described antioxidant properties. However, studies examining their effect on endothelial dysfunction in T2DM patients have yielded mixed results, some demonstrating benefit [253–259], while others have failed to show an effect [116, 260–263]. Alpha-lipoic acid, another compound with free radical-scavenging activity, was shown to improve ACh-stimulated FABF [254]. Despite the potential for vascular benefit with the

polyphenolic antioxidants present in red wine [264, 265], benefit has not been demonstrated in T2DM patients [266]. Supplementation with L-arginine, a principal substrate for eNOS, improved both brachial artery FMD and post-ischemic forearm hyperemia in T2DM women [259]. Oxidation of tetrahydrobiopterin (BH_4) may lead to uncoupling of eNOS, reducing NO production and further generating oxidant species. Intra-arterial BH_4 infusion was shown to improve FABF response to ACh in T2DM subjects [267]. Folic acid, a strong peroxynitrite scavenger, may also protect BH_4 from oxidation, reversing eNOS uncoupling [268]. Folic acid has been shown to improve FABF and brachial artery FMD in T2DM patients [269–271].

Miscellaneous Therapies

Phosphodiesterase Inhibitors

The vasorelaxation effect of NO on vascular smooth muscle is mediated by cyclic GMP (cGMP), which is catabolized by phosphodiesterase (PDE). PDE inhibitors, which are used to treat erectile dysfunction, increase the bioavailability of cGMP, which activates protein kinase G, thereby promoting vasodilatation and a penile erection. Sildenafil, a selective PDE-5 inhibitor, has been shown to increase brachial artery FMD in a double-blind, placebo-controlled, crossover study in T2DM men with erectile dysfunction [272].

Estrogens

Epidemiological studies have suggested a protective effect of estrogen on cardiovascular risk, but intervention trials of sex hormone replacement in post-menopausal women have reported no benefit, and even a possible initial adverse effect, on cardiovascular outcomes [273, 274]. Although estrogen therapy may protect endothelial function by up-regulating endothelial NO production, reducing the formation of COX-derived endothelium-derived contracting factors and have favorable effects on lipids and blood pressure, it may also have adverse effects in increasing vascular inflammation and cell adhesion [8, 275]. The effect of hormone replacement therapy on endothelial function in post-menopausal T2DM women has been inconsistent [276–279].

Testosterone

In men with testosterone deficiency brachial artery, FMD has been reported to be both increased [280, 281] and impaired [282–284]. Testosterone deficiency is associated with elevated triglyceride and low HDL-cholesterol concentrations [285], and this could explain its association with impaired endothelial function. Evidence for the effect of testosterone replacement on endothelial function is inconsistent,

however. Testosterone replacement for 12-weeks reduced FMD in hypogonadal men [281]. In a small study, hypogonadal men were found to develop impaired FMD 4-weeks following testosterone pellet implantation [286]. In contrast, in a randomized placebo-controlled study, 12-weeks of testosterone replacement improved brachial artery reactivity in men with CAD [287]. A 1-year double-blind, randomized placebo-controlled study in obese hypogonadal men with T2DM found reductions in HOMA-IR and HbA1c and an increase in FMD with testosterone treatment compared to placebo [288]. Studies of the effect of testosterone replacement therapy on endothelial function in men with diabetes are warranted. The cardiovascular benefits of testosterone replacement remain contentious [289].

Anti-cytokine Agents

Disruption of the balance between pro-and anti-inflammatory cytokine signaling pathways in atherosclerosis promotes plaque ruptures [290, 291]. Pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, IL-18, and TNF-α, are pro-atherogenic [290, 291], and accordingly have been associated with increased risk of coronary disease in a meta-analysis of prospective cohort studies [292]. Thus, the central NOD-like receptor protein 3 (NLRP3) inflammasome to IL-1 and IL-6 pathway is currently being investigated as potential therapeutic target of atherosclerosis [293]. Although hs-CRP, IL-1, and IL-6 are all useful inflammatory markers of endothelial dysfunction, hs-CRP is a downstream biomarker of the inflammatory cascade and unlikely to be an active target, whereas inhibition of IL-1 and IL-6 shows promising results as anti-atherosclerotic strategy in high-risk populations [293]. The CANTOS study showed that inhibition of IL-1β using canakinumab (monoclonal antibody IL-1β inhibitor), in patients with a history of myocardial infarction and raised hs-CRP ≥2 mg/dL significantly reduced major adverse cardiovascular events [294]. Through inhibition of IL-1β, there was a consequent reduced levels of downstream IL-6 and CRP [294]. In the CANTOS trial, greater cardiovascular event benefit was seen in those with greater reductions in both IL-6 or hs-CRP [293, 295, 296]. In a study of 556 patients with well-controlled diabetes and high cardiovascular risk, canakinumab also markedly reduced plasma IL-6 and hs-CRP levels [297].

Among the possible anti-cytokine agents, agents targeting IL-6 inhibition are of particular interest because Mendelian randomization studies show that genetic variants in IL-6 receptor signaling are associated with coronary artery disease risk, suggesting the causal importance of this pathway [298, 299]. In a phase 2 randomized, placebo-controlled trial (RESCUE) of a monoclonal antibody against IL-6 ligand (ziltivekimab) in patients with moderate-to-severe chronic kidney disease and elevated hs-CRP of ≥2 mg/dL, ziltivekimab reduced hs-CRP and other inflammatory markers (fibrinogen, serum amyloid A, haptoglobin, secretory phospholipase and Lp(a)) [300]. Approximately 70% of participants of this study had diabetes at baseline [300]. Tocilizumab, a IL-6 receptor antagonist, reduced troponin levels and hs-CRP in a small randomized study of patients with acute non-ST-elevation

myocardial infarction (NSTEMI) [301]. However, in a small study of 42 patients with NSTEMI, the group that received a single dose of tocilizumab had higher VCAM-1 levels and there was no significant difference in coronary flow reserve compared with placebo [302]. The reason for raised VCAM-1 levels with tocilizumab remains unclear and requires further study. A selective inhibitor of NLRP3 inflammasomes (upstream of IL-1 signaling pathway) was shown to reduce the ICAM and VCAM mRNA expression in apolipoprotein E-knockout mice [303], and reduced markers of oxidative stress and inflammatory genes expression in streptozotocin-induced diabetic apolipoprotein E-knockout mice [304], colchicine is a microtubule inhibitor and an indirect inhibitor of NLRP3. The colchicine cardiovascular outcome trial (COLCOT) showed that colchicine (0.5 mg/day) compared with placebo, reduced cardiovascular events in patients with recent myocardial infarction (primary composite endpoint hazard ratio 0.77) [305]. A relative risk reduction of cardiovascular events of 31% with colchicine (0.5 mg/day) when compared to placebo was also observed in patients with chronic coronary disease (LoDoCo2 randomized-controlled trial) [306]. In the subgroup analysis of patients with diabetes, the benefits of colchicine were observed in the COLCOT study [305] but not the LoDoCo2 study [306]. However, interpretation of this contrasting finding needs to be interpreted with caution owing to the small number of patients with diabetes in both studies (20% and 18%, respectively).

The effect of the aforementioned anti-cytokine agents on endothelial dysfunction specifically in diabetes is less clear owing to a lack of studies. In a randomized crossover study, pentoxifylline, an inhibitor of TNF-α production, did not alter ACh-stimulated FABF in T2DM subjects, despite reduction in serum TNF-α levels [307]. Further studies on anti-cytokine agents on endothelial function in diabetes are required.

Xanthine Oxidase Inhibitors

Xanthine oxidase is an enzyme present in endothelial cells that when activated increases oxidative stress. In a small randomized placebo-controlled trial allopurinol, an inhibitor of xanthine oxidase, was shown to improve ACh-stimulated FABF, and hence resistance artery function in mildly hypertensive T2DM subjects. There was no reduction in blood pressure, however [308].

Conclusion

T2DM patients are at markedly increased risk of CVD events. Endothelial dysfunction is the earliest manifestation of vascular involvement in diabetes and heralds an increased risk of CVD. Endothelial dysfunction can be examined indirectly in the peripheral circulation by several non-invasive methods. Studies of endothelial dysfunction serve two useful purposes in cardiovascular research. First, they can help

identify agents that could be tested as monotherapy or combination therapy in clinical endpoint trials. Second, they can provide mechanisms for the cardiovascular benefits of these treatments. Clinical trials of interventions on endothelial dysfunction may be hampered by subject selection bias, statistical underpowering, and technical imprecision in measurements. These factors may account for variation in findings among some of the studies reviewed. As methodologies are refined, measurement of endothelial function could in time provide a practical clinical tool for risk stratifying patients and guiding the intensity of treatments to reverse or prevent progression of cardiovascular disease in diabetes.

Therapeutic interventions, including lifestyle changes and lipid-regulating agents, correct diabetic dyslipidemia via several mechanisms. They have also been shown to improve endothelial dysfunction, but not all studies demonstrate a consistent benefit. Together with dyslipoproteinemia, increased oxidative stress is a major factor involved in the pathogenesis of endothelial dysfunction in T2DM. Supplementation with antioxidants may also potentially improve endothelial dysfunction in T2DM, but the reported effects on endothelial function have again not always been consistent. Other interventions including SGLT-2 inhibitors appear to have beneficial albeit inconsistent effects on endothelial function that appear to be dependent on the SGLT-2 inhibitor used and the background therapy. Other therapies including high-dose EPA, PCSK9 inhibitors, and GLP-1 agonists have shown beneficial effects on cardiovascular events and outcomes in patients with T2DM, although their direct effects on endothelial function have not been investigated. Therapeutic regulation of inflammatory pathways, with IL-6 inhibitors or colchicine, testify to its anti-atherogenic value, but currently there are paucity of studies on the effects on endothelial dysfunction in patients with diabetes. Future studies using the newly developed RNA therapeutics that target apoC-III, ANGPTL3, and apo(a), and lower triglyceride-rich lipoproteins, LDL-cholesterol, and Lp(a) levels may also provide additional benefit in this patient population and their effect on endothelial function merit further research.

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Chapter 15
Lipoproteins and Diabetic Kidney Disease

Fanny Jansson Sigfrids, Nina Elonen, and Per-Henrik Groop

Introduction

Diabetic kidney disease is a leading cause of kidney failure worldwide and has a significant impact on the quality of life and longevity. It affects up to a third of all individuals with type 1 diabetes [1–3], and approximately half of people with type 2 diabetes have signs of chronic kidney disease [4]. The clinical course varies substantially, but in a typical case, the first indications of kidney injury appear within two decades of diabetes: urinary albumin excretion increases, blood pressure rises, and gradually the glomerular filtration rate (GFR) begins to decline with a concurrent rise in mortality [5–8]. Glycemic control is the critical modifiable factor to delay and prevent diabetic kidney disease and other co-morbidities [9]. The

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detrimental vascular effects of impaired glycemic control could be mediated by lipoproteins, and therefore, the connection between serum lipoprotein lipids and diabetic kidney disease is clinically significant.

Individuals with chronic kidney disease carry a greater risk of atherosclerosis and adverse vascular events, and diabetes adds to this risk even further [10]. As kidney function declines, secondary metabolic effects and disadvantageous changes in lipoprotein metabolism follow. For instance, increased triglycerides and decreased HDL cholesterol concentrations, as well as impaired clearance of VLDL particles, are commonly seen [11]. Cardiovascular disease is the most common cause of death in individuals with kidney failure, both in those with and without diabetes. But in diabetes, the crucial changes seem to occur earlier, and individuals with type 1 diabetes and albuminuria have a dramatically increased risk of premature death even before their glomerular reserve is lost to the point of kidney failure (Fig. 15.1). In particular, the lipoprotein lipid profile is correlated with albuminuria and predicts adverse outcomes [12, 13]. However, the good news is that individuals with type 1 diabetes without signs of kidney disease show no excess mortality beyond that of the general population [7, 14].

The triad of poor glycemic control, obesity, and albuminuria indicates a high-risk vascular phenotype [15, 16]. All three risk factors overlap and are concurrently associated with dyslipidemia, particularly in the form of increased triglycerides and decreased HDL-cholesterol. This also means that it is challenging to ascertain causal relationships between serum lipoprotein lipids and diabetic kidney injury since both compartments may be parts of a larger complex of systemic atherogenic

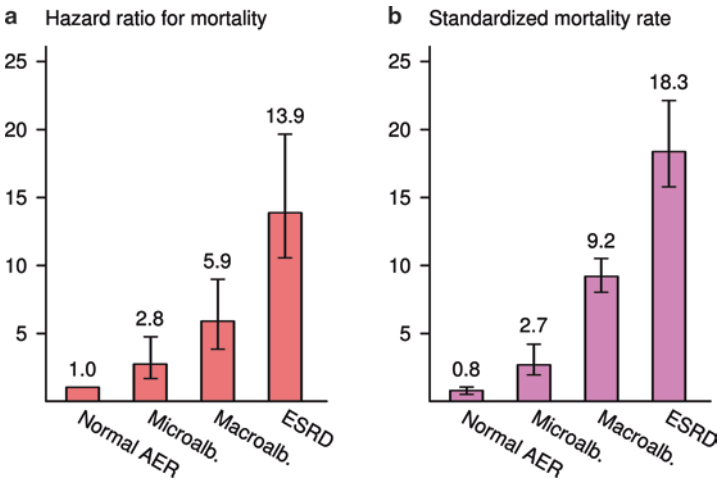

Fig. 15.1 Prospective analysis of all-cause mortality in the FinnDiane cohort of type 1 diabetes after an average of 7 years of follow-up. At baseline, 2296 individuals had normal AER, 504 had microalbuminuria, 579 had macroalbuminuria, and 293 had kidney failure. Plot **a** depicts the adjusted hazard ratios with respect to normal AER. Plot **b** depicts the standardized mortality rate with respect to the age and sex groups in the Finnish background population (reference value was set to 1.0). (The figure was adapted from [7])

perturbations. Particularly in type 2 diabetes, lipid abnormalities such as high triglycerides, excessive postprandial lipidemia, small dense LDL, and low concentrations of HDL cholesterol are frequently seen [17]. Hence, similar lipid abnormalities as in individuals with kidney failure are often observed in people with type 2 diabetes even prior to the diagnosis of diabetes. The evidence regarding the kidney pathology is also unclear: it may be confounded by age-related phenomena and seems to be less related to urinary albumin excretion or GFR than in type 1 diabetes [18]. In this section, we primarily focus on the combined diagnostic and prognostic significance of kidney disease and lipoprotein lipids in (type 1) diabetes, and briefly discuss the biological implications to the lipoprotein composition and functionality.

Conventional Lipoprotein Lipids, Albuminuria, and Kidney Function

Individuals with type 1 diabetes but without complications show no detrimental changes in their clinical lipid profile (total triglycerides, cholesterol, and HDL cholesterol), and individuals with good glycemic control often have more favorable lipids than the background population [19–21]. On the other hand, plasma lipid abnormalities have been reported in individuals with kidney disease in a number of early studies [22–24], and the association between dyslipidemia and diabetic kidney disease has since been confirmed in several larger studies (Table 15.1).

DCCT/EDIC

The Diabetes Control and Complications Trial (DCCT) was a multi-center clinical trial that compared intensive insulin therapy with the current conventional treatment (between 1983 and 1993) in a cohort of 1441 individuals with type 1 diabetes. During the trial, the intensively treated group had lower total triglycerides, total cholesterol, and calculated LDL cholesterol, but HDL cholesterol was unaffected [25]. At the same time, a significant reduction in the incidence of albuminuria was observed [26]. Specific analyses of urinary albumin excretion rate (AER) and serum lipoprotein lipids were made for the combined trial and follow-up period in the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort of 968 individuals [12]. Triglycerides, cholesterol, and calculated LDL cholesterol were increased in individuals with microalbuminuria ($40 \leq$ AER <300 mg/24 h) and macroalbuminuria (AER ≥ 300 mg/24 h) when tested for the overall trend and adjusted for age, diabetes duration, hypertension, hemoglobin A_{1c}, body-mass index, waist-to-hip ratio, and DCCT randomization group. A decreasing trend was observed for HDL cholesterol in women and in the whole dataset, but these associations could be fully explained by the aforementioned risk factors and confounders.

Table 15.1 Conventional lipid profile in individuals with type 1 diabetes and diabetic kidney disease

Study	Design	Albuminuria	Kidney dysfunction	Additional details
DCCT/EDIC [12]	Cross-sectional, $n = 968$	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	NA	
DCCT/EDIC [27]	Progression from incident micro- to macroalbuminuria, $n = 325$, 13-year follow-up	↑TG, ↑TotC, ↑LDL-C	No findings	Lower baseline TG, TotC, and LDL-C associated with regression to normal AER
DCCT/EDIC [28]	Progression from micro- or macroalbuminuria, $n = 1441$, 27-year follow-up	↑TG, ↓HDL-C	↑TG, ↑TotC, ↑LDL-C	
Estudio Diamante [120]	Cross-sectional, $n = 1822$	↑TG, ↑TotC	↑TG, ↑TotC	LDL-C not reported
EURODIAB [29]	Cross-sectional, $n = 2205$	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	NA	Sex-dependent findings on HDL-C
EURODIAB [31]	Progression from normal AER, $n = 1134$, 7.3-year follow-up	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	NA	
EURODIAB [32]	Progression from microalbuminuria, $n = 352$, 7.3-year follow-up	↑TG	NA	Lower baseline TG associated with regression to normal AER
FinnDiane [21]	Cross-sectional, $n = 2927$	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	LDL-C and HDL-C significant in macroalbuminuria and kidney failure
FinnDiane [13]	Progression to micro-, macroalbuminuria or kidney failure, $n = 2304$, 5.4-year follow-up	↑TG, ↑TotC	↑TG, ↓HDL-C, ↑TotC, ↑LDL-C	
Pittsburgh [36]	Progression from normal AER, $n = 256$, 2-year follow-up	↑TG, ↑LDL-C	NA	TotC not reported
Pittsburgh [37]	Progression to macroalbuminuria or kidney failure, $n = 485$, 10-year follow-up	↑TG, ↑TotC, ↑LDL-C	↑TG, ↑TotC, ↑LDL-C	Lipids significant only if progression within the first 5-year period

(continued)

Table 15.1 (continued)

Study	Design	Albuminuria	Kidney dysfunction	Additional details
Pittsburgh [38]	Predictors for early kidney function decline (≥3 mL/min per 1.73 m^2), $n = 98$, follow-up up to 8 years	NA	↑TG, ↑TotC	Results could not be replicated in the CACTI cohort ($n = 210$)
Swedish National Diabetes Register [39]	Cross-sectional, $n = 4795$	↑TG, ↑TotC	NA	Factors associated with microalbuminuria assessed LDL-C not reported
Swedish National Diabetes Register [40]	Progression to micro- or macroalbuminuria, $n = 12{,}350$, 8.5-year follow-up	↑LDL-C	NA	Only LDL-C reported
Nephropathy Family Study [121]	Progression from normal AER, $n = 895$, 2.3-year follow-up	↑TG, ↑TotC	NA	Higher non-HDL cholesterol associated with progression
German Diabetes Documentation System [122]	Progression to micro-, macroalbuminuria or kidney failure, $n = 27{,}805$, 2.5-year follow-up	↑TG, ↑TotC, ↑LDL-C	↑TG, ↑TotC, ↑LDL-C	Dyslipidemia (TotC >200 mg/dL, LDL-C > 160 mg/dL, or TG >150 mg/dL) associated with progression
Angers cohort [123]	Progression to micro-, macroalbuminuria or kidney failure, $n = 297$, 7-year follow-up	↑TG, ↓HDL-C	↑TG, ↓HDL-C	Elevated plasma creatinine was used as an additional diagnostic category
Steno Diabetes Center [124]	Cross-sectional, $n = 669$	↑TG, ↓HDL-C	NA	
Steno Diabetes Center [125]	Rate of GFR decline, $n = 301$, 6.7-year follow-up	NA	↑TotC	Only TotC reported
Steno Diabetes Center [3]	Progression to micro- or macroalbuminuria, $n = 277$, 18-year follow-up	See details	NA	Lower baseline TotC associated with regression to normal AER Only TotC reported
Joslin Study [126]	Regression from microalbuminuria, $n = 386$, 6-year follow-up	See details	NA	Lower baseline TG and TotC associated with AER reduction

Associations from univariate analyses are reported in the table
Abbreviations: *AER* albumin excretion rate, *TG* triglycerides, *TotC* total cholesterol, *HDL-C* HDL cholesterol, *LDL-C* estimated LDL cholesterol, *NA* not available

Risk factors for long-term kidney outcomes after the onset of persistent microalbuminuria (30< AER <300 mg/24 h) were examined among 325 DCCT/EDIC study participants [27]. The median follow-up time after a diagnosis of microalbuminuria was 13 years, and the examined endpoints comprised regression to normal AER (10-year cumulative incidence 40%), progression to macroalbuminuria (10-year cumulative incidence 28%), and/or impaired kidney function defined as estimated GFR (eGFR) <60 mL/min per 1.73 m^2 (10-year cumulative incidence 15%). Total triglycerides, cholesterol, and calculated LDL cholesterol were associated with progression to macroalbuminuria (increased concentrations) and regression to normal AER (decreased concentrations). No associations were detected for incident impaired eGFR, and HDL cholesterol failed to predict the renal outcomes altogether.

Another recent, prospective, comprehensive analysis of the DCCT/EDIC cohort explored recognized and putative risk factors for advanced kidney outcomes after a mean of 27 follow-up years. A higher mean triglyceride concentration was the second most significant risk factor for incident impaired kidney function (eGFR <60 mL/min per 1.73 m^2), surpassed only by poorer glycemic control (as hemoglobin A_{1c}). Glycemic control, male sex, and mean triglycerides were the three most powerful predictors of incident macroalbuminuria. The other lipid components (total cholesterol, HDL cholesterol, and LDL cholesterol) showed no significant associations in multivariable analyses [28].

EURODIAB

Cross-sectional associations between conventional lipoprotein measures and albuminuria were also seen in the EURODIAB IDDM Complications Study [29]. The set of 3250 individuals with type 1 diabetes were recruited from 16 European countries and represent age groups from 15 to 60 years. Increased concentrations of triglycerides, total cholesterol, and calculated LDL cholesterol were observed for individuals with macroalbuminuria (AER >200 μg/min) in comparison to those with normal AER (<20 μg/min) for both sexes. HDL cholesterol was decreased only in women with macroalbuminuria. In men and women with microalbuminuria ($20 \leq$ AER ≤ 200 μg/min), the only significant abnormal lipid variable was increased triglyceride concentrations.

When the eGFR level was considered in addition to albuminuria for the stratification of EURODIAB Prospective Complication Study participants ($n = 774$) into four chronic kidney disease phenotypes (no chronic kidney disease, albuminuria alone, reduced eGFR alone, both albuminuria and reduced eGFR), total cholesterol and LDL cholesterol in both sexes combined and triglycerides in women displayed between-group differences in cross-sectional analyses. No between-group difference was seen for HDL cholesterol [30].

A set of 1134 individuals with normal baseline AER were followed for a mean of 7.3 years in the EURODIAB Prospective Complication Study. The incidence of microalbuminuria was 12.6%, which corresponds to 18 new cases per 1000

person-years. In a multivariable model, baseline hemoglobin A_{1c}, AER, triglycerides, and waist-to-hip ratio predicted the progression to microalbuminuria [31]. A sub-study of 352 individuals with baseline microalbuminuria identified increased AER, sub-optimal metabolic control, excess body fat, and peripheral neuropathy as significant risk factors for the progression to macroalbuminuria [32]. During an average of 7.3 years, 51% regressed to normal AER, 36% remained microalbuminuric, and 14% progressed to macroalbuminuria. Overall, the lipoprotein lipids were not associated with the progressive kidney phenotype. However, fasting triglyceride concentration at baseline was a weak predictor of progression, and the lowest concentration was seen in the group that regressed.

Furthermore, based on the comprehensive phenotypic data in the EURODIAB Prospective Complication Study, a prognostic model for major vascular endpoints in type 1 diabetes, encompassing kidney failure (dialysis or kidney transplantation), was developed [33]. Several modifiable and non-modifiable characteristics were considered to be potential prognostic factors for the endpoints, among them triglycerides, HDL cholesterol, LDL cholesterol, and non-HDL cholesterol to represent the lipoprotein lipids. The best-performing prognostic model included HDL cholesterol—but no other lipid component—along with age, hemoglobin A_{1c}, waist-to-hip ratio, and albumin-creatinine ratio (ACR).

FinnDiane

The Finnish Diabetic Nephropathy Study (FinnDiane) represents a population-based sample of long-standing type 1 diabetes in Finland. In cross-sectional analyses of 2927 individuals, those with normal AER (<30 mg/24 h) had the lowest, and those with macroalbuminuria (AER >300 mg/24 h) had the highest triglyceride concentrations [21]. eGFR was also associated with lipid abnormalities: individuals with impaired kidney function (eGFR <60 mL/min per 1.73 m^2) had higher triglycerides, total cholesterol, and lower HDL cholesterol than those with normal kidney function (eGFR >90 mL/min per 1.73 m^2) or mildly impaired kidney function ($60 \leq$ eGFR ≤ 90 mL/min per 1.73 m^2).

In the prospective part of the FinnDiane Study, 2304 individuals with type 1 diabetes, followed for a mean of 5.4 years, were examined [13]. Baseline triglycerides predicted the progression of kidney disease at all stages, including progression to micro- and macroalbuminuria and to kidney failure (dialysis or kidney transplantation). These associations could not be fully explained by conventional risk factors other than baseline AER. Several lipid variables predicted progression to kidney failure, but when eGFR was included in the model, total cholesterol was the only significant lipid predictor.

The definition of the kidney disease phenotype may influence the results. In a model in which normal AER and microalbuminuric groups were pooled, the triglycerides predicted the progression to macroalbuminuria, and the results could not be fully explained by either baseline AER or eGFR. When the patient set was divided

into quartiles of triglycerides, the highest quartile had consistently higher hazard ratios for progression at all stages of diabetic kidney disease. From a practical point of view, however, no clear threshold could be observed for triglycerides and the progression of diabetic kidney disease [13]. Moreover, interactions between diabetic kidney disease, retinopathy, and most of the lipid variables were observed in the FinnDiane Study participants. When stratifying for retinopathy status, no associations between AER and lipid variables were observed in the individuals without signs of retinopathy, whereas the correlations between AER and lipid variables were much stronger in the individuals with proliferative diabetic retinopathy than in those with only mild non-proliferative diabetic retinopathy. In other words, the associations between kidney disease and lipid variables are influenced by the retinopathy status, suggesting the existence of shared pathophysiological mechanisms between the different microvascular diabetes complications [34].

Besides the conventional lipoprotein lipids listed above, the importance of remnant cholesterol—defined as the non-HDL and non-LDL cholesterol, corresponding to the cholesterol content of the chylomicron remnants, VLDL, and IDL particles—has been highlighted in the context of diabetic kidney disease in the FinnDiane cohort [35]. In a cross-sectional analysis, the remnant cholesterol concentration was found to increase with advancing stage of kidney disease. Furthermore, the remnant cholesterol concentration was higher among those whose kidney disease status progressed, and the risk of progression rose step-wise with increasing concentration of remnant cholesterol at baseline, as Fig. 15.2 illustrates.

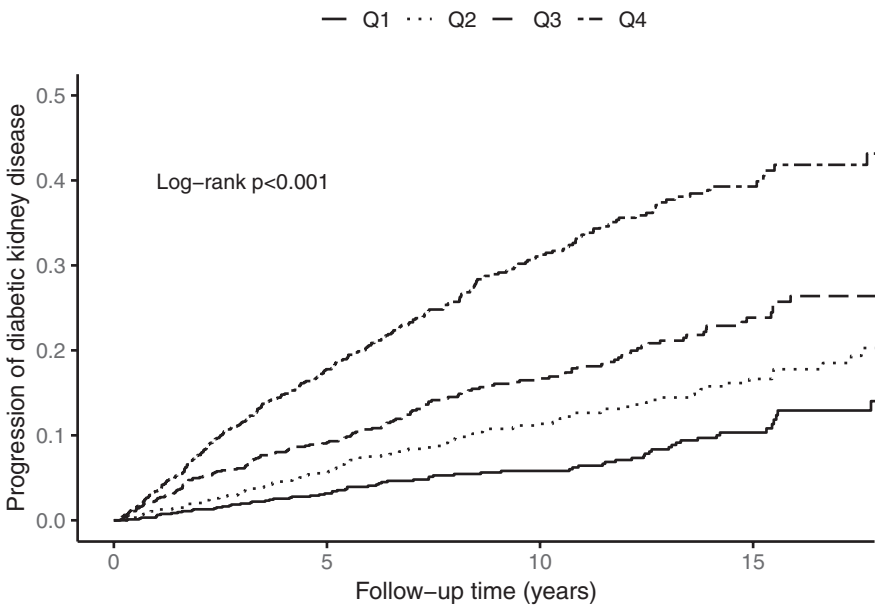

Fig. 15.2 Cumulative incidence curves for quartiles of remnant cholesterol concentration at baseline to illustrate the progression of diabetic kidney disease among 3808 participants of the FinnDiane Study. (The figure was adapted from [35])

In multivariable analyses, remnant cholesterol was associated with the progression of kidney disease independently of several well-established conventional risk factors, including diabetes duration, hemoglobin A_{1c}, systolic blood pressure, and smoking. The association was observed at all steps of progression of kidney disease except for the progression of macroalbuminuria to kidney failure, which was independent of all other included risk factors but eGFR.

Pittsburgh EDC

A total of 658 individuals with childhood-onset type 1 diabetes were included in the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study. A 2-year follow-up study of 256 participants indicated that poor glycemic control, increased LDL cholesterol, long duration of diabetes, and high systolic blood pressure at baseline were predictive of incident microalbuminuria, defined as AER >20 μg/min [36]. Glycemic control was a significant predictor in all sub-group analyses. In men, age and AER were also important predictors, whereas the duration of diabetes and triglycerides were important in women. Calculated LDL cholesterol was significant in those with a type 1 diabetes duration <20 years, but triglycerides and systolic blood pressure predicted progression in those with at least 20 years of duration. In a more recent study, 485 individuals with or without overt kidney disease at baseline (AER <200 μg/min) were followed for 10 years [37]. Estimated glucose disposal rate (a surrogate marker for insulin sensitivity) was predictive of overt kidney disease during the entire follow-up. White blood cell count, triglycerides, calculated LDL cholesterol, non-HDL cholesterol, and systolic blood pressure predicted progression during the first 5 years of follow-up.

Predictors of early kidney function decline, defined as an annual decrease in eGFR of ≥3 mL/min per 1.73 m^2, were also assessed in the Pittsburgh EDC cohort [38]. In univariate analyses, the mean concentrations of total cholesterol and triglycerides were higher in the individuals with rapid eGFR decline than in those without. However, these results could not be replicated in the *Coronary Artery Calcification in Type 1 Diabetes (CACTI) cohort*. HDL and LDL cholesterol concentrations did not differ between the groups in either cohort.

Swedish National Diabetes Register

The Swedish National Diabetes Register was launched in 1996 with the aim to collect clinical patient data from outpatient clinics and primary health care centers nationwide to monitor changes in the treatment and risk factors of diabetes, as well as the epidemiology of diabetic complications. A cross-sectional analysis including 4795 individuals with type 1 diabetes from the register was performed to assess factors associated with established microalbuminuria [39]. Multivariable logistic regression analyses revealed independent associations for hemoglobin

A_{1c}, diabetes duration, systolic blood pressure, body-mass index, smoking, and triglycerides. Despite a univariate difference between the normo- and microalbuminuria groups, total cholesterol was not associated with the outcome in the multivariable analysis. HDL cholesterol did not reach statistical significance in the analyses.

The association between kidney disease and LDL cholesterol was scrutinized in another study from the register comprising 12,350 young individuals with type 1 diabetes (duration ≤10 years at baseline), followed for up to 28 years after diagnosis [40]. The study concluded that young individuals exposed to high LDL cholesterol (above 3.4 mmol/L) have a heightened risk of albuminuria, even after consideration of hemoglobin A_{1c}, the other conventional lipoprotein lipids, eGFR category, and many other well-known vascular risk factors.

Kidney Disease and Dyslipidemia in Type 2 Diabetes

Type 2 diabetes itself is strongly linked to similar lipoprotein abnormalities that are seen in individuals with type 1 diabetes and microvascular complications, and it is therefore problematic to isolate the kidney disease-related changes from the background dyslipidemia. Nevertheless, more adverse lipid profiles distinguish individuals with kidney disease; yet, whether the unfavorable lipid profiles observed in people with type 2 diabetes and chronic kidney disease are secondary to the kidney pathologies or vice versa is unclear. As will be discussed below, interventional studies on lipid-lowering therapies for diabetic kidney disease have given rise to partly inconsistent results, and observational studies cannot conclusively answer these questions. However, many observational studies during the past decades have provided important knowledge about the link between lipoprotein lipids and chronic kidney disease in type 2 diabetes. Although not all studies published within the topic could be included, the main findings on conventional lipoprotein lipids and diabetic kidney disease from some large type 2 diabetes trials will briefly be reviewed next.

The Early Treatment Diabetic Retinopathy Study (ETDRS) was initiated in 1979 to assess the effects of laser photocoagulation and aspirin therapy on diabetic retinopathy and has since provided vast and valuable knowledge and basis for the management of diabetic eye complications. In addition, risk factors for kidney replacement therapy were assessed [41]. Among the 1292 study participants with type 2 diabetes, 150 required kidney replacement therapy during the study. Total cholesterol and triglycerides concentrations were independent risk factors of the kidney endpoint.

The U.K. Prospective Diabetes Study (UKPDS) examined individuals with newly diagnosed type 2 diabetes to identify clinical risk factors associated with the development of kidney disease [42]. Multivariable analyses were limited to the 2167 individuals without albuminuria or impaired kidney function at baseline but

with available data for all studied covariates. The median follow-up time was 15 years. Results from the multivariable analyses revealed that increased plasma triglycerides and LDL cholesterol were associated with incident macroalbuminuria but not with microalbuminuria. The triglyceride concentrations, but neither the LDL nor the HDL cholesterol concentrations, were independently associated with the doubling of serum creatinine during the observation period.

Also a cross-sectional analysis of the Italian multi-center Renal Insufficiency And Cardiovascular Events (RIACE) Study provided a comprehensive outlook on the role of the triglycerides in kidney disease in people with type 2 diabetes [43]. The results showed that high triglyceride concentrations, defined as concentrations above 1.7 mmol/L, were associated with microalbuminuria, macroalbuminuria as well as mildly to severely reduced eGFR in the large cohort (n = 15,773)—irrespective of the use of statin therapy.

The Action in Diabetes and Vascular Disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) Study enrolled study participants from 20 countries worldwide to evaluate the consequences of efficient glycemia and blood pressure control in individuals with type 2 diabetes at high vascular disease risk. A sub-analysis on HDL cholesterol was published after a median observation period of 5 years [44]. In an adjusted analysis comparing the lowest with the highest baseline HDL cholesterol tertile, an association with new microalbuminuria, new macroalbuminuria, and doubling of serum creatinine was observed. For a composite kidney outcome, the hazard ratio (lowest vs. highest HDL cholesterol tertile) was 1.19 (95% confidence interval 1.08–1.32).

A number of studies besides the trials listed above have published data that support these results. For instance, a set of 3667 individuals with type 2 diabetes with normal AER and kidney function were examined in the Swedish National Diabetes Register for incident diabetic kidney disease [45]. Increased triglycerides and decreased HDL cholesterol at baseline predicted incident albuminuria, and both were also predictive of impaired eGFR. On the other hand, total or LDL cholesterol was not significant predictor. Along similar lines, evidence in favor of the association between high triglycerides and low HDL cholesterol with kidney disease appeared from a multinational case-control study of 2535 individuals with type 2 diabetes [46]. Cases and controls were matched, for instance, based on LDL cholesterol. Kidney disease was defined either as albuminuria/proteinuria, eGFR <60 mL/min per 1.73 m^2, or both. The odds ratio for kidney disease was 1.23 (95% confidence interval 1.16–1.31) per 0.5 mmol/L increase in triglycerides, and 0.86 (0.82–0.91) for a 0.2 mmol/L increase in HDL cholesterol. These conclusions were analogous to those drawn from a longitudinal, Italian multi-center study comprising over 15,000 participants, where the adjusted hazard ratio for kidney disease (eGFR <60 mL/min per 1.73 m^2 or albuminuria) was 1.08 (1.03–1.12) for triglycerides (by 50 mg/dL) and 0.94 (0.90–0.97) for HDL cholesterol (by 10 mg/dL) [47]. Furthermore, a higher TG:HDL-C ratio was shown to be associated with the incidence and progression of CKD in a large (n = 124,700 in some analyses) Japanese cohort [48].

Interpretation of the Epidemiological Data

The cross-sectional analyses suggest that increased triglycerides, total cholesterol, and LDL cholesterol, and a reduction in HDL cholesterol is typically seen in individuals with diabetes and kidney disease. The dyslipidemia is more evident in those with advanced kidney disease, but this could be a mere consequence of poor glycemic control that, by itself, promotes the development of microvascular injuries. Furthermore, altered nutritional status and secondary effects of kidney failure may curb the increase in cholesterol but simultaneously cause an imbalance between triglycerides and HDL cholesterol when the individuals approach kidney failure [49, 50].

Interestingly, when both hemoglobin A_{1c} and body-mass index are high in type 1 diabetes, the lipid profile resembles that of the dyslipidemia typically observed in type 2 diabetes and in individuals with the metabolic syndrome [15, 51]. The weight-adjusted insulin dose tends to be similar or even higher in these individuals [52], which suggests that the dyslipidemia in today's type 1 diabetes could be at least partially related to increased insulin resistance rather than inadequate insulin administration. This fits to the concept of "double diabetes" and insulin resistance as a major pathogenetic contributor to diabetic kidney disease [53, 54].

The classical linear analyses may hide the inherent complexity of the lipoprotein lipid profile. Figure 15.3 depicts the same dataset that was introduced in Fig. 15.1, but this time dissected by a multivariable non-linear visualization method. Details of the self-organizing map (SOM) are available in supplements of previously published articles [52, 55]. Briefly, the method assigns a two-dimensional coordinate on the map for each individual based on the observed biochemical profiles. The map can then be colored according to a trait such as cholesterol concentration or prevalence of diabetic kidney disease in a given area. The idea is similar to coloring the world map based on average income or prevalence of diabetes—in that case, the coordinates represent geographical locations. In Fig. 15.3, the map is always the same, so if an individual is located on the top-left corner in Plot a, he or she is also located in the top-left corner in every other plot.

The connection between diabetic kidney disease and mortality is obvious (Fig. 15.3a, b), as one would expect based on Fig. 15.1. The top part of the map contains most of the individuals with kidney disease, older age, and a longer duration of diabetes. The patterns of lipids are more complicated: low concentrations of triglycerides, total cholesterol, and LDL cholesterol consistently characterize the individuals with no diabetic kidney disease, but greater diversity can be observed in the upper half. In linear analysis, triglycerides and cholesterol would emerge as positive regressors, but it is possible that only a subset of individuals actually shows this positive relationship. Furthermore, HDL cholesterol appears to show a completely perpendicular pattern with respect to diabetic kidney disease, which could represent an additional independent modulating effect on vascular risk.

Figure 15.3 was created from a single cohort, the non-linear method can lead to over-interpretation, and further work is needed to validate the observed patterns.

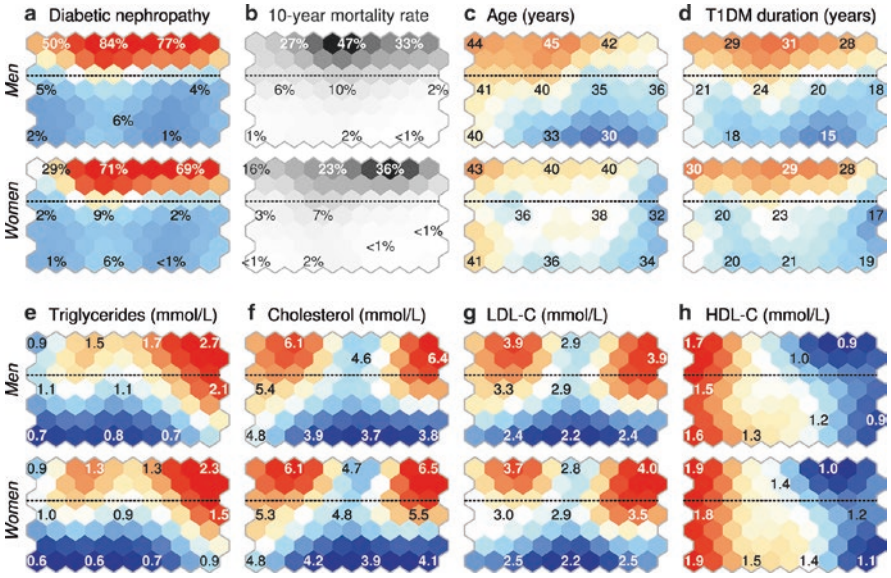

Fig. 15.3 Self-organizing map analysis of 4197 individuals with type 1 diabetes from the FinnDiane Study. The figure can be interpreted the same way as a geographical map. Suppose the rectangular area is a map of a city, and the city is divided into hexagonal neighborhoods. In each neighborhood, the residents correspond to individuals who are similar with respect to their serum and urine biochemical profiles. The locations of individuals were mathematically optimized by the self-organizing map algorithm (in a geographical map the locations would be physical coordinates). For the visualization, the rectangular map is colored based on the average characteristics in a neighborhood. For example, individuals in the top part of the map show a high prevalence of diabetic kidney disease, which is indicated by the red color (Plot **a**). Diabetic kidney disease was defined as macroalbuminuria or kidney failure. (The figure was adapted from [15])

Nevertheless, these results highlight the inherent biological complexity that may be missed by traditional approaches. For a practicing clinician, the heterogeneity is a challenge: those individuals that have complications also show the greatest lipoprotein diversity. It is also possible that elevated cholesterol in one individual is more dangerous than in another, so additional information on the causal links is of great interest.

Can Dyslipidemia Cause Kidney Disease?

In prospective analyses, the picture is similar and conventional lipids are a part of the overall risk profile that is linked to poor glycemic control. In particular, the total triglyceride concentration has been a predictive marker at different stages of albuminuria in multiple studies: higher values have been associated with progression and lower values with regression of albuminuria. Furthermore, dyslipidemia is associated with a faster decline in kidney function [29, 37, 56–58]. Although it is

difficult to ascertain causal links based on the current clinical data, the practical message is clear: if lipids are abnormal, particularly in combination with smoking, hypertension, and obesity, the prognosis is considerably worse.

Atherosclerosis and glomerulosclerosis exhibit similar features [59], and it has been hypothesized that a compensatory increase in hepatic output of circulatory lipids follows the urinary loss of albumin. This, in turn, initiates a self-perpetuating cycle of glomerular and tubular events that aggravate and maintain the progressive decline in kidney function [60]. There is some experimental evidence to support this theory. When guinea pigs and rats were fed cholesterol-rich food in a number of studies, they developed various forms of glomerular and other injuries, and the effects could be modulated by partial or unilateral nephrectomy and hypertension [61, 62]. On the other hand, cholesterol alone may not be sufficient to initiate the disease processes since not all hyperlipidemic animals develop glomerular lesions. Moreover, non-diabetic human individuals with elevated cholesterol or triglycerides rarely develop kidney disease, so it is plausible that hyperglycemia (particularly in type 1 diabetes) and/or hypertension (particularly in type 2 diabetes) are necessary causative partners of hyperlipidemia on the path to diabetic kidney injury.

Lipoprotein Subclasses and Albuminuria in Type 1 Diabetes

Impaired kidney function is associated with multiple lipoprotein abnormalities; yet, also at the preceding albuminuric stages, dysfunctions in lipid transfer proteins, lipoprotein formation, and clearance may be present. There is also evidence of mechanistic links to lipotoxicity in the nephrons [60], and the epidemiological findings of increased triglycerides and cholesterol in the circulation suggest that lipoproteins provide the fuel for these lipotoxic processes. Lipoprotein particles comprise a heterogeneous group of lipid transport vehicles with diverse tasks and multiple characteristics such as size, density, and composition. In this respect, the conventional lipoprotein lipids are summary measures—more specific measurement techniques may reveal subtle lipoprotein defects that contribute to diabetic microvascular injury.

VLDL Subclasses

In the DCCT/EDIC Study, lipoprotein subclasses were measured by a proton NMR spectroscopic method for 958 individuals with type 1 diabetes [12, 63]. The strongest signals for albuminuria were obtained for the VLDL and HDL subclasses, whereas the LDL subclasses were weaker indicators of kidney disease. The total lipid contents in medium and small VLDL subclasses were significantly associated with AER, and the two were the only lipoprotein measures that were significant in women after adjusting for other risk factors. In men, all VLDL subclasses were

increased in those with increased AER, and differences were also observed in other lipoprotein measures. On the other hand, VLDL size was not associated with albuminuria.

Lipoprotein subclasses were measured by NMR in a subset of 325 type 1 diabetic individuals from the FinnDiane cohort [55], although the methodology to extract subclass data from the NMR spectra was different from the one used in the DCCT/EDIC. The extremely large and large VLDL subclasses were significantly different between individuals without and with macroalbuminuria. The strongest positive correlations with continuous AER were observed for large VLDL cholesterol.

A prospective and extended (n = 3544) study from the same cohort revealed cholesterol- and triglyceride-enrichment of the large VLDL subclass in those who developed incident microalbuminuria as compared to those whose AER remained normal throughout the observation period. Moreover, progression from microalbuminuria was associated with enrichment of medium VLDL lipids, and progression from macroalbuminuria to kidney failure with higher triglycerides in all VLDL subclasses except the extremely large and higher cholesterol in the four largest VLDL particles [64].

Another study by Thomas et al. investigated the progression of diabetic kidney disease in type 1 diabetes in relation to VLDL particles from ultracentrifugation among 152 individuals [65]. No associations (when adjusted for other risk factors) were detected between VLDL measures and progression from normal AER, nor between VLDL and eGFR decline in the macroalbuminuric group. On the other hand, VLDL triglycerides predicted progression from microalbuminuria.

IDL and LDL Subclasses

The calculated Friedewald LDL cholesterol, which also includes cholesterol from IDL, was a significant covariate of AER in the DCCT/EDIC Study [12]. At the subclass level, the lipid mass within IDL was increased in men with macroalbuminuria but not in women. Regarding the lipid mass in the LDL particles, only that of the small LDL particles was significantly increased in individuals with kidney disease; however, not in the adjusted analyses, and there was also evidence of gender interaction with AER. Men showed a decrease in LDL size, and both sexes showed an increase in LDL particle concentration in the macroalbuminuric group. Oxidation of LDL was investigated via fluorescence ratio and delta absorbance, but there were no differences between the AER categories. Of note, Lp(a) was also similar between the AER groups.

The Pittsburgh EDC Study Group employed the same NMR method as the DCCT/EDIC to examine 42 matched pairs of progressors and non-progressors with respect to overt diabetic kidney disease (AER >200 μg/min or serum creatinine >153 μmol/L or kidney failure). Decreased LDL particle size emerged as the most important lipoprotein measure [66], and the results also suggested that lipoprotein

lipids are less important during the initial increase in AER, with larger effects at the later stages of albuminuria.

LDL-subclass lipids were not significant indicators of albuminuria in the cross-sectional analysis of the subset from the FinnDiane Study, nor were there any other measures related to IDL other than the IDL triglycerides [55]. In the prospective analysis encompassing the whole cohort, the development of incident microalbuminuria showed no association with the cholesterol- or triglyceride-contents of IDL or the LDL subclasses. Progression from microalbuminuria was related to triglyceride-enrichment of IDL, large LDL, and medium LDL, as well as cholesterol-enrichment of medium- and small-sized LDL. Progression from macroalbuminuria was related only to triglyceride-enrichment of IDL, large LDL, and medium-sized LDL particles [64].

Thomas et al. reported that LDL cholesterol, LDL-free cholesterol, and LDL mass, measured by ultracentrifugation, predicted the progression from normal AER. Furthermore, IDL triglycerides predicted the progression from microalbuminuria, whereas only decreased LDL size was associated with declining eGFR in the macroalbuminuric group [65].

Apolipoprotein B

Each lipoprotein particle in the VLDL-IDL-LDL cascade contains a single apolipoprotein B-100 molecule (apoB); thus, the determination of apoB works as a pooled measure of the circulating particle concentrations for these lipoproteins [67]. In the DCCT/EDIC Study, apoB was a significant covariate of AER and creatinine clearance, but only for men in the adjusted and sex-stratified analyses [12, 68]. Findings in the FinnDiane Study were similar: apoB was increased in microalbuminuric individuals, even more in macroalbuminuric individuals, and apoB was also associated with progression across the albuminuria categories [13, 21]. A nested case-control approach within the EURODIAB Prospective Complications Study found that in 224 individuals with type 1 diabetes, apoB was significantly increased both in the micro- and macroalbuminuric groups [69].

HDL Subclasses

HDL cholesterol is decreased in individuals with type 1 diabetes and macroalbuminuria, and the size, function, and composition of the HDL particles are altered during the course of diabetic kidney disease. In the DCCT/EDIC, the HDL subfraction was split into large HDL (assumed cardioprotective) and small HDL (non-cardioprotective). The total lipid content of the large HDL particles was decreased

in those with macroalbuminuria, but small HDL was increased in both men and women, which fits to the assumed roles. Furthermore, HDL particle size was inversely correlated with AER [12].

The HDL sub-fraction was divided into four subclasses in the FinnDiane cohort with available serum NMR data. Individuals with macroalbuminuria had decreased cholesterol and other constituent lipids in the large HDL (the second largest subclass), and weaker inverse associations were also detected for medium HDL lipids, esterified cholesterol in the largest HDL, and total lipids in small HDL [55]. In a prospective sub-cohort analysis, depletion of large HDL cholesterol was observed in individuals who progressed at a shorter duration. Surprisingly, the largest HDL subclass was positively correlated with LDL lipids and elevated in individuals at risk for progression from normal AER or microalbuminuria [52].

The HDL sub-fractions can also be divided according to buoyancy: the HDL_2 subclass represents large buoyant particles, whereas the HDL_3 denotes smaller and denser particles. HDL_3 cholesterol was investigated by an enzymatic method in the main FinnDiane cohort. In a cross-sectional analysis, both HDL_2 (estimated as non-HDL_3) and HDL_3 cholesterol were decreased in individuals with macroalbuminuria and in individuals with impaired eGFR [21]. In a prospective analysis, the pattern was similar when progressors were compared with non-progressors for each baseline kidney disease category [13]. However, the progressor groups were different with respect to gender and diabetes duration. When traditional risk factors were taken into account, HDL_3 cholesterol was positively associated with progression from normal AER to microalbuminuria.

Apolipoproteins A-I and A-II

ApoA-I and apoA-II are major structural components of the HDL particles, and their concentrations are correlated with HDL lipids [70]. Kahri et al. compared HDL particles between 52 individuals with normal AER, 37 with microalbuminuria, and 64 with macroalbuminuria. HDL_2 cholesterol was higher in those with normal AER, but no differences were detected with respect to apoA-I or apoA-II, or HDL particles with or without apoA-II [71].

Surprisingly, apoA-I was a borderline positive covariate of albuminuria in men in the DCCT/EDIC, and there was also a positive association with creatinine clearance [12]. Results from the FinnDiane Study were also somewhat unexpected: neither A-I nor A-II showed a clear trend for AER or eGFR in cross-sectional analysis, but increased apoA-II and decreased apoA-I/A-II ratio predicted progression from normal AER in prospective analysis [13, 21]. In the subset of 325 individuals from the main FinnDiane cohort, apoA-II was correlated with total cholesterol and serum phosphatidylcholine in a network model and increased in patients with a high risk of incident albuminuria [52].

Apolipoprotein C-III

ApoC-III is present on circulating apoB-containing triglyceride-rich lipoproteins, HDL particles, and to a smaller extent on LDL particles. It is a key regulator of triglyceride homeostasis acting through multiple pathways, such as inhibition of lipoprotein lipase and impairment of the remnant particle clearance, thus amplifying the plasma resident time of these particles [72]. ApoC-III has been associated with macrovascular endpoints in individuals with type 1 diabetes, but also with microvascular co-morbidities such as kidney disease. This was initially shown in a cross-section analysis of the DCCT/EDIC cohort that demonstrated a strong correlation between the concentration of apoC-III and the severity of albuminuria [73]. The finding was replicated among 3085 study participants of the FinnDiane Study, and furthermore, apoC-III was independently and positively associated with the progression of kidney disease, even after controlling for sex, diabetes duration, initial DKD category, blood pressure, hemoglobin A_{1c}, smoking status, LDL cholesterol, remnant cholesterol, and lipid-lowering medication [74].

Lipoprotein Abnormalities in Impaired Kidney Function and Their Relevance to Diabetic Kidney Disease

Loss of kidney function results in multiple systemic effects on metabolism, and lipoproteins are also affected [75, 76]. The most marked changes can be summarized as (1) reduced clearance of apoB-containing lipoproteins and their remnants, (2) accumulation of small and dense and oxidized LDL particles, and (3) impaired maturation of HDL particles. Some of these effects may depend on the kidney replacement therapies. For instance, peritoneal dialysis causes plasma albumin loss and is linked to increased LDL and total cholesterol due to increased cholesterol biosynthesis, whereas hemodialysis seems not to have similar adverse effects [77, 78]. Of note, excess apoB-containing lipoproteins have been observed in nephrotic-range proteinuria, where depletion of plasma albumin is also common [79].

Chylomicrons are large triglyceride-rich lipoprotein particles with a single apolipoprotein B-48, and they deliver dietary fatty acids from the intestine to the rest of the body. The VLDL particles are the hepatic counterpart with a single apolipoprotein B-100 molecule, and the triglyceride-poor remnants of both classes are taken up by the liver [80]. The release of the triglyceride content from VLDL particles requires apolipoproteins E and C-II from mature cholesterol-rich HDL particles [75]. In chronic kidney disease, however, HDL fails to mature properly [81], which then disrupts the normal release of triglycerides from the VLDL and their subsequent conversion to IDL and ultimately to triglyceride-free LDL that can be cleared by the liver.

The lipoprotein subclass data on diabetic kidney disease support the concept of impaired clearance of VLDL, as elevated VLDL subclass lipids were observed in

multiple studies and at different disease stages. However, individuals with type 1 diabetes and albuminuria show signs of "double diabetes," and both impaired clearance and increased VLDL synthesis are likely to be responsible for the dyslipidemia [17, 82]. It is possible that the balance between VLDL synthesis and clearance changes as kidney injuries advance. Therefore, although increases in VLDL subclasses can be observed during the entire course of diabetic kidney disease, the causes may be different for low-grade albuminuria, for proteinuria with a sufficient glomerular reserve, and for kidney failure.

Small and dense LDL particles are considered highly atherogenic and have been linked with an increased risk of cardiovascular disease [83]. In addition, oxidation of LDL makes the particles more prone to infiltrate vascular walls and promote the inflammatory cascade that leads to intima-media thickening and accumulation of atherosclerotic plaque [84]. Decreased LDL size was a significant predictor of the progression of diabetic kidney disease in some studies reviewed above, and increased concentrations of small LDL lipids among the progressors were also observed. This modification of LDL subclass distributions is probably connected to the clearance of the entire VLDL-IDL-LDL pool [85, 86]. In the DCCT, oxidation of LDL was not found to be different between AER categories [12], but more studies are needed to ascertain if LDL oxidation is essential in the pathogenesis of diabetic kidney disease.

The HDL sub-fraction contains a complex set of multi-functional particles at different stages of maturation [87]. In general, HDL particles are protective against vascular diseases: they are able to remove excess cholesterol from peripheral tissues, attenuate oxidative stress, and may have anti-inflammatory properties [88–90]. When kidney function declines, HDL fails to mature properly to its cholesterol-rich form and remains as a small lipid-poor particle, and this may explain the inverse association with the conventional HDL cholesterol [91]. As reviewed above, decreased HDL subclasses have been detected in a number of studies on diabetic kidney disease, whereas the results on apolipoproteins A-I and A-II (the major structural proteins) are conflicting; thus, it is difficult to say if the number of particles is affected. Nevertheless, the observed inverse correlation between HDL size and AER fits to the concept of impaired HDL maturation as a significant defect also in diabetic kidney disease.

Lipid Medications and Diabetic Kidney Disease

In the previous sections, we have discussed the various lipoprotein defects that are associated with kidney injury. Several pharmacological agents are available to correct atherogenic changes in lipoprotein metabolism, and their beneficial effects in the general population have been established in numerous studies. The most widely used—and most widely studied—are statins, which are effective cholesterol-lowering drugs due to their direct inhibitory effect on the HMG-CoA reductase, a central enzyme in hepatic cholesterol synthesis. Fenofibrates are synthetic ligands

to the peroxisome proliferator-activated receptor alpha (PPARα), and the lipid-lowering mechanisms include the activation of lipoprotein lipase, reduced production of apolipoprotein C-III, and the subsequent increased clearance of VLDL and IDL particles. We will also cover ezetimibe, which is a selective inhibitor of cholesterol absorption in the gut. The discussion here is centered on the potential renoprotective effects of the drugs, and therefore, explicit results on cardioprotection are omitted. It is, however, important to stress that in many of the studies, a significant reduction in cardiovascular events has been seen, especially in individuals with early stages of kidney disease.

Statins

The first statin (lovastatin) became commercially available in the late 1980s, and the first studies investigating the potential kidney effects of statin treatment among individuals with diabetes were published in the early 1990s [92, 93]. Since then, a number of studies on the topic have appeared, but the results remain to some degree inconclusive. It is noteworthy that the studies have almost exclusively been confined to individuals with type 2 diabetes, whereas less is known about the potential kidney benefits of statin therapy in type 1 diabetes. We will next review the available data connecting diabetes, statin therapy, and kidney disease, with focus on the large trials.

In 2008, The Collaborative Atorvastatin Diabetes Study (CARDS), including 2838 individuals with type 2 diabetes without a history of cardiovascular disease, concluded that atorvastatin treatment (10 mg/day vs. placebo) was associated with a modest improvement in the annual change in eGFR—a finding most apparent in those with albuminuria, while no significant influence on the incidence of albuminuria was seen [94]. Similarly, in a sub-study of the Treating to New Targets (TNT) trial in individuals with coronary artery disease, both 10 and 80 mg of atorvastatin increased eGFR in those with diabetes, with or without moderate chronic kidney disease, with a higher increase in eGFR in those treated with the 80 mg atorvastatin dose [95]. The renal effects of atorvastatin (dose titrated between 10 and 80 mg/day) were also evaluated in a post hoc subgroup analysis of the Greek atorvastatin and coronary heart disease evaluation (GREACE) cohort of which 20% had diabetes and all had normal kidney function at baseline. The GREACE Study demonstrated a significant increase of creatinine clearance (mean increase 11.6%) in the group allocated to structured care with atorvastatin (10–80 mg/day) but deterioration of kidney function (mean reduction 5.3%) in those allocated to usual care without a lipid-lowering agent over 48 months [96]. Albumin-/proteinuria was not reported in the two latter atorvastatin studies.

The Heart Protection Study (HPS), including individuals with diabetes (3% type 1, 26% type 2, and the rest without a history of diabetes) or occlusive arterial disease, found that simvastatin treatment was associated with a smaller

decrease in eGFR than placebo, and the effect was slightly larger among those with diabetes [97].

The kidney effects of pravastatin vs. placebo were evaluated in the Prospective Pravastatin Pooling (PPP) project, consisting of three randomized controlled trials in individuals with moderate CKD at baseline (30 ≤eGFR <60 mL/min per 1.73 m^2), with and without diabetes [98]. In a post hoc subgroup analysis, pravastatin modestly reduced the rate of kidney function loss. Adjusted for covariates that may influence the kidney function, pravastatin therapy was associated with a 34% slower rate of kidney function than the placebo group; however, the absolute clinical magnitude was rather small (0.22 ± 0.07 mL/min per 1.73 m^2/year slower than placebo).

Neither the HPS nor the PPP project study assessed the association between statin treatment and effect on albumin-/proteinuria, whereas this was carried out for rosuvastatin therapy in a small cohort (n = 52) of individuals with type 2 diabetes [99]. The study demonstrated a significant reduction (−40.1 ± 24%) in the urinary albumin-to-creatinine ratio over an observation period of 6-months.

Lastly, fluvastatin treatment was evaluated during a 5-year follow-up of 2102 kidney transplant recipients in the Assessment of Lescol in Renal Transplantation (ALERT) study [100]. Fluvastatin was shown to have no significant effect on the incidence of kidney graft loss, doubling of serum creatinine, decline in GFR, or major adverse cardiac events [101, 102]. Of note, fewer non-fatal myocardial infarctions and cardiac deaths were observed in the fluvastatin group. In a subsequent analysis of the study population after a 7-year follow-up, open-label fluvastatin treatment reduced the risk of the first major cardiac event by 21%, but no significant difference in graft loss or total mortality was seen.

Studies comparing different types of statins with respect to kidney disease outcomes have also been conducted [103]. The Prospective Evaluation of Proteinuria and Renal Function in Diabetic Patients with Progressive Renal Disease (PLANET I) trial was carried out to assess kidney-specific effects of two statins, atorvastatin (80 mg/day) and rosuvastatin (10 mg and 40 mg/day), among individuals with diabetes and proteinuria. Study participants (n = 353) were enrolled from 147 research centers and followed for 1 year. The PLANET I trial showed that high-dose treatment with atorvastatin was associated with a significant decrease in the protein excretion rate and a stable eGFR over the follow-up year. In contrast, high-dose treatment with rosuvastatin did not affect the protein excretion rate, while the eGFR was significantly decreased from baseline, and doubling of serum creatinine and acute kidney injury were more common than in the other treatment groups. Thus, the study concluded that although high-dose rosuvastatin improved the lipid levels more efficiently than what atorvastatin did, the latter may be more renoprotective in this high-risk group of individuals.

Atorvastatin was compared with pravastatin in a small study (n = 35 and 28, respectively) comprising individuals with diabetes and manifest kidney disease [104]. Alike PLANET I, the study participants were followed for 1 year. A significant decrease from baseline in the urinary albumin-to-creatinine ratio was observed within the atorvastatin group; however, at 12-months, the level was not significantly different than the one in the pravastatin arm. Cystatin C and cystatin C-based eGFR

were more beneficial in the atorvastatin group vs. the pravastatin group at 12-months; however, no between-group difference was seen in the creatinine-based eGFR after 1 year.

Another study compared pravastatin (20 mg/day) against pitavastatin (2 mg/day) among 83 individuals with type 2 diabetes and manifest kidney disease [105]. The study concluded that after 1 year, pitavastatin therapy had resulted in a greater reduction of urinary albumin-to-creatinine ratio than pravastatin in the initially macroalbuminuric group, whereas no difference was seen in those with initial microalbuminuria. A significantly different change in eGFR was neither observed between the two agents in any of the albuminuria groups.

Moreover, there are also studies that have assessed the relationship between the use of any statin with kidney disease outcomes in populations with diabetes. For instance, in a study with 197,551 veterans (27% with diabetes), statin treatment was associated with a 13% decrease in the development of renal dysfunction, possibly by other than lipid-dependent mechanisms [106]. In a population-based Danish study including 15,679 individuals who had used statins regularly until their diagnosis of diabetes, matched to 47,037 individuals who had not used statins before the diagnosis, statin-use was associated with a lower incidence of diabetic retinopathy, neuropathy, and gangrene of the foot. However, no difference in the incidence of diabetic kidney disease was seen over the median follow-up time of 2.7 years (range 0–13 years) [107].

Fenofibrate

Fenofibrate treatment of individuals with type 2 diabetes reduced the progression of microalbuminuria in the Diabetes Atherosclerosis Intervention (DAIS) [108] and in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) studies [109, 110]. However, the effect sizes were modest. A combination of fenofibrate and simvastatin modestly reduced progression to micro- or macroalbuminuria compared to simvastatin treatment alone in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [111]. In a meta-analysis of fibrate studies including albuminuria data from the studies above (15,731 individuals), fenofibrate reduced the risk of albuminuria progression by 14% [112].

In the ACCORD post-trial follow-up ACCORDION, concerns were raised as fenofibrate treatment was associated with a significantly higher risk of doubling of serum creatinine than the non-fibrate group [113]. However, it is presumable that this finding is at least partly attributable to characteristics of the study design, such as the low number of creatinine measurements during follow-up [114]. Similarly, in an additional study of the FIELD cohort with a washout period, an initial and reversible increase was seen in plasma creatinine, but during a follow-up of 5-years, fenofibrate slowed eGFR loss, and greater benefit of eGFR preservation with fenofibrate treatment was seen in those with baseline dyslipidemia [115].

Ezetimibe

Combination therapy with simvastatin and ezetimibe reduced the number of major atherosclerotic events by 17% in 9270 individuals in the Study of Heart and Renal Protection (SHARP) trial [116]. A subgroup analysis comprising 6245 individuals with CKD but not on dialysis was performed (median observation time 4.8 years). Allocation to simvastatin and ezetimibe had no significant effect on the incidence of kidney failure (defined as the initiation of maintenance dialysis or kidney transplantation), the outcome of kidney failure or death, or on kidney failure or doubling of serum creatinine in comparison to the placebo arm [117]. The findings persisted in the sensitivity analysis, including only those with a history of diabetes (23% of the cohort).

Clinical Utility of Lipid Treatment in Diabetic Kidney Disease

Overall, lipid-modifying treatments seem to have a modest effect on the development of albuminuria and the decline of eGFR. As discussed in the previous sections, the causal links between lipoprotein lipids and kidney injury are tentative, which may explain the lack of strong effects. Of note, improving glycemic control and aggressive treatment of hypertension is effective in protecting the kidneys, and lifestyle interventions have beneficial effects on the total systemic metabolism, including lipids. Most of the evidence on lipid drugs comes from individuals with detectable vascular problems; it is not known whether lipid-based interventions at an earlier stage could provide benefits that are lost at later stages of diabetic kidney disease. Furthermore, trials with hard renal endpoints and direct GFR measurements (not only estimated GFR which is dependent on creatinine production and excretion) are needed to clarify the situation.

Should the dyslipidemia in individuals with diabetic kidney disease be medicated? There is a consistent body of evidence that reducing the atherogenicity of lipoproteins is beneficial in most population groups. Subsequently, diabetic individuals with albuminuria but without kidney failure should be medicated, perhaps even more aggressively than the general population. Unfortunately, kidney failure with or without diabetes seems to be a tough problem to solve. Attenuation of the reduction in cardiovascular endpoints and mortality with statin-based treatment as eGFR declines is established, and in fact, there is little evidence of benefit in individuals on maintenance dialysis [118]. This is likely due to the physiological and metabolic disturbances that develop secondary to the advanced kidney disease, leading to a cardiovascular pathophysiology distinct from the “traditional” one. Accordingly, cardiovascular events in individuals on maintenance dialysis are largely driven by non-atherosclerotic events, such as heart failure, arrhythmias, and hemorrhagic stroke [119]. Therefore, the usefulness of lipid medication should be carefully assessed for these individuals.

Concluding Remarks

Clinical and other research have established a strong link between lipoprotein metabolism and cardiovascular disease. At the same time, the sequence of events from the first signs of albuminuria, followed by persistent proteinuria and culminating in cardiovascular death and/or kidney failure, has been described in individuals with diabetes. Therefore, it is plausible that the interplay between dyslipidemia and diabetic kidney disease may form the basis for the excess mortality in diabetes.

Figure 15.4 depicts a multivariable summary of the FinnDiane cohort. This data-driven visualization is essentially the clinical picture of Finnish individuals with

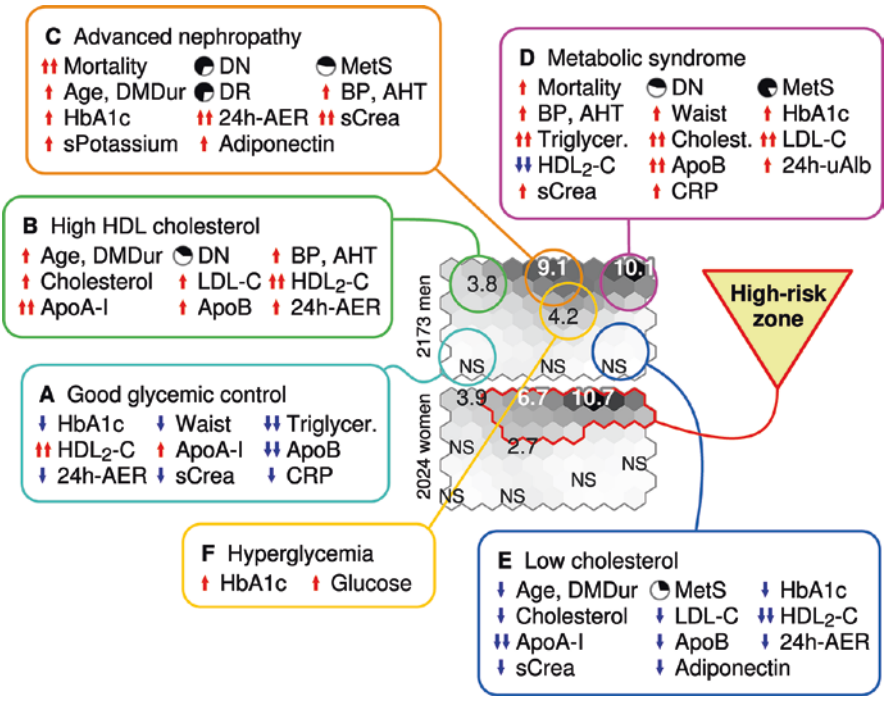

Fig. 15.4 Exploratory analysis of 4197 individuals with type 1 diabetes by a self-organizing map (SOM) of biochemical measures [15]. The SOM algorithm produces a two-dimensional layout of the individuals based on their biochemical profiles: the distance on the map is proportional to the similarity of the biochemical profiles, which means that a specific region on the map contains individuals with mutually similar metabolic features, whereas the individuals on opposite sides are metabolically different. The map itself is just the layout, but this layout can be colored with respect to different clinical traits, or subgroups of individuals. Here, men and women were visualized separately (although both were analyzed with the same map). The grayscale on the two colorings was determined based on the vitality status of the individuals during an average of 8-year follow-up. The numbers on the map depict the relative mortality rate compared with the background population of similar age. The results show that individuals with the characteristics of the metabolic syndrome (Phenotype D) and individuals with advanced nephropathy (Phenotype C) are at high risk of premature death. Individuals with favorable lipids show lower mortality compared to the metabolic syndrome phenotype despite a higher age and similar prevalence of diabetic nephropathy (Phenotype B vs. D). *AER* albumin excretion rate, *AHT* anti-hypertensive treatment, *BP* blood pressure, *CRP* C-reactive protein, *DMDur* type 1 diabetes duration, *DN* diabetic nephropathy (kidney disease), *DR* diabetic retinopathy, *MetS* metabolic syndrome, *sCrea* serum creatinine

long-standing type 1 diabetes, and the six model phenotypes from A to F could be real individuals walking into the clinic for a check-up. Advanced kidney disease is associated with the highest absolute mortality (Phenotype C), but the age-adjusted risk for premature death is, in fact, equally high in younger individuals with the dyslipidemic, metabolic syndrome characteristics (Phenotypes D). In contrast, individuals with the opposite pattern have an overall favorable metabolic profile without any excess mortality (Phenotype A). How much of the differences between A and D are due to an individual's life choices and the quality of care, and how much of it comes from genetic heterogeneity? At this point, our knowledge is insufficient to answer this question. Nevertheless, Phenotype A and the lipid profile therein may represent an ideal treatment target that protects from long-term complications, and any means from lifestyle interventions to new pharmacological agents should be employed to achieve it.

Observational data support the connection between micro- and macrovascular complications, and lipids are the prime candidates for the connecting agents. However, specific trials on dyslipidemia as a predictor or causative factor to the onset of diabetic kidney disease are sparse. In particular, most lipid drug trials have focused on late vascular events such as myocardial infarctions, and at that point, it may be too late to investigate diabetic kidney disease. Primary prevention is most effective before significant atherosclerotic lesions develop, and in this respect, the potential links between serum lipid profile and early stages of diabetic kidney disease—as a proxy for a vulnerable vascular phenotype—should be investigated more thoroughly.

Finally, individuals with diabetic kidney disease may be more vulnerable to the effects of dyslipidemia than the general population. For instance, the commonly used threshold for triglycerides may be too high for those with type 1 diabetes since the majority of these individuals are below the recommended limit while still having a high incidence of cardiovascular disease and microvascular complications. It is also important to remember that cholesterol in the modern world is typically twice as high as in hunter-gatherer communities, regardless of diabetes status. Tighter lipidemic control is therefore warranted in situations of impaired glycemic control to avoid a double hit on vascular health.

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Chapter 16
Lipids and Diabetic Retinopathy

Alicia J. Jenkins

Introduction

Diabetes is pandemic. Globally, an estimated 537 million people have diabetes, about 10% of the adult population, with the incidence and prevalence of both common types of diabetes, Type 1 and Type 2 diabetes, increasing [1]. The epidemiology of diabetes is discussed in more detail in the chapter herein by Drs Bloomgarden and Handelsman. With chronic forms of diabetes comes the risk of microvascular complications, including diabetic retinopathy (DR). People who develop DR are also at higher risk of the other microvascular complications of diabetic nephropathy and diabetic (peripheral and autonomic) neuropathy and of the macrovascular complications of cardiovascular, cerebrovascular, and peripheral vascular disease, likely related to common risk factors [2]. Some groups, such as indigenous peoples, are at higher risk not only of diabetes, but also of its chronic complications than their non-indigenous peers [3, 4]. The incidence and prevalence of Type 2 diabetes in youth, which is also common in indigenous peoples, are also increasing substantially [1, 5] and are associated with even higher rates of long-term complications, including retinopathy, than people with similar duration of Type 1 diabetes [6–8]. Potential reasons for this may relate to higher rates of risk factors in youth with Type 2 diabetes, including obesity, dyslipidemia, and hypertension, and often lower engagement with the healthcare system [5, 6].

DR, a serious and most-feared complication of diabetes [9], is the third leading cause of vision loss globally and the commonest cause of adult-onset blindness [10, 11]. The risk of blindness for a person with diabetes is 25-fold that of a person without diabetes, yet with appropriate management over 90% of vision loss due to DR

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is preventable [12]. Over the last century, the rates of DR, and in particular of sight-threatening diabetic retinopathy (STDR), are generally declining, at least in advantaged areas, due to better diabetes care [13]. However, due to the greatly increased prevalence of diabetes, 80% of whom live in disadvantaged regions, the number of people at risk of and with DR is, and will likely, remain high [1]. Multiple aspects of care including regular eye screening, systemic risk factor control, and ocular treatments for late-stage DR are needed to prevent and treat the personally and socioeconomically costly consequences of DR.

Epidemiology of Diabetic Retinopathy

The first and largest major attempt to estimate the global prevalence of DR was published in 2012 [14]. A systematic review and pooled analysis of individual patient data from 22,896 people with diabetes (52% female, 44% Caucasian), mean age 58.1 years (range 3–97), median diabetes duration 7.9 years (interquartile range [IQR] 3–16), and median HbA1c 8.0% (6.7–9.9%) (50–85 mmol/mol) from 35 studies (1980–2008) were conducted. Another 23 studies identified in the systematic review chose not to participate. The goal was to estimate the global prevalence and major systemic risk factors for DR. DR status was ascertained by grading of retinal photographs, with the endpoints being any DR, proliferative DR (PDR), sight-threatening DR (STDR), and diabetic macular edema (DME). STDR includes PDR and sight-threatening DME. Pooled prevalence estimates were age-standardized to the 2010 world diabetes population aged 20–79 years. Globally, there was an estimated 93 million people with DR, 17 million with PDR, 21 million with DME, and 28 million with STDR. The overall prevalences were any DR 34.6% (95% CI 34.5–34.8); PDR 6.96% (6.87–7.04); DME 6.81% (6.74–6.89); STDR 10.2% (10.1–10.3). Their data supported a decline in DR prevalence post-2000. The prevalence of any DR and of all types of DR increased with longer diabetes duration, higher HbA1c, and blood pressure. Total cholesterol was a risk factor for DME only. The prevalence of all DR types was higher in people with Type 1 diabetes than in those with Type 2 diabetes. People with over 20 years of Type 1 diabetes were 2.7 times more likely to have any DR (relative risk (RR) 2.69 [96% CI 2.47–2.93]), 15 times more likely to have PDR (15.3 [11.3–20.8]), 5 times more likely to have DME (4.83 [3.71–6.30]), and 8.7 times more likely to have STDR (8.69 vs. the reference group of those with Type 2 diabetes for less than 10 years) [14]. Study strengths include the large sample size, inclusion of studies from diverse geographical and ethnic areas, consideration of major risk factors, and the use of retinal photos to ascertain DR status. Study limitations include studies that were not included and lack of data from many regions, including the high diabetes incidence regions of Africa, the Middle East, and South America. While retinal imaging was used in all subjects, different methods were used, including types of retinal cameras and number of photos taken (hence area of retinal coverage). Study heterogeneity may also impact data accuracy (but not precision). There may be some misclassification as to diabetes type, due to different ascertainment methods (e.g., self-report, blood tests, based on age of diabetes onset) [14].

A second systematic review of 59 population-based studies (up to the year 2020) and their meta-analysis published in 2021 estimated the global burden of DR and predicted the burden till 2045 [15]. Projections of DR, STDR, and clinically significant DME (CSDME) burden were based on population data from the IDF Atlas 2019. For people with diabetes, the global prevalence of DR was 22.27% (95% CI 19.73–25.03%), 6.17% (5.43–6.98%) for STDR, and 4.07% (3.42–4.82%) for CSDME. In 2020, the number of adults worldwide with DR, STDR, and CSDME was estimated to be 103.12 million, 28.54 million, and 18.83 million, respectively. By 2045, these numbers are projected to increase to 160.50 million, 44.82 million, and 28.61 million, respectively. This ongoing high burden of DR through 2045 was predicted to disproportionately affect people in the Middle East, North Africa, and the Western Pacific regions [15]. These are already areas of high rates of diabetes, predominantly Type 2 diabetes, and some areas in these regions already struggle to provide equitable access to comprehensive diabetes care, screening, risk factor control, and ocular treatments for late-stage DR. The negative impacts of the COVID pandemic on healthcare systems, on communities and on individuals may increase the challenges related to DR prevention and care in some regions. Much more public health funding, risk factor and eye screening, prevention, and treatment are needed to reduce the personal and societal burden of DR.

Diabetic Retinopathy in Pre-diabetes

There is a continuum of glucose levels from normal to pre-diabetes to diabetes, with major diabetes-related specialty organizations, such as the American Diabetes Association, deciding diagnostic cut-points for the presence of diabetes mellitus and for pre-diabetes and normoglycemia. Although hyperglycemia is essential for the development of DR, pre-diabetes is usually thought not to be associated with risk of diabetic microvascular complications, including DR, while increased risk of macrovascular complications in people with pre-diabetes is recognized. Indeed, over the years, the cut-points of measures of glycemia, predominantly glucose levels, for diabetes diagnosis have been at least partially informed by the levels of glucose at which DR develops.

A recent systematic review (published 2022) including predominantly (79%) population-based studies until 2020 included 24 studies and 8759 people with pre-diabetes. DR prevalence rates were median 7.1% (IQR 2.4–9.7%) and range 0.3–14.1%. As the studies included both people with pre-diabetes and normoglycemic subjects, the median DR prevalence in pre-diabetes was 6.6% (IQR 1.9–9.8%) vs. 3.2% (IQR 0.3–7.3%) in those with normal glucose tolerance. The authors recognized that differences in diabetes screening methods, retinopathy grading protocols, and study populations would have lowered the certainty of evidence by the GRADE criteria [16]. Another systematic review based on nine community-based cross-sectional studies with 14,751 adult participants, including 3847 (26.1%) with pre-diabetes, also showed that pre-diabetes was associated with higher DR prevalence compared to normoglycemia [odds ratio (OR): 1.55, 95% CI: 1.10–2.20, $p = 0.01$, $I^2 = 34\%$]. A sensitivity analysis by excluding one study at a time showed

consistency of results (OR: 1.35–1.73, p all<0.05) and subgroup analyses showed that study country, definition of pre-diabetes, sample size, mean participant age, or univariate or multivariate statistical analyses were unlikely to impact the association (all $p > 0.05$) [17]. Ideally, prospective cohort studies are needed to validate these findings. Nevertheless, these data support that DR can occur in pre-diabetes, and that even low-level hyperglycemia and the related milieu are harmful to the retina. This supports consideration of further alternation of the diagnostic cut-points for diabetes to lower levels.

In a 2011 publication [18], data from 44,623 20–79 year old adults from nine studies in five countries was reported. Colaguiri et al. related retinal photo diagnosed DR (graded as moderate or severe) with glycemia (fasting plasma glucose in n = 41,411); 2-h post-oral glucose load plasma glucose (n = 21,334), and HbA1c (n = 28,010). A curvilinear relationship between DR and fasting glucose and HbA1c was identified. DR prevalence was low for fasting plasma glucose levels <6.0 mmol/L (108 mg/dL) and HbA1c <6.0% (42 mmol/mol) and increased above these levels. Suggested cut-points at which DR could be present, hence potential diabetes diagnosis points, were estimated using two different statistical techniques. First, by dividing the group into 20 equal-sized groups based on the three glycemic measures, DR was noted over the ranges of 6.4–6.8 mmol/L (115–122 mg/dL) for fasting plasma glucose; 9.8–10.6 mmol/L (176–191 mg/dL) for 2-h post-glucose load plasma glucose; and 6.3–6.7% (45–50 mmol/mol) for HbA1c. Thresholds for DR estimated from receiver-operating characteristic (ROC) curve analyses were: 6.6 mmol/L (119 mg/dL) for fasting plasma glucose; 13.0 mmol/L (234 mg/dL) for 2-h post-glucose load plasma glucose; and 6.4% (46 mmol/mol) for HbA1c. The wider range of 2-h post-glucose load and costs of an oral glucose tolerance test strengthens the case for using diagnostic tools of fasting plasma glucose and HbA1c. These data support a diabetes diagnostic level of fasting plasma glucose of 6.5 mmol/L (117 mg/dL), which is not in clinical use, and for a HbA1c of ≥6.5% (48 mmol/mol), [18] which is in clinical use [19].

Development and Staging of Diabetic Retinopathy

DR develops slowly and painlessly over years, even decades, and is clinically silent until late-stage DR or complications thereof, such as a retinal bleed or retinal detachment, results in impaired vision. Because of this, screening is key for the early detection of DR, which also signals risk of, and perhaps presence of, other chronic complications. As Type 2 diabetes can be asymptomatic or has symptoms, such as tiredness, that are often misattributed, such as due to aging, diabetes can be present for years pre-diagnosis, hence DR may be present even at Type 2 diabetes diagnosis. In people with Type 1 diabetes DR does not usually become evident until at least 5 years post diabetes onset [20, 21]. As gestational diabetes usually resolves

within a few weeks to months of the end of pregnancy [20], and is of months, not years duration, DR does not usually develop in previously normoglycemic women who develop diabetes during their pregnancy. However, gestational diabetes may actually be previously undiagnosed Type 2 diabetes or pre-diabetes may have been present before pregnancy, and as reviewed above, DR may occur in "pre-diabetes" that is based on current diagnostic cut-points [16, 17, 19].

Types and Assessment of Diabetic Retinopathy

DR is characterized by changes in both retinal blood vessels and nerves, which are initially not detectable by routine clinical examination and imaging. The processes underlying DR include inflammation, oxidative stress, thrombosis, and disturbed angiogenesis, with the latter including retinal overexpression of pro-angiogenic Vascular Endothelial Growth Factor (VEGF) and suppression of anti-angiogenic Pigment Epithelium Derived Growth Factor (PEDF). Implicated cell signaling processes include increased Protein Kinase C (PKC), and the Wnt pathway and β-catenin. These pathways are thought to be driven by traditional risk factors such as hyperglycemia, hypertension, dyslipidemia, smoking, and obesity, all of which are also pro-inflammatory states [21, 22] (Fig. 16.1).

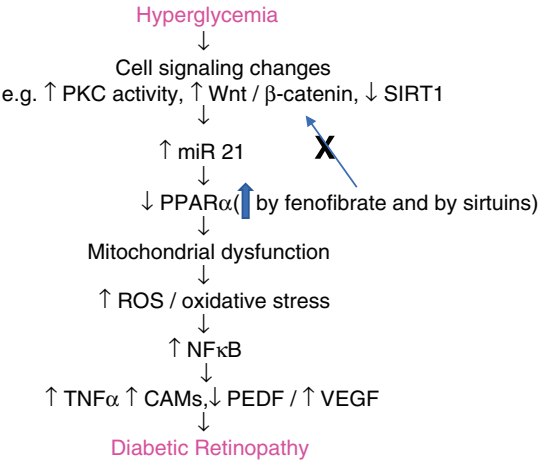

Fig. 16.1 Schema of some pathogenic pathways of DR. *CAMs* cell adhesion molecules, *miR* microRNA, *PEDF* pigment epithelium derived growth factor, *PKC* protein kinase C, *Wnt* Wingless-related integration site, *PPAR* peroxisome proliferator-activated receptor, *ROS* reactive oxygen species, *SIRT1* sirtuin 1, *TNF* tumor necrosis factor, *VEGF* vascular endothelial growth factor, ***X*** blocked, ↑ induced. (Original figure by Dr. A. Jenkins)

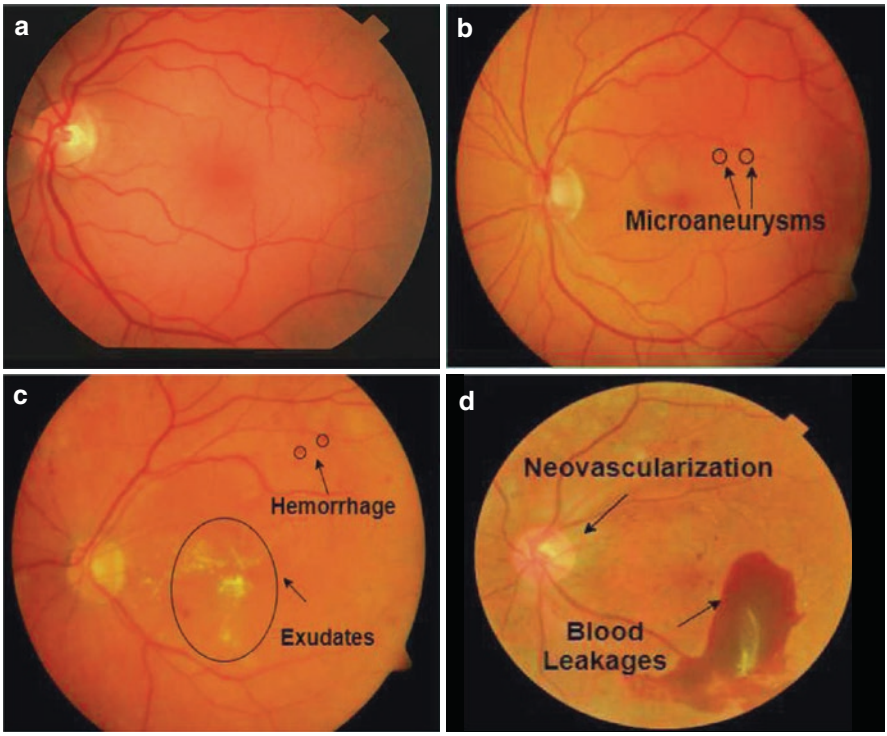

Fig. 16.2 Examples of different classes of diabetic retinopathy images. (**a**) Normal; (**b**) Mild DR; (**c**) Moderate DR; (**d**) PDR. (Reproduced with permission from Deepa et al., https://doi.org/10.1007/s13246-022-01129-z)

The clinically visible features of DR by which it is staged and monitored clinically include micro-aneurysms, exudates (hard and soft), hemorrhages, macular edema, and late-stage neovascularization (new blood vessel formation) [21–24]. Soft exudates are retinal infarcts, and hard exudates are extravasated lipids. Figure 16.2 shows images of the stages of DR. Usually lesions will progress sequentially from mild to moderate to severe non-proliferative DR (NPDR) then to PDR. Early-stage DR lesions may regress, particularly with improvement in glucose control, although as shown in the Diabetes Complications and Control Trial (DCCT), there may be an initial worsening of DR prior to improvement [25, 26]. DME may occur at any stage of DR, even in children with Type 1 diabetes [27] and ideally is urgently assessed and treated with input by an ophthalmologist as it can lead to vision loss.

DR status can be assessed by clinical examination by a clinician using a handheld or slit lamp ophthalmoscope or by grading of retinal photos [28], which used to be paper-photo or slide-based, and but are now usually digital. Other diagnostic modalities for DR includes fundus fluorescein angiography, including ultra-widefield fluorescein angiography and OCT, and OCT angiography. The gold

standard for detecting and monitoring macular edema is now OCT [29]. OCT non-invasively visualizes the layers of the retina and is particularly good for detecting and monitoring macular edema, which can occur at any stage of DR and any diabetes duration [27].

Predominantly for research purposes, rather than for clinical practice, more detailed numerical grading scales for DR are used, such as the scale developed by the Early Treatment Diabetic Retinopathy Study (ETDRS), which are also based on the presence or absence of micro-aneurysms, exudates, neovascularization, and macular edema. OCT metrics also include quantitative measures such as macular area and volume [21].

It is preferable that both eyes be evaluated as DR can be asymmetrical. Some minor variation is not uncommon, but major differences should flag further clinical consideration and investigations. A recent systematic review of 84 original or review articles or case reports from 1965 to 2020 reported that 5–10% of diabetes patients with PDR have asymmetric DR, defined as PDR in one eye and no DR, NPDR, background DR, or pre-proliferative DR in the other eye that persists for at least 2 years [30]. Causes may relate to vascular lesions, such as carotid obstruction; inflammation, such as uveitis; degenerative disorders such as retinal detachment; operations, such as cataract surgery or vitrectomy, or other eye conditions such as optic atrophy or glaucoma. Many of these ocular and systemic conditions require specific treatment, so asymmetric DR merits further investigation, and consideration of referral to an ophthalmologist.

Subclinical Retinal Changes and Diabetic Retinopathy

Changes in retinal vessel caliber (central retinal arteriole narrowing and central retinal venule dilation) and retinal vessel geometry have been suggested as early changes in DR. Clinical research studies based on retinal photos acquired for DR screening have shown associations with, and predictive power of, these subtle retinal changes for DR and for diabetic nephropathy, including in people with Type 1 diabetes and with Type 2 diabetes [31–39]. As yet these measures are not ready for clinical use as normal ranges and validated diagnostic levels for risk of future DR status are not available.

Eye Screening

Regular eye screening is recommended for all people with diabetes, with the suggested frequency of screening previously having been annually, with shorter intervals recommended if there is DR. This regular screening enables the early detection of diabetes-related eye damage, in particular any STDR, the treatment of systemic risk factors and the detection and treatment of any related complications such as

diabetic kidney disease and cardiovascular disease. Eye screening is usually recommended from Type 2 diabetes diagnosis and about 5 years after Type 1 diabetes onset [19, 20]. As pregnancy can accelerate DR, eye screening prior to a planned pregnancy or early in the pregnancy is recommended [19, 20, 40] and any significant DR treated by an ophthalmologist with ocular therapies (e.g., retinal laser, intraocular anti-VEGF, or corticosteroid injections) to reduce the risk of vision loss.

Screening is usually by digital retinal imaging and should include assessment of visual acuity, intraocular pressure, and cataracts, with glaucoma and cataracts being more common in people with diabetes. Retinal photos can be graded by ophthalmologists, optometrists, trained retinal image graders, and increasingly by artificial intelligence [41–43]. The use of artificial intelligence may be particularly helpful in remote or disadvantaged regions, and in some ethnic groups, particularly if the software is available on local computers rather than in "the cloud" due to internet connectivity or cultural issues [43].

Traditional and Novel Risk Factors for Diabetic Retinopathy

Traditional risk factors for DR, and also for other microvascular and macrovascular diabetes complications, are increasing age, diabetes duration, poor glycemic control (usually reflected by high HbA1c levels), hypertension, smoking, and dyslipidemia [19, 21, 44]. Many of these risk factors for DR and its other chronic complications are impacted by modifiable lifestyle choices, such as poor diet, lack of physical activity, and suboptimal mental health [45–48], which are unfortunately common in high-risk groups, such as Indigenous Australians, with diabetes [46, 47]. Mental health conditions such as anxiety, depression, and diabetes distress are also important considerations [46–48] that are often not well addressed in the primary and secondary prevention of diabetes and its complications.

Novel risk factors include inflammation, a pro-thrombotic tendency, endothelial dysfunction, imbalance of pro- and anti-angiogenic growth factors, oxidative stress, Advanced Glycation End Products (AGEs), genetic risk factors, and qualitative changes in lipoproteins and other disturbances in lipoprotein metabolism [21].

As well as hyperglycemia being a major risk factor for, and key driver of, DR, improving glycemia, particularly if glycemic control is fair or poor, usually substantially reduces DR onset and progression in both Type 1 and Type 2 diabetes [25, 49]. Furthermore, metabolic memory lasting for many years (1–2 decades) exists for glycemia and DR [50–52]. While not as strong or consistent as the links between glucose and DR and between lipids and cardiovascular disease, many epidemiologic studies show associations and predictive power of traditional lipid profiles for DR [53]. There is also evidence of metabolic memory for lipids and lipid-lowering drugs [54], including for DR [55], likely modulated by epigenetics and effects on sirtuins [55, 56], which are an intracellular family of signaling proteins involved in metabolic regulation. Sirtuin 1 (SIRT1), a class III histone deacetylase is a multifunctional enzyme with key regulatory roles in processes implicated in DR and

other diabetes complications, including inflammation (via suppression of NFκB signaling), cellular metabolism, DNA repair, cell stress responses, and survival. SIRT1 has also been shown to induce resistance to high glucose induced cellular metabolic memory and to be activated by the PPARα agonist fenofibrate [55, 56].

Lipid Variability and Detailed Lipid Analysis

Traditional lipid levels (total cholesterol, triglycerides, LDL-C, HDL-C) are low-cost, widely available, part of clinical care guidelines [19], but particularly if measured just once, may be considered a rather blunt instrument for association studies of lipids with DR and for relating responses of DR to lipid treatments. Multiple lipid measures over long periods of time are desirable as lipid levels may fluctuate related to changes in diet, exercise, smoking, glycemic control, age, weight, hormonal status, and medications. As discussed in another chapter herein, lipid and lipoprotein variability, like glycemic variability, may be associated with increased risk of diabetic microvascular and macrovascular complications [57–60]. For example, in a study of 25,186 diabetes patients (mean age 63 years, 50% male) HbA1c and lipid variability were significant predictors of eye and kidney complications, of cardiovascular disease and of all-cause mortality. Inflammation is implicated as a mediator. Further studies including pre- and post-lipid drug interventions, detailed lipid measures, and DR assessments are of interest.

Novel lipoprotein related measures may provide more insight. Such measures are currently research tools such as remnant lipoprotein levels, NMR determined lipoprotein subclass profiles, and levels of modified lipoproteins, such as oxidized LDL or lipoprotein-immune complexes.

In a cross-sectional study of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Control (DCCT/EDIC) cohort, 988 adults (440 women, 548 men) with Type 1 diabetes were evaluated for associations between DR and traditional and novel risk factors, in particular lipoprotein related factors [61]. DR was characterized by ETDRS scores, hard exudate scores, and ETDRS scores minus the hard exudate component. Lipoproteins were characterized by traditional lipids, nuclear magnetic resonance determined lipoprotein subclass profile (NMR-LSP), apoA1, apoB, lipoprotein(a), and the susceptibility of isolated LDL to copper-induced oxidation. Data were analyzed with and without adjustment for risk factors: age, sex, diabetes duration, HbA1c, kidney function, hypertension, BMI, waist-hip ratio, smoking, and DCCT treatment group. The severity of DR was positively associated with triglycerides (both sexes) and negatively associated with HDL-C (men and both sexes). NMR-LSP provided more detail than traditional lipid levels alone. DR was positively associated with small and medium VLDL and negatively with VLDL size. In men only, DR was positively associated with small LDL, LDL particle concentration, apoB concentration, and small HDL and was negatively associated with large LDL, LDL size, large HDL, and HDL size. No associations were found with apoA1, Lp(a), or susceptibility of LDL to oxidation. All three

measures of DR revealed the same associations [61]. Not all studies are positive. In a cross-sectional study in 921 adults with Type 2 diabetes from the Multi-Ethnic Study of Atherosclerosis (MESA) study, DR was assessed from retinal photographs and NMR-LSP determined. After controlling for age, race/ethnicity, study center, and diabetes, and vascular risk factors, no consistent patterns of associations between DR and detailed NMR-defined lipoprotein particle concentrations and subclasses were evident [62]. Longitudinal studies, including in relationship to lipid drug treatments, are of interest.

In another Type 1 diabetes cohort study, the FinnDiane study, associations between highly atherogenic remnant cholesterol levels and STDR and diabetic nephropathy were determined in 5150 subjects with diabetes [63]. Remnant cholesterol was calculated as total cholesterol–LDL-C–HDL-C and variability as the coefficient of variation. Diabetic nephropathy category was based on consensus albuminuria reference limits and progression status confirmed from medical records. STDR was defined by need for retinal laser photocoagulation therapy. In addition, in a substudy DR was graded using the ETDRS scale. Median (IQR) follow-up time was 8.0 (4.9–13.7) years for nephropathy and 14.3 (10.4–16.3) years for DR. Remnant cholesterol (mmol/L) was higher with increasing baseline nephropathy category ($p < 0.001$) and was significantly higher in nephropathy progressors vs. non-progressors (0.55 [0.40–0.85] vs. 0.41 [0.32–0.55]), $p < 0.001$. In a Cox regression analysis, remnant cholesterol predicted DN progression, independently of diabetes duration, sex, HbA1c, systolic blood pressure, smoking, BMI, estimated glucose disposal rate, and estimated glomerular filtration rate (eGFR) (hazard ratio (HR): 1.51 [1.27–1.79]). Remnant cholesterol was also higher in those who did vs. did not develop STDR (0.47 [0.36–0.66]) (0.40 [0.32–0.53]), $p < 0.001$, and the concentration increased stepwise with increasing DR severity ($p < 0.001$). The HR for STDR for remnant cholesterol was 1.52 (1 [0.26–1.83]). Unlike nephropathy, remnant cholesterol variability was not independently associated with the DR outcomes. Larger studies with assessment in relationship to other measures of DR such as ETDRS scores and OCT metrics and at different times of follow-up are of interest. As with any association study, even if associations are found, it remains to be elucidated whether the associations are causal or not.

There are also clinical trials and observational data that suggest that lipid drugs may retard DR, however most trials have DR as a secondary or tertiary endpoint [53]. There are many factors to consider in evaluating the potential role of lipids and of lipid drugs in DR (discussed below).

Considerations in Evaluating Roles of Lipids and Lipid Drugs in Diabetic Retinopathy

Table 16.1 lists factors which make it challenging to discern the relative importance of lipids and lipid-lowering drugs in the development and progression of DR. The selected study population, its size, and duration of follow-up, the means of defining diabetes type, and DR status are important. As with atherosclerosis, the levels of

Table 16.1 Challenges to elucidating relationships between lipids and lipid drug effects and DR

• Interactions between lipids and other DR risk factors
• Metabolic memory for traditional risk factors and some related drugs
• Slow development of DR
• Study selection bias, inadequate study size, diabetes duration, or length of follow-up
• Differences in definitions of DR
• Differences in means of DR assessment
• Different associations between lipids and various stages of DR and its treatment
• Lipid measures used: choice of lipoprotein related measures
• Circulating lipids may not reflect intra-retinal lipids diabetes, endpoints, diagnostic criteria
• Direct and indirect effects of lipid drugs
• Confounding effects, e.g., unmeasured genetics, epigenetics

lipoprotein related factors measured in blood may not reflect well the quantity and quality of lipids and lipoproteins in the tissue being damaged, in this case the neurovascular retina with its specialized blood–retina barrier. This is an important issue given the toxicity of modified lipoproteins such as LDL modified by glycation and/or oxidation to retinal cells [64–68] and the leakiness of the retinal vasculature in diabetes and DR. The effects of modified lipoproteins and related lipoprotein-immune complexes are discussed in more detail in other chapters in this book. It is likely, but not extensively studied, that effects on macrovascular and microvascular cells are similar.

Also of importance to consider in evaluating the role of lipid drugs in modulating DR are the direct, and indirect or pleiotropic effects of lipid-lowering drugs, summarized in Table 16.2. The pleiotropic effects are often, but not always, common to most lipid drug classes, in spite of different mechanisms of action and predominant lipid targets [69–73]. Where possible these factors should be considered in study design, conduct, and the interpretation of study results.

Associations between lipid levels and DR have been reported since the 1950s, with recognition of the association between lipid levels and vascular complications, including retinal hard exudates [74], yet clinical study results provide contrasting results, which may be partly related to factors such as those listed in Table 16.1. Some representative cross-sectional and longitudinal studies and meta-analyses are summarized in Table 16.3 (originally published in [53]). Most studies are based on readily available traditional lipid levels, and some associations between lipid levels and DR are observed.

Another way to evaluate the relationships between lipid levels and DR is Mendelian randomization study. In these ideally very large studies, the impact of genes for high or low lipid levels is related to the condition of interest [75]. An international consortium pooled genome-wide association studies (GWAS) summary statistics from 18 studies in people with Type 2 diabetes and performed a meta-analysis. DR was assessed clinically or by modified ETDRS grading of retinal photos, and severe DR was defined as severe NPDR and/or PDR. They evaluated associations between 157 lipid (total cholesterol, triglycerides, LDL, and HDL) associated SNPs and the presence of any DR ($n = 2969$ cases and 4096 controls) and

Table 16.2 Potential lipid drug effects which may modulate DR

Anti-inflammatory
Anti-oxidant
Anti-thrombotic/pro-fibrinolytic
Anti-platelet
Anti-angiogenic effects
↓ VEGF
↑ PEDF
Vasodilatory
↑ Nitric oxide synthesis
Anti-apoptotic effects, including of retinal microvascular cells
Increase in endothelial progenitor cells
Immunomodulatory effects
Cell signaling effects
PPARα activation (fibrates)
Wnt pathway inhibitor
AGE-RAGE/VEGF inhibitor
SIRT1 activation (fibrates)
Telomere related
Telomerase activation
Retarding telomere shortening
Genetic effects
Epigenetic effects, e.g., microRNA21
Neuroprotective effects, e.g., fenofibrate
Increased insulin sensitivity (especially in liver)
Effects on diabetes incidence and/or glycemia
Worsening: statins, nicotinic acid
Improvement: resins
Research CETP inhibitors and rHDL improve glycemia

the presence of severe DR (n = 1277 cases and 3980 controls). Controls were Type 2 diabetic subjects with no DR. There was no statistically significant change in odds ratios of having any DR or severe DR for any of the lipid SNPs evaluated. However, the study had limited statistical power to detect odds ratios less than 1.23 per SD in the genetically induced increase in plasma lipid levels. More modest effect sizes would have been missed. Larger studies with a wider range of DR endpoints and SNPs are of interest.

Associations between traditional lipid levels, even if present and strong, do not necessarily imply causation. Basic science studies, such as retinal cell cultures, are supportive of retinal damage, particularly by modified lipoproteins [64–68], which exist in low levels in blood and are difficult to quantify. Basic science studies can also be very helpful in elucidating mechanisms of lipid damage and protection and in pre-clinical drug testing [70, 75–84], but results do not always translate to the

Table 16.3 Clinical studies demonstrating the diversity of association between circulating traditional lipid profiles and diabetic retinopathy

Study	Diabetes type	Type of diabetic retinopathy	*n*	Blood lipids evaluated	Results
Cross-sectional studies					
Brown et al. [106]	T1D + T2D	Exudative DR	31	TC, TG	Increased serum TG in participants with vs. without DR
Sacks et al. [96]	T1D + T2D	DR (PDR, moderate or severe DME) or ETDRS scale ≥20	2535	TC, TG, LDL-C, HDL-C	Higher TC and lower HDL with vs. without DR, but not once adjusted for hypertension and HbA1c
Raman et al. [107] (SN-DREAMS)	T2D	CSME, non-CSME	1414	TC, TG, LDL-C, HDL-C	High serum LDL-C, non-HDL-C, and HDL-C ratio related to non-CSME; High serum TC related to CSME
Benarous et al. [108]	T1D + T2D	NPDR (mild, moderate, severe), PDR, DME (mild, moderate, CSME)	500	TC, TG, LDL-C, HDL-C, non-HDL-C	Serum lipids independently associated with CSME only; no associations with DR, mild or moderate DME, or macular thickness
Wong et al. [109] (MESA)	T1D + T2D	DR, DME, CSME, STDR	778	TC, TG, LDL-C, HDL-C	No associations with DR, DME, or CSME
Cetin et al. [110]	Not specified	NPDR, PDR, DME	199	TC, TG, LDL-C, VLDL-C HDL-C	Serum lipid levels not associated with severity of DR or DME
Tan et al. [111] (SEED)	T2D	DR, NPDR (severe), DME, CSMO, STDR	2877	TC, LDL-C	Higher TC and LDL-C associated with lower risk of any type of DR
Guerci et al. [112]	T1D	DR, NPDR, PDR	341	Lp(a)	Higher Lp(a) levels associated with more severe DR; Lp(a) > 300 mg/L (30 mg/dL) associated with higher PDR

(continued)

Table 16.3 (continued)

Study	Diabetes type	Type of diabetic retinopathy	*n*	Blood lipids evaluated	Results
Longitudinal studies					
Dodson and Gibson [113] (7 years)	T2D Hypertension	DR with exudative maculopathy	52	TC, TG, LDL-C, VLDL-C, HDL-C, HDL_2	Higher HDL_2 subfraction with exudative maculopathy
Klein et al. [114] (WESDR, 30 years)	T1D	PDR, DME	903	TC, HDL-C	Serum lipids not associated with incidence of PDR or DME, nor was statin use
Chew et al. [115] (ETDRS, 5 years)	T1D + T2D	Hard exudate	2709	TC, TG, LDL-C, VLDL-C HDL-C	High TC, TG, and LDL-C associated with higher risk of hard exudate
Miljanovic et al. [116] (DCCT, 6.5 years)	T1D	CSME, hard exudate, DR progression, PDR	1441	TC, TC, LDL-C, HDL-C, TC/HDL	Higher serum lipids associated with higher risk of CSME and retinal hard exudate; no lipids associated with DR progression or development of PDR after adjustment for Hb1Ac
Klein et al. [117] (WESDR substudy, 5 years)	T1D	DR severity, PDR, hard exudate incidence and progression, DME	251	TC/HDL	Univariate analyses: TC/HDL associated with all incident retinal lesions; multivariate analyses: no significant association
Morton et al. [118] (ADVANCE, 5 years)	T2D	New or worsening DR	11,400	Baseline HDL-C	HDL-C levels not related to DR
Lloyd et al. [119] (EDC, 2 years)	T1D	DR progression (Airlie House classification), PDR	657	TC, TG, LDL-C, HDL-C	TG and LDL-C predictive of DR progression and development of PDR
Singh et al. [120] (DiaGene, 6.97 years)	T2D	NPDR, PDR	1886	Plasma Lp(a) levels, two SNPs modulating Lp(a) levels	No association between Lp(a) levels or SNPs with incident or prevalent DR

(continued)

Table 16.3 (continued)

Study	Diabetes type	Type of diabetic retinopathy	*n*	Blood lipids evaluated	Results
Meta-analyses					
Yau et al. [14] (META-EYE)	T1D + T2D	DR, PDR, STDR, DME, CSME	22,896	TC	Higher TC associated with higher prevalence of DME
Zhou et al. [121]	T1D + T2D	DR	4366	TC, TG, LDL-C, HDL-C	TC, TG, HDL-C: no difference between DR vs. no DR LDL-C: higher in DR vs. no DR

CSME clinically significant macular edema, *DR* diabetic retinopathy, *DME* diabetic macular edema, *EDC* (Pittsburgh) Epidemiology of Diabetes Complications, *HDL-C* HDL-cholesterol, *LDL-C* LDL-cholesterol, *Lp(a)* lipoprotein(a), *MESA* Multi-ethnic Study of Atherosclerosis, *META-EYE* Meta-Analysis for Eye Disease, *NPDR* non-proliferative diabetic retinopathy, *SEED* Singapore Epidemiology of Eye Diseases, *SN-DREAMS* Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study, T1D type 1 diabetes, *T2D* type 2 diabetes, *TC* total cholesterol, *WESDR* Wisconsin Epidemiologic Study of Diabetic Retinopathy

human setting. Also challenging to assess is the role of intraocular lipids, including those extravasated into the retina and also those produced and metabolized in the retina [53].

Retinal Lipid Metabolism

The retina is a highly metabolically active organ with high cell turnover and energy demands, for which it obtains some cholesterol from the circulation and predominantly by local biosynthesis. All retinal cells contain enzymes for cholesterol biosynthesis, with the highest levels in Muller cells and photoreceptor inner segments. LDL receptors and scavenger receptors in the retinal pigment epithelium (RPE), part of the blood–retinal barrier, control the uptake of lipids from the circulation, which are then transferred to the neural retina by ATP-binding membrane cassette transporters. Lipids are removed from the retina by reverse cholesterol transport involving ABCA1 and ABCG1 transporters on RPE and endothelial cells [53, 85]. Cholesterol is also removed from the retina by conversion to more soluble oxysterols which can diffuse into the ocular then systemic circulation for removal by the liver [53]. As recently reviewed, diabetes disrupts cholesterol metabolism in the retina, with reduced oxysterol production and decreased LXR activity, reducing cholesterol removal and promoting cholesterol crystal formation in the retina [53], similar to atherosclerosis. Spectral OCT has detected lesions consistent with cholesterol crystals in the retina in people with diabetes and other eye diseases such as age-related macular degeneration [53].

Effects of Lipid Drugs on Diabetic Retinopathy

Unlike for cardiovascular disease, there is a relative paucity of, and sometimes inconsistent evidence, regarding the effects of lipid-lowering drugs on DR. Most evidence related to the effects of lipid-lowering drugs on DR stem from cardiovascular disease trials for which DR was a secondary or tertiary endpoint. The results of some lipid drug DR related studies are now summarized below.

Triglyceride Lowering Agents

Fibrates

In a small early trial (23 adults with severe DR and 25 controls), 3 years of clofibrate use significantly reduced severity of retinal exudates but did not improve other DR lesions or visual acuity. There was no association between retinal exudate severity or its improvement and serum lipid levels of changes thereof [86], which was also seen in subsequent larger fibrate trials [87, 88]. Since then, there have been two major fenofibrate trials in Type 2 diabetes, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial [87] and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Lipid Eye Trial [88], which had cardiovascular endpoints but also evaluated DR.

The FIELD Trial

In the FIELD study, microvascular complications, including DR, were pre-stated endpoints, but not the primary endpoint. Not all participants had retinal imaging, OCT was not available, and the standard therapy for STDR was retinal laser therapy rather than anti-VEGF injections. In the FIELD trial (n = 9795), a median of 5 years once-daily 200 mg co-micronized fenofibrate significantly reduced STDR incidence by 31%, with similar benefit for PDR and DME. In the FIELD ophthalmology substudy (n = 1012) in which (slide-based) retinal imaging was performed, the composite exploratory endpoint (2-step progression of ETDRS Severity Scale grade, DME or laser) was significantly reduced by 34%, with results driven by those with pre-existent DR [87]. A substudy (n = 208 subjects) in which baseline and 2-year digitized retinal photos were suitable for grading of central retinal arteriolar and venule caliber was recently reported [89]. Central retinal venule caliber was significantly reduced by fenofibrate and unchanged by placebo. Arteriole metrics did not change. Study power to determine relationships to subsequent STDR was too low [89]. Larger and longer studies relating retinal vessel caliber to future DR complications and responses to longer therapy are merited.

The ACCORD-EYE Trial

In the ACCORD-EYE Lipid Arm study (n = 1593) with statin (simvastatin) background therapy a mean of 4 years of 160 mg fenofibrate significantly reduced DR progression (by ≥3 ETDRS steps or need for laser or vitrectomy) by 40% [88]. In both these randomized controlled trials, results were consistent regarding fenofibrate benefit for DR, which was of similar magnitude, and without visual acuity benefit, and was independent of traditional lipid levels and of changes thereof [87, 88]. Fenofibrate is approved for use in Type 2 diabetes patients with existent DR in 19 countries, independent of lipid levels, but not by the US Food and Drug Administration (FDA) or major European Medicines Agency. As yet there are no fibrate trials with DR as the primary endpoint, but several are in progress [90–93].

Basic science studies are supportive of fibrate benefits. In animal models of DR and retinal angiogenesis intraocular fibrate, including by nanoparticle ocular delivery, was beneficial [78, 94], but as yet there are no human studies using this route of administration. Ocular delivery may increase retinal drug levels, reduce toxic systemic drug levels and side-effects, and enable treatment in those for whom the systemic drug is not tolerated or is contraindicated (e.g., end-stage kidney disease). Cultured retinal cell and diabetic animal models support the conclusion that the protective mechanisms of fibrates are due to a combination of anti-inflammatory effects, suppression of VEGF, and of the PPARα/Wnt/β-catenin signaling pathway, neuroprotection and anti-apoptotic effects on retinal microvascular cells [76–84]. Fenofibrate has also been shown to reverse the adverse effects of hyperglycemia-induced metabolic memory in endothelial cells [54, 55] through a SIRT1-dependent mechanism [56].

Omega-3 Fatty Acids

Another predominantly triglyceride lowering agent are the omega-3 fatty acids, such as in fish, nuts, fish oils, or purified EPA supplements. Most studies focus on CVD, but a Spanish trial also evaluated effects on STDR [95]. In the PREDIMED study testing Mediterranean diets supplemented with extra virgin olive oil or nuts vs. a control diet in 3482 adults with Type 2 diabetes, of whom 75% met the dietary long-chain omega-3 polyunsaturated fatty acid recommendation (≥500 mg/day) over 6 years of follow-up, there were 69 new-onset STDR cases. On adjusted analyses those who met the dietary recommendations vs. those not doing so had a 48% reduced risk of incident STDR [95] However, further larger trials with primary DR endpoints and high dose omega-3 supplements are merited.

HMG CoA Reductase Inhibitors

As yet there are no major statin trials with DR as the primary endpoint. Early statin trials, usually with 1–50 participants showed that statins could reduce late-stage DR complications and loss of visual acuity [96, 97]. In earlier large statin CVD trials

such as the primary CVD prevention Collaborative Atorvastatin Diabetes Study (CARDS) trial, no DR benefit was evident [98]. However some more recent large observational studies, usually with more potent statins, support benefit for DR. In a prospective study (n = 37,894) from the Longitudinal Health Insurance Database in Taiwan, adults with Type 2 diabetes with dyslipidemia were matched with patients without dyslipidemia. Over a mean of 7 years of follow-up dyslipidemia was associated with significantly increased risk of DR, including NPDR, PDR, and DME. Statins were protective against NPDR (HR 0.83; 95% CI 0.76–0.90) to a similar extent in patients with and without dyslipidemia but did not reduce PDR or DME [99].

In another Taiwanese population-based cohort (n = 219,359) with Type 2 diabetes and dyslipidemia followed for a mean of 7 years, statins significantly decreased DR (HR 0.86; 95% CI 0.81–0.91) and the need for STDR treatment [100]. Statin use was associated with significantly decreased rates of all DR: NPDR (HR 0.92; 95% CI 0.86–0.99), PDR (HR 0.64; 95% CI 0.58–0.70), vitreous hemorrhage (HR 0.62; 95% CI 0.54–0.71), retinal detachment (HR 0.61; 95% CI 0.47–0.79), and DME (HR 0.60; 95% CI 0.46–0.79). Statins were also associated with significantly lower rates of STDR interventions, including laser (HR 0.71; 95% CI 0.65–0.77), intravitreal injections (HR 0.74; 95% CI 0.61–0.89), and vitrectomy (HR 0.58; 95% CI 0.48–0.69) [100].

A large USA health insurance claims database confirmed that 269,782 adults with Type 2 diabetes, of whom 37% (n = 99,233) were taking lipid-lowering medications, were less likely to develop NPDR, PDR, or DME and to receive intravitreal injections of anti-VEGF medication, laser, or vitrectomy [101]. In adjusted time-to-event analyses, patients who took lipid-lowering drugs prior to developing diabetes were less likely to progress to any DR (HR 0.60; 95% CI 0.55–0.65) and to need STDR treatment for (HR 0.81; 95% CI 0.78–0.84). Results were also significant for each level of DR (NPDR, PDR, MDO) and for each type of STDR treatment (anti-VEGF, laser, and vitrectomy).

A Japanese prospective clinical practice study of 40–75 year old Type 1 and Type 2 diabetes patients (n = 363 and 5489, respectively) identified factors associated with NPDR. Only in Type 1 diabetes was any lipid (HDL-C) associated with NPDR, but statin and fibrate use, and the number of lipid-lowering drugs, were associated with significantly lower risk of NPDR [102].

In another observational study (192 eyes) including both Type 1 and Type 2 patients, pre-operative statin use was associated with better outcomes for primary vitrectomy for STDR. Statin users had a significantly better 1-month best correct vision acuity improvement than non-users and the need for repeat vitrectomy over 12 month follow-up was significantly less (HR 0.28, 95% CI 0.08–0.93) [103]. However, not all observational studies evaluating statins and risk of DR are positive [104].

Meta-analysis of Statin and/or Fibrate Trials for Diabetic Retinopathy

A systematic review and meta-analysis evaluated statin and/or fibrate randomized placebo-controlled trials for prevention and progression of DR using the Cochrane guidelines, the PRISMA statement and GRADE approach re evidence certainty [105]. There were four fibrate trials, three statin trials, one fibrate plus statin trial, with eight evaluating existent DR and four evaluating DR prevention. Fibrates were associated with a 45% reduction of DME incidence, but this was of low certainty. There were no other positive outcomes for fibrates or statins. There was no evidence of harm. Trial quality was not ranked highly in this meta-analysis [105]. As further trials accrue further meta-analyses will be of interest.

Future Directions

There is a major need for DR trials of all lipid drug classes, including statins, fibrates, ezetimibe, PCSK-9 inhibitors, bempedoic acid, omega-3 fatty acids, and emerging molecular based therapies. Ideally DR will be the primary endpoint with detailed assessments of a large area of the retina, OCT, measures of retinal vessel caliber and geometry, neural function (such as by electroretinograms), visual acuity, and color vision. Large observational studies and meta-analysis are merited. Subtle retinal changes such as in retinal vessel caliber that may predict early future DR and the response to lipid drug therapies should be evaluated.

Basic science studies to better understand the mechanisms of lipid and lipid drug effects in the eye are merited. Results of such studies may also guide the development of novel DR therapies. Ocular delivery of effective lipid drugs in animal than in human studies is of interest.

As translation of effective therapies into clinical practice is key, and 80% of people with diabetes live in disadvantaged regions [1], it will be important to map the availability of any effective lipid related therapies for DR and their efficacy for DR prevention widely in clinical practice.

Conclusions

Lipids are implicated in the pathogenesis of DR, and lipid drugs may be protective, though large robust clinical trials and observational studies are still merited, including studies of existent and new lipid drugs and ocular drug delivery. The value of

traditional and novel lipoprotein related tests and measures of DR in the prediction of DR and response to lipid drugs is of interest. Basic science to understand the role of lipids in DR and of lipid drugs to protect against DR is needed. Importantly, lipid treatments with positive outcomes must be widely translated into clinical practice.

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Chapter 17
Roles of Extravasated and Modified Plasma Lipoproteins in Diabetic Retinopathy

Timothy J. Lyons

Introduction: Diabetic Retinopathy (DR)

With sufficiently long survival, almost everyone with diabetes mellitus eventually develops some degree of retinal damage (diabetic retinopathy (DR)), but the rate of progression and its severity vary greatly among individuals. This is true even when conventional risk factors such as duration of diabetes and severity of long-term hyperglycemia are taken into account. DR is one of the most feared complications of the disease [1]. Globally, it is the only cause of blindness whose standardized prevalence has increased from 1990 to 2020, with the burden falling most heavily on developing regions of Asia and sub-Saharan Africa [2]. In contrast, in high-income nations with effective screening programs, better glycemic management, and resources to support specialized treatments, the outlook is improving [3], and in these nations, DR is no longer the most common cause of blindness in working-age people as it has been for decades. In 2007, according to a report from the (US) National Eye Institute, about 50% of people with diabetes had at least some degree of retinopathy, and in the USA, approximately 1 person in 400 had sight-threatening retinal disease caused by diabetes [4]. Over the past 20 years in the USA, the increasing prevalence of early-onset type 2 diabetes in African American and Native American youths [5] has been a cause for grave concern, especially in view of disparities in access to high-quality care according to race and income. In type 2 diabetes, the time of onset is often ill-defined, whereas in type 1 diabetes, with no prolonged asymptomatic phase, the duration of diabetes is clear-cut. Thus, type 2 patients should be considered at risk from the time of diagnosis. In contrast, in type 1 patients, clinical DR typically does not

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develop within the first 5 years, yet even during this phase, it is evident that subclinical retinal injury is taking place. This was elegantly demonstrated in a dog model of DR by Engerman and Kern [6]. Once present, DR may broadly be classified into three stages: background disease, non-proliferative diabetic retinopathy (NPDR), and an advanced, sight-threatening phase, proliferative diabetic retinopathy (PDR). Fortunately, the latter develops in only a minority of patients [7]. PDR is characterized by the growth, in response to retinal ischemia, of abnormal new, fragile blood vessels. These vessels invade the vitreous humor and are leaky and prone to hemorrhage.

The normal retina is a highly specialized tissue bounded on the inside by the inner limiting membrane and on the outside by the basement membrane of the retinal pigment epithelium (RPE) (Bruch's membrane) (Fig. 17.1). Counterintuitively, the light-sensing rods and cones are located in the outer retina, immediately inside the RPE, so that light must pass through the overlying retinal "circuitry" (ganglion cell nuclei and plexiform layers) and blood vessels to reach them. Signals to the brain pass inward from the rods and cones, through the ganglion cell layers, to axons which travel across the innermost layer of the retina to reach the optic disc and optic nerve. "Müller cells" are glial cells which span all the layers of the retina vertically: they are essential to retinal health and perform

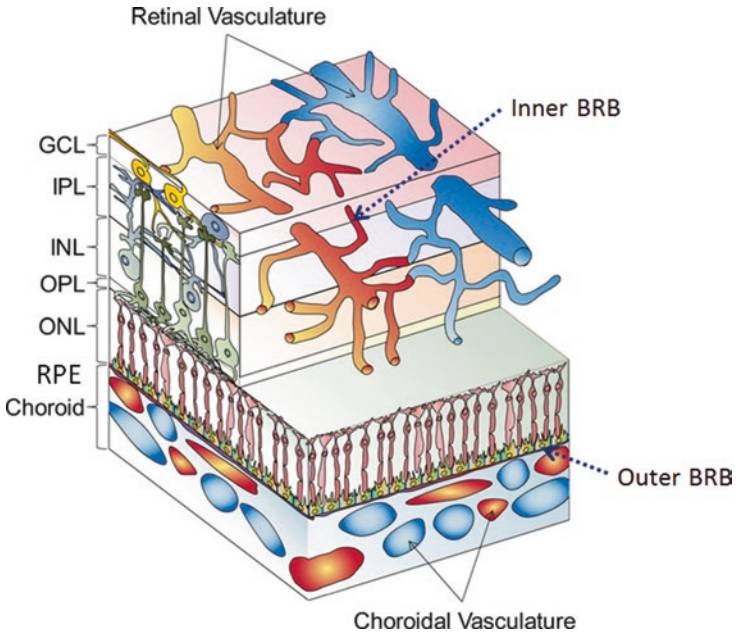

Fig. 17.1 Retinal cells and blood supply. (Modified from *Fulton, A.B. et al., Retinal degenerative and hypoxic ischemic disease. Doc Ophthalmol. 2009;118:55–61*; and reproduced with permission). *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *RPE* retinal pigment epithelium, *BRB* blood-retinal barrier

a macrophage-like function. Sooner or later, all types of retinal cells may be injured in diabetes. Most, but not all, investigators believe that the initial lesion is in the microvasculature.

The retina has a dual blood supply. The central retinal artery, a branch of the ophthalmic artery, enters the eye at the optic nerve head and branches across the inner retina. It is readily visualized with an ophthalmoscope, and in diabetes, perhaps because of this easy visual access, abnormalities of its vascular tree serve to define the severity of DR. The capillaries of this inner retinal circulation are highly specialized: endothelial cells have tight junctions which form the inner blood-retinal barrier (IBRB), while pericytes are more numerous than in any other capillary bed, equaling the number of endothelial cells. The pericytes are contractile and regulate retinal blood flow (blood flow and oxygen consumption in the retina are the highest in the body per gram of tissue); they also regulate the growth and maintain the function of the endothelial cells and IBRB. On the outside of the retina, the ophthalmic artery supplies the choroidal circulation, which lies between Bruch's membrane and sclera. This vascular bed is separated from the neural retina by the outer blood-retinal barrier (OBRB), which is formed by tight junctions between the RPE monolayer. This "outer" choroidal circulation provides a majority (65–70%) of the oxygen and nutrients consumed by the retina [8], but it is not visible with the ophthalmoscope, and so in this location, the effects of diabetes are less well defined.

As mentioned, early DR is defined by damage to the readily observed inner retinal capillaries and involves pericyte loss and leakage of the IBRB. Ophthalmoscopically, "microaneurysms" appear and are thought to be the result of proliferation of endothelial cells following loss of pericytes. Other features include "hard exudates," which are accretions of lipid-rich material following vascular leakage, and "soft exudates," areas of retinal edema resulting from ischemia. Later in the disease process, macular edema and neovascularization may be evident, and both are major causes of vision loss as a result of retinal detachment and/or hemorrhage.

For decades, two assumptions dominated DR research. First, it was viewed as a "microvascular complication of diabetes": one in which the retinal insult is primarily or entirely vascular in both its origin and its progression, specifically involving damage to the highly specialized inner retinal capillaries. Second, hyperglycemia has been viewed as the primary cause of both early and advanced disease. While both of these contentions hold strong elements of truth, it has also become clear that they are oversimplifications. The injury in DR is not confined to the capillaries (and consequent ischemia), but rather affects many (perhaps all) varieties of retinal cell. Hyperglycemia is now regarded as necessary, but not sufficient, for DR to develop: other factors modulate disease severity, and understanding these will bring new opportunities for prevention and therapeutic intervention. This chapter describes the development of a lipoprotein-related concept for the propagation of DR, which is consistent with a generalized retinal injury and which adds a "secondary mechanism" that comes into play once leakage of the blood-retinal barriers develops.

The Initiation of DR

The earliest preclinical events in the evolution of DR are unclear. They are likely to vary from one person to another, to involve several simultaneous insults, and to be scattered in space and time across the retina. Breakdown of the IBRB is an established early feature [8–11]: it may result from metabolic or osmotic injury from high (and fluctuating) plasma glucose levels, or intermittent exposure of the capillary endothelium to the severe metabolic stresses that accompany uncontrolled diabetes ("diabetic ketoacidosis"). Such stresses include acidosis, osmotic stress, and elevation of plasma ketone bodies and free fatty acids. Supporting this, it is thought that recurrent diabetic ketoacidosis may be a risk factor for retinopathy [12]. Another early feature of DR, pericyte loss, may occur independently of, or as a result of, injury to endothelial cells and/or the IBRB, but regardless, it will itself lead to endothelial injury and IBRB leakage [11]. Such leakage can be detected by fluorescein angiography and occurs at the preclinical phase [13]. Furthermore, studies using microspheres show that particles as large as 100 nm diameter can leak from retinal capillaries in the early, preclinical stages of DR in vivo in animal studies [14]. This is of relevance to our present subject, since all major classes of plasma lipoproteins are smaller than these microspheres (HDL: ~9 nm; LDL: ~20 nm; VLDL: 50–70 nm) and therefore are likely to become extravasated from capillaries into the retinal tissue, from which they are normally rigorously excluded, early in the course of DR.

In summary, metabolic stresses of diabetes, including exposure to elevated glucose, free fatty acids, osmotic stress, and other factors, may initiate inner retinal capillary leakage. As a secondary stage, this allows the retina to be flooded with plasma constituents that normally are excluded, initiating new (formerly inoperative) mechanisms for the propagation of DR. It is also possible that the earliest stages of diabetes, prior to IBRB leakage, may lead directly to dysfunction of other cell types (Müller cells, neurons, RPE, choroidal circulation), but these effects are not yet well defined. I contend that while hyperglycemia and inner retinal capillary damage may indeed be dominant initial causal factors and features of retinal injury in DR, they are soon followed by a cascade of events where these "secondary mechanisms" come into play, and where extravasated, glycated and oxidized lipoproteins are important promoters of a vicious cycle of generalized vascular and neuronal injury. As detailed in this chapter, there is evidence that these processes are well advanced by the time clinical retinopathy becomes evident: detection and intervention in the preclinical phases are of paramount importance.

Treatment Considerations for DR

An ideal treatment for DR would arrest its development in the preclinical phase. Efforts in this regard currently focus on the control of modifiable risk factors, most notably hyperglycemia, and indeed it appears that true normalization of plasma

glucose would completely prevent DR. Unfortunately, for the foreseeable future, normalization of glucose levels is unlikely for all but a small proportion of people with diabetes worldwide. Established specific treatments for DR address only advanced disease. Laser treatment entirely ablates ischemic areas of the retina, removing the angiogenic stimulus that drives PDR in neighboring regions, but often sacrifices peripheral vision to save central vision. Antiangiogenic therapies given by intermittent intravitreal injection can inhibit PDR, but by definition are effective only when an ischemia-induced angiogenic stimulus is already present. Enhanced knowledge of disease mechanisms could yield specific measures to block progression even in the presence of hyperglycemia. For example, treatments to preserve the integrity of the blood-retinal barriers might hold promise.

Challenges in Defining the Role of Plasma Lipoproteins in DR

Numerous studies have sought to define associations between lipoprotein levels and severity of DR, either cross-sectionally or prospectively. There are many challenges: large numbers of subjects must be studied, the plasma lipoprotein system is highly complex, DR severity and progression over time must be assessed objectively (even the fact that a person has two eyes creates challenges), disease progression takes years, and there are numerous confounding clinical variables to be considered (age, sex, diabetes duration, long-term glycemia, renal function, medications over time, and many others). Despite this, a consistent message has emerged from studies over the past 50 years [15–39] (including some recent large cohort studies reviewed below [37–39]), revealing significant associations between adverse lipoprotein levels and DR as detailed below. Nevertheless, interest has been muted because the strength of these associations has been weak compared with (a) associations between DR and hyperglycemia [40, 41] and (b) those between plasma lipoproteins and risk for atherosclerosis [42, 43].

The term “dyslipidemia” requires definition: here it is used to describe not only quantitative but also qualitative alterations of lipoproteins found in plasma. The former usage is the standard one and refers to altered levels of simple measures of plasma lipids, e.g., total or LDL cholesterol, HDL cholesterol, and triglycerides. The latter usage includes modification of lipoprotein particles (e.g., by glycation of apolipoproteins and phospholipids, and/or oxidation of any component, but especially unsaturated fatty acids), structural changes, altered distribution of subclasses defined in various ways, and compositional changes in the ratios of component lipids and individual apolipoproteins. Many of these qualitative changes result from or are enhanced by the presence of diabetes, most obviously glycation and oxidation; however, while enhanced glycation of lipoproteins occurs in plasma in diabetes [44], oxidation predominantly occurs outside the circulation, after extravasation and sequestration in vessel walls, as is established in atherogenesis. Strictly speaking, these “extravascular” modifications and effects of lipoproteins are distinct from properties found in plasma; that is, they are not “dyslipidemia” but instead are tissue-based risk factors, and they are not detected in the analyses of plasma samples.

Studies of the Associations Between Plasma Lipoproteins and DR

In the past, many cross-sectional studies in type 1 and type 2 diabetes have described correlations between retinopathy and standard measures of plasma cholesterol, including total and LDL cholesterol, and LDL:HDL cholesterol ratio [16–26]. Others found no such association [45–47], and some found correlations with plasma triglycerides [19, 20, 27, 30].

Some studies have used more detailed measures of plasma lipoprotein profiles, i.e., beyond "conventional" lipid profiles. We studied 988 type 1 diabetic patients (440 women and 548 men) from the Diabetes Control and Complications Trial (DCCT) [39]. We measured detailed lipoprotein characteristics, including not only conventional lipid profiles, but also nuclear magnetic resonance lipoprotein subclass profile (NMR-LSP), apoA1, apoB, lipoprotein(a), and susceptibility of LDL to oxidation [39]. We assessed associations of these parameters with DR as defined by the rigorous DCCT protocol (serial seven-field stereo-retinal photographs read centrally [41]). In brief, plasma lipid/lipoprotein parameters that were positively associated with DR included serum triglycerides, serum concentrations of low-density lipoprotein (LDL), LDL particle concentration, and ApoB. The severity of retinopathy was negatively associated with HDL cholesterol. In men, but not in women, higher levels of small dense LDL and small HDL and lower levels of large buoyant LDL and large HDL were associated with severe DR. In general, an atherogenic plasma lipoprotein profile was associated with more severe retinal disease (and of note, DR is a known risk factor for atherosclerosis in people with diabetes [48]). The Hoorn study [37], which included 2484 50- to 74-year-old Caucasians, yielded similar findings in type 1 and type 2 diabetes (including newly diagnosed and known diabetes) in a population-based cross-sectional study. The prevalence of retinopathy was positively associated with serum cholesterol and triglyceride levels, and elevated plasma total and LDL cholesterol levels showed associations with retinal hard exudates. Furthermore, the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study [38] of a large type 1 diabetes cohort demonstrated that serum triglycerides and, to a lesser extent, higher levels of LDL cholesterol were associated with the progression of retinopathy. Progression to proliferative retinopathy was related to higher LDL cholesterol, serum triglycerides, as well as albumin excretion rate and glycated hemoglobin. Another report demonstrated that apoAI, apoB, and apoB:apoAI ratio were significantly and independently associated with DR in a cross-sectional study of 224 diabetic patients (85 type 1; 139 type 2) [45]. Although these population studies show significant associations between DR and plasma lipids and lipoproteins, the prognostic value for individual patients, regarding the risk for DR, is very limited.

Fibrate Drugs May Be Effective Against DR: But Not Because They Lower Plasma Triglycerides

Importantly, evidence has emerged that fibrate drugs appear to have significant protective effects against DR; however, these effects are unrelated to their long-established effects on plasma lipids. The first evidence came from a series of studies in the 1960s suggesting that an early fibrate, clofibrate, reduced retinal hard exudates [30–36]. This was erroneously assumed to be related to lowering of plasma triglycerides, and then largely forgotten. Much more recently, two large and important prospective studies aiming to define the efficacy of fenofibrate against cardiovascular disease in type 2 diabetes, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study [49] and Action to Control Cardiovascular Risk in Diabetes (ACCORD)-Lipid [50], demonstrated an unexpected secondary outcome: a very significant reduction in the need for laser treatment for DR. This beneficial effect of fenofibrate has received further circumstantial clinical support [51, 52], and randomized prospective studies specifically addressing its effects on inhibiting DR in people with type 1 and 2 diabetes are under way.

So too are studies to define the mechanism of action. As mentioned, the beneficial effects of fenofibrate in DR are unrelated to its effects on plasma triglycerides. Interestingly, and consistent with the hypothesis that extravasated lipoproteins promote DR, fenofibrate may act, at least in part, by protecting the integrity of blood-retinal barrier [53, 54]. Consistent with a specific intraocular effect, the drug may be more effective if administered locally as eye drops than if taken orally [53].

Plasma Lipoproteins as "Secondary Mediators" of DR

Overall, several points are notable. The associations between the plasma lipoprotein characteristics and DR are, in general, statistically highly significant, but only moderate in magnitude. Also, in people who do not have diabetes, dyslipidemia does not cause retinal disease. Finally, dyslipidemia is very clearly associated with atherosclerosis not only in the presence [46, 47, 55–57] but also in the absence of diabetes. Taken together, the evidence suggests an indirect effect of plasma lipoproteins in the retina, one which is contingent upon unique properties of that tissue and upon unique effects of diabetes. I posit that this relates to the presence of the blood-retinal barriers and their breakdown in diabetes.

Extravasated, Modified LDL in the Pathogenesis of DR

In atherosclerosis, elevated plasma levels of LDL and/or modified LDL (oxidized LDL: ox-LDL) are associated with cardiovascular disease [46, 47, 55–57], but the modification of LDL and its consequent toxicity are tissue, not plasma, phenomena.

We have developed a new concept: that LDLs (and, by extension, other plasma lipoproteins) mediate a significant proportion of retinal injury in diabetes, but do so indirectly, not by initiating vascular damage, but rather by becoming extravasated through leaking inner and outer blood-retinal barriers, subsequently being modified by glycation and oxidation, thereby becoming toxic towards any cells in the vicinity. Initially, such damage is patchy and vascular cells (e.g., pericytes) may be the first to be damaged, but later with more severe leakage, extravasated lipoproteins could permeate throughout all layers of the retina, which is only ~249 μm in total thickness [58], establishing vicious cycles of further leakage and retinal injury.

Effects of Modified LDL on Retinal Capillary Vascular Cells

We have accrued considerable evidence of injurious effects of modified LDL towards a variety of retinal cell species in culture. For in vitro work, we generally have employed two control conditions: not only native (N-)LDL (i.e., unmodified LDL), comparing its effects to modified forms, but also serum-free medium, recognizing that in the healthy retina, no extravasation of plasma lipoproteins occurs. We utilized degrees of in vitro modification and concentrations of LDL designed to simulate conservatively the stresses present in diabetes in vivo. Initially, we investigated the effects of normal and mildly modified human LDL (from healthy donors) on bovine retinal capillary endothelial cell and pericytes. These modified LDLs, prepared in vitro, were intended to simulate characteristics of circulating, plasma lipoproteins, not those that had undergone more severe oxidation after extravasation. This work was intended to address the question of whether mild glycation and/or oxidation of plasma LDL might contribute to the initiation of retinal capillary injury. We found that indeed, survival of both endothelial cells and pericytes decreased with exposure to low levels of modified LDL, and that toxicity increased in the following order: glycation < mild oxidation < combined glycation/mild oxidation [59].

In subsequent cell culture work, we employed more severe degrees of LDL modification, again imposed in vitro on LDL from healthy donors, to simulate lipoproteins that have been damaged after extravasation. To prepare "highly oxidized glycated" LDL (HOG-LDL), N-LDL was first glycated (as would happen in plasma in diabetes) and then copper-oxidized to simulate its fate after extravasation [60–62]. In all of this work, we strove to maintain conditions that are pathophysiologically relevant. It is of interest that antibodies raised against copper-oxidized LDL (that had been prepared by a similar protocol to our own) recognized in vivo-oxidized LDL in atheromatous plaque, and in our hands, in human diabetic retinae (see below). The concentration of LDL employed is also critical. In our cell culture work, we used a range of concentrations up to about half of typical plasma levels. Tissue levels in the diabetic retina are unknown, but estimates of ApoB levels in atheromatous plaque suggest that they may be 2–79 times *higher* than in plasma [63, 64]. This surprising finding may be explained by extensive sequestration of LDL in vessel walls as a result of covalent cross-linking, and it is reasonable to

expect a similar effect in the diabetic retina. Also, "average" tissue concentrations of extravasated lipoproteins conceal the effects of uneven tissue distribution, with high localized concentrations at sites of leakage.

Modified LDL Mediates Apoptosis of Retinal Capillary Endothelial Cells and Pericytes

As detailed above, our early studies demonstrated that mild modification of LDL resulting from separate or combined processes of glycation and oxidation is implicated in chronic retinal capillary injury and thus perhaps in the initiation of DR [59], acting in concert with hyperglycemia. Using more severely modified HOG-LDL, we showed that oxidative stress and inflammation are associated with LDL-induced retinal cell death. HOG-LDL enhanced intracellular reactive oxygen species (ROS), 3-nitrotyrosine (3-NT), tyrosine nitration of prostacyclin synthase, peroxynitrite ($ONOO^-$) formation, inducible nitric oxide synthase (iNOS) expression, and nitric oxide (NO) production, in parallel with the induction of monocyte chemoattractant protein-1 (MCP-1) secretion and nuclear factor-kappa B (NF-kappa B) activation in human retinal capillary pericytes [65, 66]. It activated endoplasmic reticulum stress, apoptosis, and autophagy [67]. Thus, HOG-LDL has pro-inflammatory and prooxidant effects on retinal pericytes. HOG-LDL also induced DNA fragmentation, activated the caspase cascade, and inhibited cell proliferation in pericytes, consistent with a possible contributory role in the apoptotic pericyte loss that occurs in vivo in DR [62, 68]. Exposure to HOG-LDL versus N-LDL induced similar phosphorylation of mitogen-activated protein kinase (MAPK) signaling pathways including extracellular signal-regulated kinase (ERK), p38, and Jun N-terminal kinase (JNK), and blockade of the ERK, p38, and JNK pathways did not inhibit apoptosis of pericyte induced by HOG-LDL, suggesting that apoptosis induced by HOG-LDL is independent of the activation of MAPK signaling pathways [61]. Subsequently, we implicated Wnt signaling pathways in DR [69, 70]. Wnt signaling regulates cell proliferation and differentiation, apoptosis, stem cell maintenance, angiogenesis, inflammation, fibrosis, and carcinogenesis [71]. In our studies, modified LDL activated Wnt signaling via oxidative stress [70]. In conclusion, this body of cell culture work indicates that modified LDL, if it comes in contact with specialized retinal cells, can activate multiple intracellular pathways, inducing effects similar to those characteristic of DR.

Modified LDL Influences Gene Expression in Human Retinal Capillary Pericytes

Complementing the studies described above, we used gene array studies to investigate the effects of 24-h exposure to HOG-LDL versus N-LDL in human retinal pericytes [72]. This revealed 60 genes that were altered, including members of

functional pathways involving fatty acid, eicosanoid, and cholesterol metabolism; fibrinolytic regulation; cell growth and proliferation; cell stress responses; kinin system; and angiogenesis, indicating that HOG-LDL elicits gene expression in retinal pericytes that may contribute to pericyte loss and other retinal abnormalities in DR. Pro-apoptotic and pro-angiogenic responses to HOG-LDL may be of particular importance in this regard [72]. Microarray analysis also showed that matrix metalloproteinase 1 (MMP1), MMP2, MMP11, MMP14, and MMP25 and tissue inhibitor of metalloproteinase 1 (TIMP1), TIMP2, TIMP3, and TIMP4 were expressed in pericytes. Of these, only TIMP3 mRNA showed altered regulation, being expressed at significantly lower levels in response to HOG-LDL versus N-LDL [72]. Quantitative PCR and immunoblotting of cell/matrix proteins confirmed the reduction in TIMP3 mRNA and protein in response to HOG-LDL. In contrast to cellular TIMP3 protein, analysis of secreted TIMP1, TIMP2, MMP1, and collagenase activity indicated no changes in their production in response to modified LDL [60]. Thus, HOG-LDL selectively influences tissue inhibitor of metalloproteinase-3 gene expression and protein production among pericytes and might contribute to microvascular abnormalities in DR.

Aminoguanidine Mitigates Toxicity in Human Retinal Capillary Pericytes Exposed to HOG-LDL

Much evidence suggests beneficial effects of aminoguanidine in experimental DR, including prevention of abnormal endothelial cell proliferation [73], reduction of pericyte dropout [73, 74], inhibition of the development of retinal microaneurysms [74] and acellular capillaries [74], prevention of arteriolar thrombosis [73], and reduction of retinal capillary-associated basement membrane thickening [75]. These benefits have been found in various animal models including diabetic dogs [74], streptozotocin-induced diabetic rats [73, 76], and diabetic and hypertensive rats [75]. Typically, aminoguanidine was administered by intraperitoneal (i.p.) injection (~25–50 mg/kg) or by being added to diet (~3.0 g/kg) or drinking water (~50 mg/100 mL). In vitro, we found that remarkably low concentrations of aminoguanidine (in the nanomolar range) blocked cytotoxic modification of LDL exposed to stresses including oxidation and glycation that simulate the diabetic environment [77], thus protecting retinal capillary cells from previously modified LDL. This action may contribute to the beneficial effects of aminoguanidine observed in experimental DR. The efficacy of aminoguanidine at nanomolar concentrations suggests an action through scavenging reactive carbonyls (whether generated by oxidative or metabolic processes) rather than by NOS inhibition that occurs at higher concentrations [77, 78]. Unfortunately, in a clinical trial of oral aminoguanidine (300 mg/day), three patients developed glomerulonephritis [79], and further human studies have not taken place. However, local administration of this drug to the eye could still represent a potential intervention, potentially bringing its beneficial effects while avoiding systemic side effects.

Effects of Pigment Epithelium-Derived Factor

PEDF is a glycoprotein with neurotrophic, antioxidative, and antiangiogenic properties. Previous studies have shown that decreased ocular levels of PEDF are associated with DR [80–82]. Intravitreal injection of PEDF reduced vascular leakage in rat models of diabetes and oxygen-induced retinopathy (OIR), likely resulting from the decreased levels of retinal inflammatory factors including VEGF, VEGF receptor-2, MCP-1, tumor necrosis factor alpha (TNFα), and intercellular adhesion molecule 1 (ICAM-1) [83]. In cultured retinal capillary endothelial cells, PEDF treatment decreased TNF-alpha and ICAM-1 expression under hypoxia. Downregulation of PEDF expression by siRNA leads to increased levels of VEGF and TNF-alpha secretion in retinal Müller cells. Taken together, PEDF is a novel endogenous anti-inflammatory factor in the eye. As stated above, HOG-LDL, but not N-LDL, significantly increased ONOO(−) formation, NO production, and iNOS expression in human retinal capillary pericytes [66]. These changes were alleviated by PEDF. Moreover, PEDF significantly ameliorated HOG-LDL-induced ROS generation through upregulation of superoxide dismutase 1 expression [66]. Overall, PEDF is a potential candidate for the prevention or inhibition of DR, operating at least in part by inhibiting the effects of oxidized LDL [66, 83].

Effects of Modified Lipoproteins on Retinal Müller Cells and RPE

Retinal Müller cells are glial cells that support retinal neurons and span the whole thickness of the retina. In tissue culture experiments, modified LDL induces Müller cell apoptosis mediated by oxidative and endoplasmic reticulum stress [84]. Berberine, a plant alkaloid with effects on insulin resistance and AMPK activation that has garnered much recent interest, was able to protect Müller cells from the toxic effects of modified LDL in cell culture models [85].

RPE cells exist as a monolayer whose tight junctions constitute the outer blood-retinal barrier. These remarkable cells act as the “lungs, liver, and kidneys” of the protected intraretinal microenvironment and are the most metabolically active tissue in the body. Enormous quantities of cholesterol, needed for retinal neuronal function, cross the RPE. Not surprisingly therefore, these cells can withstand substantial stress and, in our tissue culture work, tended to be more robust than other retinal cell types when exposed to modified LDL. Nevertheless, they too succumbed to apoptotic cell death at concentrations of LDL likely to be present in vivo [86]. These data suggest that modified lipoproteins in the retina may be derived from, and mediate, outer as well as inner blood-retinal barrier leakage and are toxic not only to endothelial cells and pericytes but to other key retinal cell species as well.

Immunologic Consequences

An intriguing possibility is that extravasated and modified LDL may trigger an immune response, and the resulting modified LDL immunocomplexes might also mediate retinal injury (Fig. 17.2). Such effects have been implicated in atherogenesis, and increased levels of oxidized LDL immune complexes are associated with the development of coronary calcification [82, 83]. In addition, oxidized LDL and advanced glycation end product-modified LDL (AGE-LDL) in circulating immune complexes are associated with progression and increased levels of carotid intima-media thickness (IMT), demonstrating that ox-LDL immune complexes have pro-inflammatory and pro-atherogenic properties in type 1 diabetes [87, 88]. A study of the DCCT/EDIC cohort demonstrated that concentrations of LDL immune complexes are predictive of the severity of DR many years in the future [89], and immune complex formation appears to amplify the toxicity of oxidized LDL towards retinal pericytes [90].

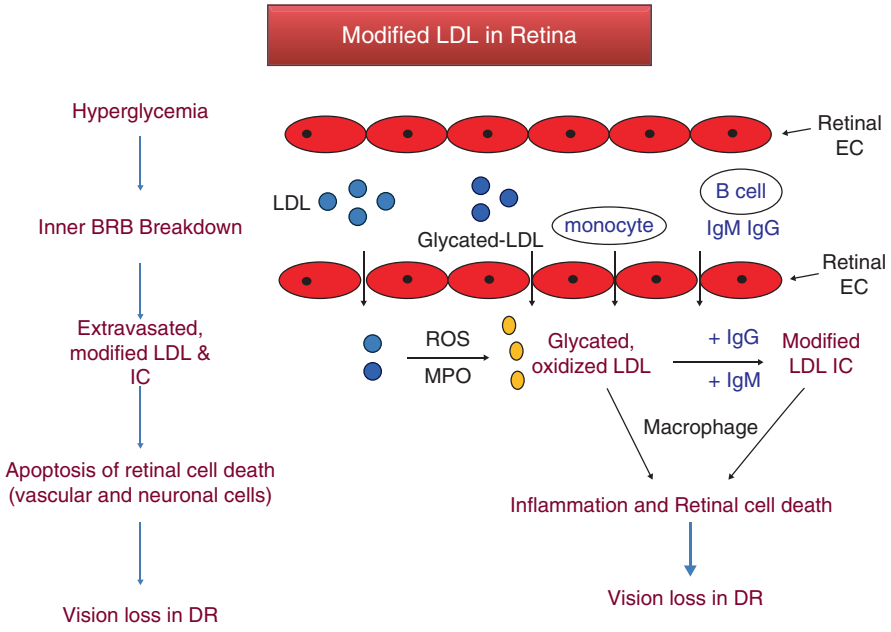

Fig. 17.2 Potential consequences of extravasated LDL in the progression of retinal injury in diabetic retinopathy: After extravasation, LDL becomes severely modified by glycation and oxidation and, as a result, toxic towards numerous types of retinal cells. In addition, extravasated and modified LDL may trigger an immune response, and the resulting modified LDL immunocomplexes may mediate retinal injury

Evidence for the Presence of Modified Lipoproteins in the Diabetic Retina

Clearly, validation of our hypothesis requires demonstration of the actual presence of modified lipoproteins in the diabetic retina, and their absence in the healthy retina. We obtained human retinae postmortem from nondiabetic and type 2 diabetic individuals with varying degrees of DR [62] (Fig. 17.3) and performed immunohistochemistry to detect oxidized LDL and ApoB (ApoB100, a marker of LDL and VLDL). Staining was absent in nondiabetic subjects, but present in those with diabetes, correlating with the severity of retinopathy across three categories (no clinical retinopathy, non-proliferative DR, and PDR). Thus, lipoprotein extravasation in diabetic retinas was clear-cut and was present even in subjects with no clinical DR (consistent with a causative role for future DR), but entirely absent in healthy retinae from nondiabetic individuals. Ox-LDL was prominent in inner retina (ganglion

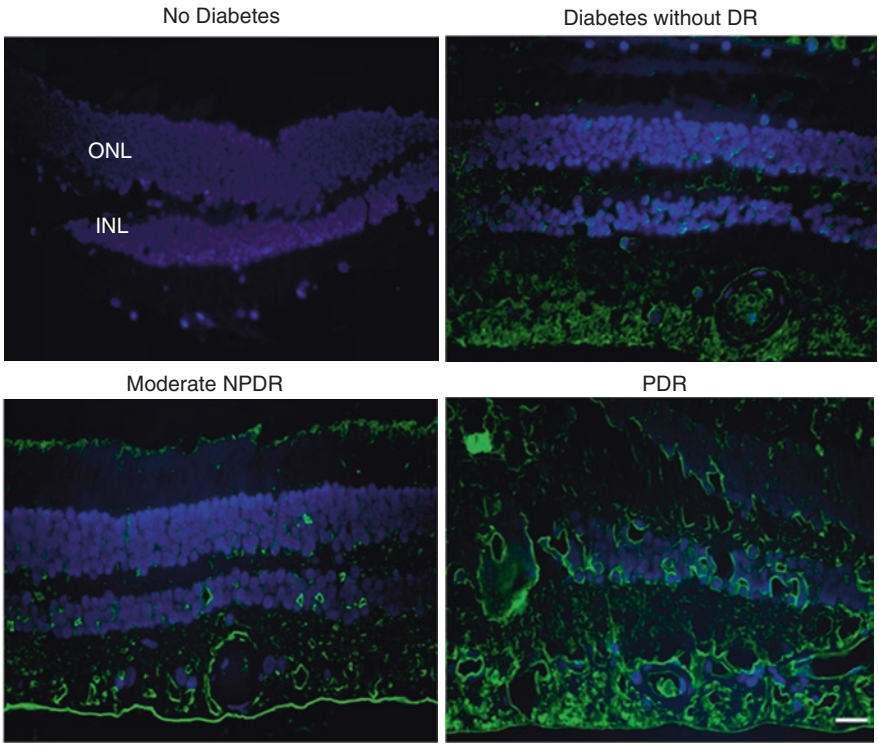

Fig. 17.3 Immunostaining for ox-LDL in retinae from type 2 diabetic patients: Staining was observed in all diabetic groups, even before the onset of clinically detectable DR. The fluorescent signal intensity increased with DR severity but was absent in nondiabetic retinae. (Image reproduced with permission from *Wu, M., et al., Intraretinal leakage and oxidation of LDL in diabetic retinopathy. Invest Ophthalmol Vis Sci, 2008; 49: 2679–85*). *ONL* outer nuclear layer, *INL* inner nuclear layer layer. Ox-LDL staining: green; Dapi: blue. Scale bar: 20nm

cell layer (GCL)) where most blood flow is from the central retinal artery. In PDR, ox-LDL was also present in the outer retina, near the RPE, which is supplied by the choroidal circulation. This could represent permeation of extravasated LDL from the inner to all retinal layers, or it could suggest leakage of the OBRB as an additional mechanism for DR. Besides ox-LDL, intraretinal immunofluorescence of ApoB was also present in diabetic human retinae, paralleling the findings with ox-LDL and correlating with the severity of DR [62]. In addition, in retinal sections from subjects with PDR, macrophage infiltration was prominent—suggesting significant inflammation and another parallel with atherogenesis.

An Animal Model to Simulate Intraretinal Effects of Extravasated, Modified, Lipoproteins

If extravasated modified plasma lipoproteins are indeed important promoters of DR, an animal model to simulate their presence in the retina could facilitate mechanistic studies, accelerating the development of diabetic retinal lesions, providing a more pathophysiologically relevant disease model and a platform to test new treatments. Conceivably, it could assist in reaching the elusive goal of an animal model of proliferative DR; this advanced form of DR does not occur in diabetic rodents, and notably, LDL particles are absent in the rodent circulation. To develop such a model, we administered small quantities of normal and modified human LDL by intravitreal injection to control and streptozotocin-induced diabetic mice [91] and rats [92]. The intent was that the lipoproteins would gradually permeate into the retinal tissue. One concern was an immunologic response to the foreign protein, but no effects, retinal or otherwise, were observed in response to intravitreal normal (unmodified) LDL in either nondiabetic or diabetic animals. In contrast, LDL that had been glycated and oxidized, while having no effect on nondiabetic rodents, induced dramatic retinal lesions in the diabetic animals within 1–2 weeks: these included vascular leakage, inflammation, and apoptosis. Consistent with the finding that fenofibrate could protect the outer blood-retinal barrier in RPE cell culture [54], administration of fenofibrate was protective against the retinal effects of intravitreal modified LDL in diabetic animals [92].

Summary

In summary, the data summarized above suggest that lipoproteins from plasma may play a central, and heretofore unrecognized, role in propagating retinal injury, even though the associations of plasma lipoproteins with the severity of retinopathy are relatively weak. Modified LDL is shown to be toxic to many cell types, including vascular and neural cells. Toxic modifications of LDL occur primarily after extravasation and trapping in tissue. These observations support the concept that plasma

lipoproteins (which we can study relatively easily) may modulate disease risk, but extravasated and modified lipoproteins (much less accessible) represent a secondary mechanism driving DR as soon as vascular leakage is established. From this, it follows that effective treatments must correct not only adverse quantitative plasma lipoprotein levels, but also a spectrum of qualitative abnormalities in both plasma and tissues, and, perhaps most importantly, the processes that lead to inner and outer BRB leakage, and those by which lipoproteins and cells interact in tissues at the sites of disease.

Conclusion

Diabetes and its vascular complications, including DR, are epidemic worldwide. In many settings, the disease process proceeds unchecked for years or decades before detection. Better identification of risk factors, better understanding of disease mechanisms, and development of effective screening, prevention, and treatment strategies are critical in meeting these challenges. The described effects of modified LDL (and by extension, other lipoproteins) in the retina are analogous to the effects in atherogenesis in cardiovascular diseases but have received less attention. As stated above, extravasated LDL and subsequently-modified LDL (ox-LDL) are present in diabetic human retinae, correlating with the severity of DR. Modified LDL has toxic effects on many types of retinal cells and is likely to contribute to retinal dysfunction and vision loss. Its effects may be amplified by the formation of immune complexes. Further studies are necessary to elucidate more details regarding these mechanisms, such as involvement of the Wnt pathway and endoplasmic reticulum (ER) stress, and to explore new interventions that may prevent capillary leakage, or the effects of modified lipoproteins in the retina after extravasation. Fenofibrate, a long-established drug for hypertriglyceridemia with an excellent safety profile and relatively low cost, holds significant promise. This drug, and other future treatments that can block the preclinical stages of DR, may obviate the need for today's late-stage interventions and may have a major impact on global health and healthcare costs.

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Chapter 18
The Role of Lipids and Lipoproteins in Peripheral Neuropathy

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Overview of the Diabetic Neuropathies

According to the International Diabetes Federation, the estimated global number of adults aged 20–64 with diagnosed or undiagnosed diabetes mellitus (DM) was 352 million in 2019 [1]. Among these patients, the reported prevalence of diabetes-related neuropathy ranges from 16% to as much as 87%, making neuropathy the most common complication of DM [2].

The diabetic neuropathies (DN) can be classified into three main groups [3], namely (1) diffuse neuropathies, (2) mononeuropathies, and (3) radiculopathies or polyradiculopathies. Diffuse neuropathies are the most common type of DN and frequently manifest as distal symmetric polyneuropathy (DSPN). Patients with DSPN usually present with numbness, tingling, and/or pain that typically starts in the toes or fingers and slowly progresses proximally in a "stocking-glove" pattern [3, 4]. However, it is important to note that approximately 50% of patients with DSPN can be asymptomatic [3]. The other major subtype of diffuse neuropathy is diabetic autonomic neuropathy (DAN), which may involve the autonomic fibers that innervate multiple tissues including the heart, vasculature, gastrointestinal tract, urinary bladder, and genitalia [5, 6]. In consequence, the clinical manifestations of

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DAN are multiple and encompass resting tachycardia, orthostatism, gastrointestinal dysmotility, constipation, diarrhea or fecal incontinence, neurogenic bladder, sudomotor dysfunction, and altered pupillary responses.

The second group of DN (mononeuropathies) involves isolated cranial or peripheral nerves, most frequently cranial nerves III or VII, or the median, ulnar, radial, or common peroneal nerves. Mononeuropathies tend to resolve spontaneously over the course of a few months [3]. Finally, radiculopathies or polyradiculopathies typically affect the lumbosacral plexus causing extreme unilateral pain in a dermatomal pattern, weight loss, and/or motor weakness in a myotomal pattern [3, 4].

Epidemiology of Diabetic Neuropathy

Diabetes and its complications are increasingly common; the worldwide prevalence of diabetes in 2019 was estimated at 9.3% (463 million people) and is expected to increase to 10.9% (700 million) by 2045 [7]. Thus, diabetes can be considered a true global epidemic of the twenty-first century [2]. Even more worrying, about half of the people living with diabetes do not know that they have the disease [7].

Diabetic polyneuropathy (DPN) is the most common chronic complication of both type 1 and type 2 diabetes [8]. Among patients with diabetes, the prevalence of DPN may reach up to 30% [9]. For example, a study in two separate regions of Italy (Varese and San Giovanni Rotondo) that used door-to-door screening based on subjective symptoms reported a total prevalence for neuropathy of 1–4%, of which 40–55% of cases were secondary to diabetes [10].

Typically, DPN develops more frequently and progresses more rapidly in type 1 diabetes, causing more severe manifestations [8]. In fact, almost 100% of patients with type 1 diabetes will eventually develop DPN [5]. On the other hand, the prevalence of DPN in patients with type 2 diabetes has been estimated at 20–40% in different populations [11, 12]. These discrepancies in the epidemiology of DPN between the two types of diabetes may reflect underlying differences in their pathophysiology [8].

Among the many subtypes of DPN, the most common is by far DSPN [2]. Interestingly though, the incidence of DSPN is actually higher in patients with type 2 (approximately 6100 per 100,000 person-years) than in those with type 1 diabetes (approximately 2800 per 100,000 person-years) [3]. However, the reported prevalence of DSPN is very similar in patients with type 1 (11–50%) or type 2 diabetes (8–51%) [13]. This apparent contradiction may be due to more extensive detection efforts among patients with type 1 diabetes. Also, type 1 diabetes is usually diagnosed at an earlier age, so patients are exposed to the deleterious hyperglycemic, insulin-deficient *milieu* for a much longer time. When asymptomatic neuropathy is included, the overall prevalence of DPN reaches up to 45% in individuals with type 2 diabetes and 54% in those with type 1 diabetes [14]. The costs of DSPN and its complications are exorbitant; in the United States alone, they

represent more than 10 billion dollars annually [15]. Most importantly, DSPN stands out among diabetes complications for its impact on the patient's independence and quality of life.

Another important subtype of DPN is autonomic neuropathy, especially cardiovascular autonomic neuropathy (CAN) [16]. The prevalence of CAN displays wide variation among studies, ranging from 25% to 75% in type 2 diabetes [17]. This variability can be attributed to the lack of universal diagnostic criteria and the underdiagnosis of CAN in hospital settings [18].

Pathophysiology of Diabetic Neuropathy

Pathways in the Pathophysiology of Diabetic Neuropathy

DPN has been described as the "most enigmatic" of diabetic complications [19], because of its complex and multifactorial pathophysiology, and because its evolution does not always parallel the glycemic control status of affected patients. We will describe the known pathways of mechanisms leading to DPN and then highlight how hyperglycemia does not completely explain the genesis of this complication, and lipid metabolism may play a key role in DPN development.

One central pathway linking poor glycemic control to nerve damage involves the accelerated production of free oxygen radicals and high oxidative stress characteristic of hyperglycemia [20]. Free radicals induce lipid peroxidation, chemical modifications of DNA, simultaneous activation of multiple DNA repair systems, and exhaustion of the cellular antioxidant systems, all of which result in the induction of pro-inflammatory transcription factors like nuclear factor kappa-B (NF-kappa B) [21]. Another important pathway that connects hyperglycemia with DPN involves hyperactivation of the intracellular enzyme aldose reductase, which transforms glucose into sorbitol and fructose in the so-called polyol pathway. Sorbitol does not diffuse easily out of the nerve cells, and there is no major pathway for its degradation, so sorbitol is a metabolic dead end. The progressive accumulation of the osmotically active sorbitol leads to cellular edema and depletion of key regulators of neural activity like taurine, myoinositol, and adenosine by poorly understood mechanisms [11]. In addition, aldose reductase uses NADPH as a coenzyme, so its hyperactivation consumes and depletes cellular NADPH, impairing the regeneration of glutathione, the main defense against oxidative damage [22]. Thus, the polyol pathway has the potential to synergistically increase the damage imparted by free oxygen radicals.

The improperly constant stimulation of nociceptors of the transient receptor potential family, especially channel subfamily V member 1 (TRPV-1), plays a role in the progression of early DPN. TRPV-1 is a receptor involved in the modulation of nociceptive inputs to spinal cord and brain stem centers, as well as the integration of diverse painful stimuli [23]. Normally, TRPV-1 is stimulated only by potentially

noxious heat (≥43 °C), by protons, or by specific agonists like capsaicin [24]. However, in the altered metabolic *milieu* of diabetic patients, TRPV-1 is constantly stimulated, inducing the local release of the neurotrophins: nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). A feed-forward loop is then established, in which NGF binds to the trkA receptor, which lowers the threshold for TRPV-1, and leads to further sensitization and pain, and hence further NGF release [25]. This phenomenon is the subject of extensive study in the search for pharmacological targets for DPN.

Yet another pathway that has been implicated in the genesis of diabetes complications is the activation of atypical isoforms of protein kinase C (PKC). These atypical isoforms activate NF kappa-B and other pro-inflammatory transcription factors. This has been well demonstrated in other types of microvascular damage, especially diabetic nephropathy [26]. It is conceivable that atypical PKC activation also plays a role in DPN, but this mechanism has not been extensively studied.

Hyperglycemia may also relate to DPN via the hexosamine pathway, a common mechanism of several long-term diabetes complications [27]. In the presence of a high glucose influx, some of the fructose-6 phosphate in the glycolytic pathway is diverted by the enzyme glutamine:fructose-6-phosphate transferase to glucosamine-6 phosphate. This last compound is then used to glycate the nucleotide UDP and produce UDP-*N*-acetyl glucosamine (UDP-GlcNAc). UDP-GlcNAc is then enzymatically added to the serine and threonine residues of multiple transcription factors, modifying their activity and altering the expression of multiple target genes. When this effect occurs in nerve cells, it results in extensive damage. In addition, diabetes is characterized by the nonenzymatic glycation of cellular proteins, resulting in the formation of advanced glycation end products (AGEs), which directly hinder the function of multiple essential cellular and extracellular proteins (tubulin, actin, laminin). Naturally, this also impacts cell types of the peripheral nervous system. AGEs also bind to and activate the receptor for AGEs, or RAGE, inducing a pro-inflammatory, pro-oxidative transcriptional program in peripheral nerves [28].

Association Between Glycemic Control and Prevention of Diabetic Neuropathy

Several landmark clinical trials have examined the impact of glycemic control on the risk of developing DPN among patients with diagnosed T1DM or T2DM.

In the Diabetes Control and Complications Trial (DCCT), perhaps the most influential trial of glycemic control in T1DM, patients randomized to the intensive control arm achieved an HbA1c on average 1.8% lower than the conventional treatment arm after a follow-up period of 6.5 years. Intensive control resulted in a 69% lower incidence of DPN (defined as DSPN on physical examination plus abnormal nerve conduction in two different nerves or unequivocally abnormal autonomic test

results) [29]. These patients were then followed prospectively in an observational 8-year extension called the Epidemiology of Diabetes Intervention and Complications (EDIC) study. Despite the original HbA1c difference between groups having entirely disappeared (8.0% prior intensive group vs. 7.9% prior conventional therapy group) [30], the difference in DPN incidence persisted (cumulative incidence of 7.0% in the intensive group vs. 3.5% in the control group). Furthermore, the NeuroEDIC study extended this follow-up for up to 14 years after the DCCT closure, and the between-group difference in the risk for neuropathy not only persisted but widened (25% in the former intensive group vs. 35% in the former control group, $p < 0.001$) [31]. Nonetheless, it is noteworthy that 25% of T1DM patients who had been relatively well controlled over more than 20-year time span still went on to develop DPN.

In patients with T2DM, the Kumamoto and Action to Control Cardiovascular Risk in Diabetes (ACCORD) trials found similar results. In the Kumamoto study, patients treated with intensive insulin therapy (IIT) (three or more daily administrations) achieved better glycemic control than those under conventional insulin therapy (HbA1c 7.1% IIT group vs. 9.4% conventional therapy). This better glycemic control translated into less nerve damage after 6 years, a significant albeit modest difference (median nerve conduction velocity [NCV] 53.2 m/s in IIT vs. 50.2 m/s in conventional group, $p < 0.05$) [32]. Similarly, in the glycemic component of the ACCORD trial, patients originally randomized to strict glycemic control (mean HbA1c 6.4%) had a slower progression of DPN versus the standard treatment group (mean HbA1c 7.5%) (hazard ratio [HR] for loss of ankle jerk at study end 0.90, 95% CI: 0.84–0.97). Once again, however, the absolute difference in DPN incidence was modest (45.7 vs. 49.3%) [33].

There is also a group of glycemic control trials in T2DM in which better glycemic control has not shown benefits in DPN prevention. The first example is the Veterans Affairs Diabetes Trial (VADT), in which a solid 1.5 percentage point difference in HbA1c (8.4% control group, 6.9% intensive group) had no impact on the cumulative incidence of any type of neuropathy (43.5% control, 43.8% intensive) [34]. In the United Kingdom Prospective Diabetes Study (UKPDS), better HbA1c control (7.0% in intensive arm vs. 7.9% in standard arm) did not translate into a different incidence of DPN measured by absent ankle reflexes (35% in the intensive group, 37% in standard group, $p = 0.60$) [35]. Likewise, the Action in Diabetes and Cardiovascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) study showed no significant effect of tighter glycemic control on neuropathy in patients with T2DM [36].

The more recent cardiovascular safety/efficacy trials of specific antidiabetic agents of the GLP-1 agonist and SGLT-2 inhibitor families (ELIXA, LEADER, SUSTAIN-6, EXSCEL, REWIND, EMPA-Reg Outcomes, CANVAS, DECLARE-TIMI 58) have not examined nor reported on neuropathy outcomes.

Thus, the cumulative body of evidence supports the idea that other major factors besides glycemic control may be strong determinants of the risk of developing DPN.

Role of Lipids and Lipoproteins on Diabetic Peripheral Neuropathy

It is well known that cells from peripheral nerves express insulin receptors, whose stimulation leads to canonical insulin signaling [26]. Insulin binding to its receptor leads to its autophosphorylation in tyrosines, followed by binding of insulin receptor substrate 2 (IRS-2) to phosphotyrosine residues and phosphorylation of phosphoinositide 3-kinase (PI3K) and subsequently phosphoinositide-dependent kinase-1 (PDK1) and protein kinase B (PKB/Akt) [37]. The normal functioning of this pathway guarantees normal nerve regeneration, neurite outgrowth, and mitochondrial function [38]. In T2DM and insulin resistance, the activation of intracellular pro-inflammatory signals leads to IRS-2 serine phosphorylation and impaired insulin signaling. When sciatic nerves from obese mice have been exposed to insulin, their PKB/Akt activation response is lower than that in normal-weight animals [39]. On the other hand, studies in human patients with metabolic syndrome, a clinical precursor of T2DM, have found a positive association between insulin resistance measured as the homeostatic model assessment-insulin resistance (HOMA-IR) index and presence of peripheral neuropathy [40].

A recent systematic review of 39 clinical trials with a total of 32,668 patients showed that patients with DPN had on average higher plasma triglyceride (TG) and lower high-density lipoprotein (HDL) concentrations than controls [41].

Free Fatty Acids Mediate Insulin Resistance and Malfunction in Peripheral Nerves

Insulin resistance causes activation of adipocyte hormone-sensitive lipase and subsequently an increase in plasma levels of free fatty acids (FFAs) [42]. These FFAs are then taken up by cells and serve as a substrate for the synthesis of diacylglycerol and ceramides, which activate serine-threonine kinases that phosphorylate IRSs and reduce their signaling capacity [43] (Fig. 18.1). The type of fatty acids that partake in the composition of the phospholipid bilayer of cells is also involved in processes related to DPN. Membranes from healthy patients are characterized by high concentrations of polyunsaturated fatty acids (PUFAs), a characteristic that facilitates insertion of membrane receptors and transporters. In T2DM, increased FFA leads to high cytoplasmic saturated fatty acyl-CoA, which allosterically inhibits fatty acid desaturases and reduces the synthesis of PUFA [44]. Under these circumstances, membrane flexibility decreases, and multiple functions associated with electrical conduction and signal transduction may become affected [45]. In addition, high intracellular saturated FFA levels directly stimulate the expression of the p65 subunit of nuclear factor kappa-B (NF-kB) [46]. This pathway raises production of reactive oxygen species (ROS) and promotes oxidative stress, a central factor in the appearance and progression of DPN [47].

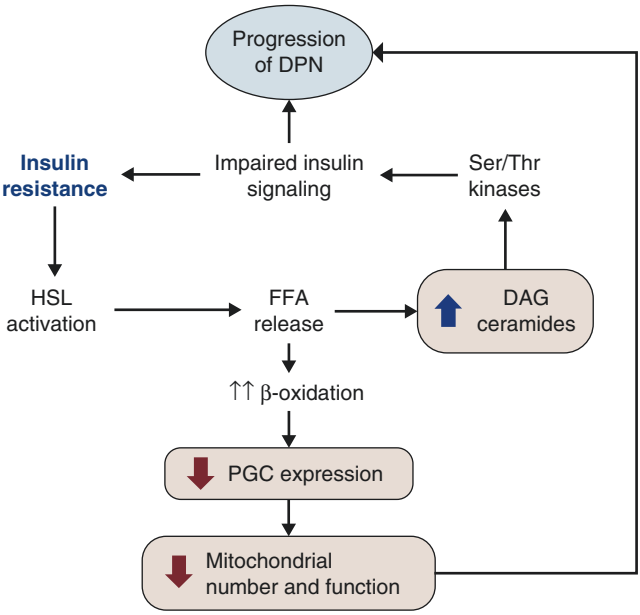

Fig. 18.1 Free fatty acids mediate the relationship between insulin resistance and progression of DPN. Insulin resistance leads to derepression of hormone-sensitive lipase (HSL), which releases free fatty acids (FFA) from intracellular stores. These FFAs are taken up by other tissues, where they are used for the synthesis of diacylglycerol (DAG) and ceramides, both of which activate Ser/Thr kinases. These kinases phosphorylate IRS-2 and other members of the insulin signaling pathway, exacerbating insulin resistance and facilitating DPN progression. Also, the excessive availability of FFA increases the flux through beta-oxidation in nerve cells, reducing the expression of PGC-1alpha and leading to impaired mitochondrial function

The relevance of the membrane composition of nerve cells is revealed by studies in streptozotocin diabetic rats, an animal model of type 1 diabetes. These studies have found that 5 weeks of supplementation with the PUFAs gamma-linolenic (omega 6) and eicosapentaenoic (omega 3) acids led to a significant improvement in sensitive and motor NCV [48]. Similar findings were reported in a multicenter clinical trial among patients with T2DM, showing a significant improvement of 13 DPN parameters (including conduction velocities, thermal sensitivity, and tendon reflexes) after supplementation with gamma-linolenic acid for 1 year [49]. A mechanistic study in humans evaluated the causality of the association between FFA and DPN by simultaneously infusing intralipid and heparin into patients with T2DM in order to intentionally raise FFA levels. Heart rate variability was measured by spectral analysis for 3 h. Plasma FFA correlated positively with the low-frequency/high-frequency variability ratio (higher values indicate lower heart rate variability) ($r = 0.57$, $p < 0.02$). After 3 months of good glycemic control, when circulating FFA had dropped to normal levels, heart rate variability measures also returned to normal [50].

Alterations of Lipid Metabolism Cause an Imbalance in Mitochondrial Bioenergetics That Promotes Neuropathy

Mitochondria are the main site of reactive oxygen species (ROS) generation. In neurons and glial cells, a dysregulation of mitochondrial bioenergetics as seen in T2DM has been associated with reductions in the number and respiratory capacity of mitochondria [51, 52].

Nerve cells from patients with insulin resistance or T2DM exhibit a proteome that reflects changes in mitochondrial substrate utilization and dysfunction of the electron transport chain [53]. FFAs have the ability to directly inhibit the respiratory chain [54], a property that has been demonstrated in Schwann cells in vitro [55]. Studies in streptozotocin diabetic rats found that insulin doses insufficient to reduce plasma glucose were still able to normalize the rates of mitochondrial coupled respiration in cells from dorsal root ganglia [56]. Peripheral nerves and dorsal root ganglia from rodents with diabetes display reduced glycolytic intermediaries, in association with increased oxidative damage of proteins and lipids [57]. The severity of this type of damage seems to be proportional to the length of the nerve involved and is particularly evident for the sciatic nerve [58]. AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 alpha) are "central hubs" of energy metabolism that appear to be involved in the pathway from fatty acids to mitochondrial dysfunction and DPN. A high-fat diet increases mitochondrial concentrations of fatty acid oxidation intermediaries and decreases PGC-1 expression [59]. Interestingly, stimulation of AMPK signaling has improved neuropathic manifestations like thermal hypoalgesia in a rodent model of diabetic neuropathy [60]. Administration of a specific PPAR-gamma agonist has demonstrated to improve NCV in diabetic obese rats, an animal model for human T2DM [61]. Thus, part of the toxicity of FFA on nerve functionality is exerted through alterations of mitochondrial homeostasis (Fig. 18.1).

Oxidized Lipids Promote DPN

In observational prospective studies of persons with T2DM, increased LDL cholesterol and TG levels predispose to a faster progression to DPN [62]. It is known that oxidation of LDL is increased in patients with diabetes compared to healthy controls [62], resulting in a pro-inflammatory state. Dorsal root ganglia express the lectin-like oxLDL receptor (LOX-1) [63]. When oxidized LDL (oxLDL) binds to LOX-1 in the nerve roots of patients with DPN, a signaling pathway is activated that increases ROS and oxidative stress, potentiating the damage initiated by other pathways.

Atypical Sphingolipids

Sphingolipids are a class of naturally occurring lipids made by subsequent modifications of a sphingoid base, mostly sphingosine. The rate-limiting step in their synthesis is the condensation of L-serine and palmitoyl-CoA, catalyzed by the enzyme serine-palmitoyl transferase (SPT) [64]. Complex lipids from this group such as ceramide and sphingomyelin are involved in cell structure and signaling. Deoxysphingolipids (DOSLs) are atypical sphingolipids characterized by the lack of an OH group in C1. Several DOSLs display neurotoxic activity [65]. DOSLs are produced when SPT activity is altered, and it uses L-alanine or glycine instead of serine as amino acid substrate. As serine and alanine are involved in carbohydrate metabolism, it is believed that DOSL synthesis is a metabolic intersection between lipid and carbohydrate pathways and oxidative stress [66], specially in patients with T2DM [67].

Plasma DOSL levels are significantly increased in patients with metabolic syndrome and/or T2DM. A study comparing the sphingolipid profile of patients with T1DM, T2DM, and controls found increased levels of DOSL only among patients with T2DM [66]. In a case-control study, patients with T2DM also had higher DOSL plasma levels compared to controls [67]. Plasma sphingolipid profiling of patients with DPN compared to other types of neuropathies and compared to patients without neuropathy revealed increased atypical sphingolipids mostly in DPN [68]. In a subgroup study from EDIC, patients who reported neuropathy at any point of follow-up exhibited higher deoxy-ceramide levels than those without neuropathy [69]. A pilot model with diabetic rats demonstrated that intentionally lowering plasma DOSL may improve neuropathy measures like mechanical sensitivity and NCV [70]. In a trial comparing treatment with fenofibrate versus niacin for 6 weeks in patients with primary hypercholesterolemia or mixed dyslipidemia, fenofibrate effectively lowered atypical sphingolipids [71], which suggests that PPAR-alpha agonists with an impact on lipid metabolism may provide a positive impact on DPN. Nonetheless, the exact mechanism by which DOSL induces damage to cells of the nervous system is not completely understood.

Current Evidence on Lipid Modification and Diabetic Neuropathy

The treatment of DPN should encompass strategies aimed at four broad targets: (1) maintenance of near-normoglycemia, (2) modification of DPN pathogenesis, (3) symptom relief, and (4) avoidance of risk factors [72]. Once DPN has been established, most current treatment algorithms do not emphasize pathogenic treatment, but rather purely symptomatic control [73]. For this reason, DPN treatment

guidelines usually recommend as first-line options the use of tricyclic antidepressants (TCA) (amitriptyline), alpha-2-delta calcium channel subunit agonists (pregabalin or gabapentin), or serotonin-noradrenaline reuptake inhibitors (SNRI) (duloxetine). The specific agent is chosen according to patient profile and contraindications [73]. Other available second-line therapies include opioids, anticonvulsants, topical capsaicin, and membrane stabilizers [74]. Only two treatments aimed at modifying DPN pathophysiology have been incorporated into clinical practice, with mixed results: epalrestat (an aldose reductase inhibitor) and α-lipoic acid.

α-Lipoic Acid (ALA)

α-Lipoic acid (ALA), also known as thioctic acid, is a fatty acid with multiple antioxidant properties, which also participates in key reactions of intermediary metabolism. Treatment with ALA increases reduced glutathione in vivo and in vitro. ALA is also a powerful lipophilic free radical scavenger on peripheral nerves and promotes fiber regeneration via production of NGF [72]. A classic meta-analysis encompassed four clinical trials that evaluated intravenous ALA 600 mg/day, 5 days/week for 3 weeks (ALADIN I, ALADIN III [*Alpha-lipoic Acid in Diabetes Neuropathy*], SYDNEY [*Symptomatic Diabetic Neuropathy*], and NATHAN II [*Neurological Assessment of Thioctic Acid in Neuropathy*]). The meta-analysis included a total of 1258 patients with symptomatic DSPN ascertained through the total symptom score (TSS). Change in TSS was the primary outcome, but other key scores were also measured. The relative difference in favor of ALA compared to placebo was 24.1% (95% CI 13.5–33.4) for TSS and 16% (95% CI 5.7–25.5) for the sign-based Neuropathy Impairment Score in the Lower Limbs (NIS-LL) [75].

Later studies focused on oral formulations of ALA. SYDNEY 2 was a multicenter, randomized, double-blind, placebo-controlled trial, in which 181 patients received once-daily oral ALA at doses of 600 mg (ALA600), 1200 mg (ALA1200), 1800 mg (ALA1800), or placebo, for 5 weeks [76]. The primary outcome was also change in the TSS. All three ALA groups displayed improvements in mean TSS, stabbing pain, and burning pain compared to placebo. Additionally, when comparing the intervention groups, there was no statistically significant difference among the three ALA doses. However, in the safety analysis, there was a dose-dependent increase in nausea, vomiting, and vertigo, implying that the 600 mg dose provides the best ratio of benefit to adverse effects and should be the one used to increase adherence [76]. A recent case series of 90 patients with T2DM in Egypt evaluated the effects of 600 mg/day of oral ALA for 3 months on peripheral neuropathy and metabolic parameters [77]. NCV increased significantly over the follow-up, with parallel improvements in neuropathic symptoms (Table 18.1). These results encourage the idea that modifications of lipid metabolism may be a potential treatment against the development and/or progression of DPN.

Table 18.1 Summary of clinical trials of lipid-modifying agents in patients with type 2 diabetes and peripheral neuropathy

Reference	Agent and dose	Duration	Main outcome	Main result
[75]	I.V. ALA 600 mg/day, 5 days/week	5 weeks	TSS	24% reduction in TSS
[76]	Oral ALA 600 mg/day	5 weeks	TSS	51% reduction in TSS
[80]	Rosuvastatin 20 mg/day	12 weeks	NSS	57% reduction in NSS
[82]	Rosuvastatin/ ezetimibe 20/10 mg/day	16 weeks	Oxidative stress markers	47% reduction in plasma MDA Doubling of plasma NO
[71]	Fenofibrate 160 mg/day	6 weeks	Plasma DOSL	Significant reductions in 8 atypical sphingolipids
[49]	GLA 480 mg/day	1 year	Median and peroneal nerves NCV	4.1 m/s increase in peroneal NCV 4.5 m/s increase in median NCV
[86]	GLA 360 mg/day	6 months	NSS	25% reduction in NSS
[89]	Coenzyme Q10 400 mg/day	12 weeks	NSS	40% reduction in NSS

Data taken from Refs. [49, 71, 75, 76, 80, 82, 86, 89]
ALA alpha-lipoic acid, *TSS* total symptom score, *NSS* neuropathic symptoms score, *MDA* malondialdehyde, *NO* nitric oxide, *DOSL* deoxysphingolipids, *GLA* gamma-linolenic acid, *NCV* nerve conduction velocities

Statins

As previously stated, modifications of serum lipids are a potential therapeutic target in DPN. Beyond their LDLc modification ability, statins have pleiotropic effects such as improvement of endothelial function, increased bioavailability of nitric oxide, and antioxidant actions [78–80]. In the Fremantle observational cohort study, the use of fibrates or statins over a mean follow-up of 6 years was associated with an HR for new DPN of 0.52 (0.27–0.98) and 0.65 (0.46–0.93), respectively [81]. A randomized, double-blind, placebo-controlled phase IIa clinical trial evaluated the effect of rosuvastatin 20 mg versus placebo over 12 weeks on DPN measures, among 34 patients with diagnosed T2DM and early DPN [80]. Patients in the rosuvastatin group experienced increases in mean NCV for the tibial, peroneal, median, and ulnar nerves, but none of them reached statistical significance vs. placebo. A more mechanistically based study evaluated the effects of ezetimibe 10 mg/simvastatin 20 mg and rosuvastatin 20 mg vs. placebo for 16 weeks on oxidative stress in 74 patients with DPN. There were significant reductions in lipid peroxidation metabolites in both statin arms [82]. Thus, it is thought that a possible mechanism of action of statins against DPN could be reduction of oxidative stress. However, no

significant changes were observed in nerve conduction studies or symptom scores in either statin group. Consequently, there is still not sufficient evidence to recommend statin therapy for the treatment of DPN.

Fibrates

The abovementioned Fremantle study provided a first insight into a potentially protective effect of fibrates against DPN [81]. No published trial has directly evaluated the effect of fibrates on DPN, but the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study provided some information in this regard. FIELD assessed the effect of fenofibrate versus placebo on major cardiovascular events in 9795 patients with T2DM, showing a significant reduction in the risk of a first amputation (HR 0.64 [0.44–0.94]) and of an amputation in patients without prior macrovascular disease (HR 0.53 [0.30–0.94]) [83]. Given that most amputations are attributable to a combination of neuropathy and angiopathy [84], it is likely that part of these improvements on amputation events are due to favorable actions on DPN. Some of the pathways we discussed earlier may be related to the potential effects of fibrates on DPN. In a study conducted in 66 dyslipidemic patients, administration of fenofibrate 160 mg/day significantly lowered serum levels of DOSL and other atypical sphingoid compounds, without affecting the concentrations of typical sphingolipids [71].

Other

Gamma-linolenic acid (GLA) is an omega-6 polyunsaturated fatty acid produced from dietary linoleic acid. GLA is one of the main components of the phospholipid bilayer in neurons, necessary for their normal structure and function [85]. Thus, GLA has been extensively studied as a potential pathophysiological therapy for DPN. Two randomized, double-blind, placebo-controlled studies evaluated the effect of GLA in patients with DPN using as outcomes symptom scores, neurophysiological tests, and nerve biopsies [49, 86]. Both trials demonstrated significant improvements in the three groups of outcomes (symptom scores, neurophysiological tests, and nerve biopsies) in the GLA group. There was a later trial which compared GLA and ALA (ALA representing the main known pathophysiological treatment), using as primary outcome measures the score in the visual analogue scale (VAS) for pain, and the TSS [87]. Mean VAS scores and TSS dropped similarly in both groups compared to baseline, but there were no statistically significant differences between the two treatments. In this study, GLA was non-inferior to ALA in terms of reducing pain, measured by either of the two outcomes.

Ubiquinone, also known as coenzyme Q10, is a key component of the electron transport chain in the mitochondria, necessary for ATP synthesis. Ubiquinone can be reduced to ubiquinol, which acts as a reducing agent, preventing lipid peroxidation and its subsequent deleterious effects [88]. Thus, it is hypothesized that supplementation with ubiquinone could help prevent the development of DPN. To date, only one trial evaluating the impact of ubiquinone on DPN has been published. Forty-nine patients with T2DM were randomized to receive ubiquinone 400 mg/day or placebo, for 12 weeks. At study end, the ubiquinone group displayed significant improvements in the neuropathy symptom score, neuropathy impairment score, sural sensory nerve action potential amplitude, peroneal motor NCV, and ulnar motor NCV [89]. Despite these promising results, further studies need to be conducted in order to establish ubiquinone as a *bona fide* treatment for DPN.

Ongoing and Future Trials of Lipid Modification for Diabetic Neuropathy

A few current trials evaluate lipid modification as a treatment for DPN. One of them is OPTIMUM (pregabalin and alpha-lipoic acid combination versus each monotherapy in patients with diabetic peripheral neuropathy), a randomized, parallel, open-label, multicenter, phase IV, 2 × 2 factorial clinical trial. The primary objective is to assess and compare the efficacy and safety of the two agents (pregabalin 150 mg/day, ALA 480 mg/day) on a VAS of pain over 12 weeks among patients with DPN (NCT04846673, 2021). Secondary outcomes will include the proportion of patients reaching a 30% or 50% reduction in pain severity in the VAS, total symptom score (TSS), brief pain inventory Korean version (BPI-K), pain detect questionnaire (PD-Q), and quality of life assessed with the three-level version of Euro-Qol-5 dimensions (EQ-5D-3L).

One particular formulation of omega-3-fatty acid ethyl esters is currently being tested for DPN. In a randomized study (NCT00931879), the commercial formulation Lovaza® or matching placebo will be administered orally at a dose of 4 g/day during 6 months to patients with T2DM, DPN, and elevated plasma triglyceride levels. The study endpoints are NCV, indices of large and small fiber nerve function (including heart rate variation, vibration and thermal thresholds, and markers of inflammation and oxidative stress), and endothelium-dependent and heat-induced vasodilation in the foot dorsum.

An interesting trial assesses the effect of L-carnitine on DPN among adult patients with DPN and an HbA1c <10%. Even though carnitine itself has limited impact on plasma lipids, at the cellular level, carnitine is involved in the transport of fatty acids to the mitochondrial matrix for their beta-oxidation. The experimental group will receive, in addition to antidiabetic therapy, oral supplementation with L-carnitine syrup, 1500 mg/day, for 10 weeks (NCT04145245). The intervention will be

compared against placebo. The primary outcome is the change in pain rating in a VAS, while the secondary outcome is change in neuropathic symptoms as reflected by the neuropathy symptom score (NSS) and neuropathy disability score (NDS).

As we can observe, most ongoing trials of lipid modification for DPN are focused on pain alleviation. However, it would be interesting to give equal attention to more objective measures such as NCV or pathophysiological mediators (e.g., lipid peroxidation, nerve growth factor concentrations). There is a remarkable degree of heterogeneity concerning inclusion criteria and outcome definition in most DPN trials, much more so in trials of lipid modification. Therefore, an international effort to harmonize trial methodology in this field is much needed. Moreover, future efforts should be centered on halting the progression of early DPN, perhaps long before symptoms are overtly evident [11].

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Chapter 19
Lipoproteins and Ischemic Stroke in Diabetes

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Introduction

Diabetes mellitus (DM) affects about one in ten adults in the United States [1]. Dyslipidemia occurs commonly in persons with DM, and both conditions are intrinsically linked to the development of atherosclerotic cardiovascular disease (ASCVD), including cerebrovascular disease [2]. About 90% of strokes are ischemic in nature, with atherosclerosis as the leading cause, followed by small-vessel disease and cardioembolism [2, 3].

The development of atherosclerosis begins with endothelial dysfunction, followed by endothelial damage and numerous inflammatory responses mediated by local and systemic cytokines that ultimately converge in a final common pathway

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leading to atherosclerosis. Once atherosclerosis has developed, plaque disruption can cause in situ thrombosis within cerebral arterial beds, or embolism from a more proximal artery such as the aorta or carotid arteries, with both thrombosis and embolism resulting in vascular occlusion, cerebral ischemia, and stroke. DM precipitates pathologic changes at the cellular level, involving changes to carbohydrate metabolism, production of advanced glycation end products (AGEs) including lipoproteins and extracellular matrix components, and activation of the protein kinase C pathway. All these changes result in mitochondrial dysfunction, increased production of reactive oxygen species (ROS), oxidative stress, and inflammation that lead to progressive arterial injury and atherogenesis [4–6].

Pathophysiology of Atherosclerosis in Stroke

Atherosclerosis in major intracranial arteries, which can occur concomitantly with systemic atherosclerosis or in isolation [7], typically leads to changes ranging from minor wall distortion to significant vessel stenosis. The pathophysiology of intracranial atherosclerotic disease begins with intimal necrosis of intracranial arteries, estimated to occur one to two decades before formation of the first fibromuscular plaques and fatty streaks [8]. Appearance of atherosclerotic plaques in the posterior circulation and particularly the basilar artery appears to precede those in cerebral arteries in the anterior circulation [9]. Intimal and adventitial fibrosis is more common in intracranial arteries than lipid infiltration; a postmortem histological analysis of plaques in the middle cerebral artery revealed that luminal stenosis, percentage of plaques containing >40% of lipid area, as well as prevalence of neovasculature and intraplaque hemorrhages were more in the plaques associated with infarct [10, 11]. Calcium deposits are also less common in intracranial arteries compared to coronary arteries [12].

Dyslipidemia plays an important role in the pathophysiology of ischemic stroke [13]. Insulin resistance and DM also render the lipid profile more pathogenic, including elevated circulating triglycerides (TG) and free fatty acids, increased production of very-low-density lipoproteins (VLDL), higher levels of low-density lipoprotein (LDL) particles, and decreased levels of high-density lipoproteins (HDL), in aggregate the so-called diabetic dyslipidemia. Levels of LDL in patients with DM are frequently normal, but the composition of LDL subtypes is altered with increased levels of small dense LDL, which is a more atherogenic form of LDL [14]. Thus, while dyslipidemia and DM each contribute independently to ASCVD and stroke risk, DM itself can promote dyslipidemia, increasing the risk of ischemic stroke independently of the direct effects of hyperglycemia and inflammation (Fig. 19.1).

In this chapter, we focus on the pathophysiology and mechanisms by which dyslipidemia leads to atherosclerosis and ischemic stroke in the presence of DM and discuss the existing evidence for lipid-lowering therapies in DM for the prevention of stroke.

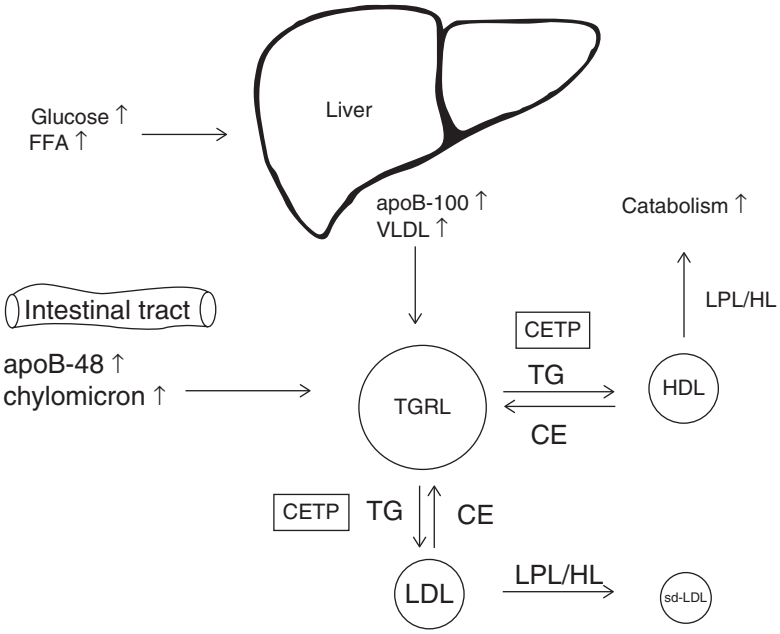

Fig. 19.1 Diabetic dyslipidemia. *apoB* apolipoprotein B, *CE* cholesteryl esters, *CETP* cholesterol ester transfer protein, *FFA* free fatty acids, *HDL* high-density lipoprotein, *HL* hepatic lipase, *LDL* low-density lipoprotein, *LPL* lipoprotein lipase, *sd-LDL* small-dense low-density lipoprotein, *TG* triglycerides, *TGRL* triglyceride-rich lipoproteins, *VLDL* very-low-density lipoprotein

Burden of Ischemic Stroke in Diabetes Mellitus

The global burden of DM (hereafter, referred to as "diabetes") was reported as 451 million in 2017 and is projected to increase to 693 million by year 2045 [15]. In 2018, DM was the seventh leading cause of mortality in the United States according to the data from the US National Health and Nutrition Examination Survey (NHANES) [16]. DM portends a major risk for both microvascular and macrovascular complications. Indeed, DM is a major risk factor of stroke for all age groups and confers a 1.6–6.0-fold increase in relative risk [2] with the highest relative risk being for individuals younger than 65 years old [17]. In the Get With The Guidelines-Stroke registry of 409,060 patients aged 65 years and older followed for 3 years post-discharge from hospital, patients with DM were younger and had a higher risk of adverse outcomes, including all-cause mortality, all-cause readmission, and ischemic stroke or transient ischemic attack (TIA) readmission regardless of initial stroke severity [18]. Indeed, similar results were replicated by Zhang et al. who performed a meta-analysis of 18 studies containing a total of 43,899 participants and showed that the risk of stroke recurrence was significantly higher among stroke patients with DM [19].

The relationship between DM and ischemic stroke does not appear to be associated with hyperglycemia. In a meta-analysis of 13 randomized control trials that included a total of 58,160 patients with type 2 DM, intensive glucose-lowering therapy was associated with a reduced risk of major cardiovascular events and myocardial infarction (MI), but there was no significant effect on the risk of total mortality or stroke [18]. Hyperglycemia in patients with DM and stroke is associated with a higher risk of in-hospital death, but this is also seen in nondiabetes, in a so-called stress hyperglycemia response [20]. Strict hyperglycemic control has not been shown to reduce the incidence of stroke in patients with DM [21]. However, the identification of other risk factors, such as hypertension and dyslipidemia, allows the early recognition of patients at risk of stroke and the implementation of successful risk reduction strategies.

Evidence for the Role of Lipoproteins in Ischemic Stroke in the General Population

LDL-C

It is very well recognized that LDL-C is a causal risk factor for the development of systemic atherosclerosis, and this relationship is dependent on the dose and duration of exposure [22]. While the relationship between high LDL-C levels and cerebral and systemic atherosclerosis is very well established, this relationship is not as defined when stroke is a result of a different etiology like cardioembolic or small-vessel cardiovascular disease [23]. Despite the fact that LDL-C lowering has not been studied extensively in these settings, it is still common clinical practice to use medications that aim at lowering its levels given the common overall high systemic burden of atherosclerosis [23].

HDL-C

The inverse association between the levels of HDL-C and the risk of coronary artery disease (CAD) and MI has been established by numerous epidemiologic studies [24]. For instance, a study from the Multi-Ethnic Study of Atherosclerosis (MESA) found an inverse association between HDL-C and incident stroke in Black individuals [25]. However, the evidence is not as strong for the outcome of cerebrovascular events. Case-control studies have found that the concentration of HDL-C is lower in patients who had a stroke even after controlling for other confounding factors [26]. Studies utilizing carotid artery ultrasound have demonstrated a similar inverse relationship between HDL-C levels and extracranial atherosclerotic disease [27]. The Northern Manhattan Stroke Study evaluated the role of HDL-C in ischemic stroke, and the findings were consistent with a reduced risk of ischemic stroke as the HDL-C levels increased [28].

TG-Rich Lipoproteins

The role of TG in ischemic stroke remains controversial. Some studies have established a strong correlation between TG and increased risk of MI, CAD, and death [29, 30], while other studies have questioned this association. On the one hand, large-scale epidemiological studies have detected a relationship between elevated non-fasting TG and risk of ischemic stroke [29]. In the Blood Lipids and First-Ever Ischemic Stroke/Transient Ischemic Attack in the Bezafibrate Infarction Prevention (BIP) registry, a large prospective trial of 11,177 patients with underlying CAD, fasting hypertriglyceridemia was found to be an independent risk factor for the development of first ischemic stroke or transient ischemic attack (TIA). In a multivariable analysis, after adjusting for traditional risk factors, the odds ratio (OR) for ischemic stroke or TIA for TG >200 mg/dL was 1.47 (95% CI 1.19–1.80) [31]. A meta-analysis of prospective cohorts by the Asia-Pacific Cohort Studies Collaboration (APCSC) that included 96,224 individuals showed that those with TG levels in the highest fifth had a HR of 1.97 (95% CI 1.52–2.55) for the risk of fatal or nonfatal ischemic stroke compared with individuals in the lowest fifth [32]. The Finnmark study, a population study that included 13,266 participants with a 14-year follow-up, reported a significant association between non-fasting TG and stroke for women only [33]. The Copenhagen City Heart Study, a prospective observational study of 19,698 participants, found a strong linear relationship between non-fasting TG levels and cerebral ischemic events [34].

However, other studies have suggested that TG levels are not a risk factor for ischemic strokes [35]. A prospective, randomized, nested case-control study among patients from the Physician Health Study, which included 296 fatal and nonfatal ischemic strokes in white male physicians and controls, showed no association between non-fasting TG and ischemic stroke; the adjusted OR for the highest vs. the lowest quartile of TG was 1.07 (95% CI 0.63–1.82) [36]. In a case-control study of 204 patients with ischemic stroke and 204 controls, the authors did not find any significant association between fasting TG measured 7 days prior to the index event and acute ischemic stroke [37]. An analysis from the Atherosclerosis Risk in Communities (ARIC) study showed only a weak and inconsistent relationship between TG levels and ischemic stroke [38].

Lipoprotein (a)

Lipoprotein (a) [Lp(a)] gained more attention as a risk factor for CAD and ischemic stroke in the past decade. It has been estimated that about 20% of the general population has elevated Lp(a) levels, which are mostly determined by variation in the LPA gene [39]. Lp(a) is discussed in more detail in another chapter in this book (K and G Kostner). The link between Lp(a) and ischemic stroke has been questioned by some researchers. For instance, studies by Hachinski [40] and Glader [41] et al. showed no association between Lp(a) levels and risk of ischemic strokes. In another prospective study in Finland, no significant relationship was found between

baseline Lp(a) and risk of cerebrovascular events [42]. On the contrary, recent meta-analyses support the role of Lp(a) in strokes. The largest meta-analysis carried out by the Emerging Risk Factor Collaboration analyzed 13 prospective cohort studies and concluded that Lp(a) is an independent risk factor for ischemic stroke [43]. Another meta-analysis by Nave et al. analyzed 20 studies and showed that elevated Lp(a) levels were associated with higher risk of stroke, especially in younger patients [44]. Interestingly, other studies suggest that Lp(a) increases the risk of large artery atherosclerotic strokes [45]. However, it is important to note that further studies in larger populations and from different ethnicities are needed to better establish the role of Lp(a) as an independent risk factor for ischemic stroke.

Pathophysiology of Ischemic Stroke in DM

The end-organ damage caused by DM is primarily mediated by oxidative stress at the cellular level. This oxidative stress promotes inflammation via several distinct pathways, all culminating in the development of atherosclerosis: endothelial injury, leukocyte recruitment, foam cell and lipid core formation within subendothelial plaques, and ultimately rupture, thrombosis, and embolism within cerebrovascular arterial beds that result in ischemic stroke.

DM results in elevated intracellular glucose levels that increase the concentration of electron donors, reduced forms of nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide ($FADH_2$), within the cell and its mitochondria. This increases flow of electrons through the electron transport chain resulting in overproduction of superoxide anion and other ROS and free radicals that overwhelm the cell's neutralization capacity. In the setting of insulin resistance, increased free fatty acid release and oxidation cause a similar increase in ROS, even in the absence of hyperglycemia. The excess of ROS inhibits the glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), increasing the concentration of all glycolytic intermediates upstream of this enzymatic step. The overproduction of ROS leads to five putative inflammatory pathways: (1) increased flux through the polyol pathway; (2) increased formation of AGEs; (3) increased expression of the AGE receptor and its activating ligands; (4) activation of the protein kinase C (PKC) pathway; and (5) overactivity of the hexosamine pathway (Fig. 19.2) [6].

Increased flux through the polyol pathway depletes nicotinamide adenine dinucleotide phosphate (NADPH), reducing the cell's ability to neutralize ROS and increasing intracellular oxidative stress. Intracellular ROS and AGEs directly damage proteins and organelles and interfere with normal cellular functions. The binding of AGEs to their receptors causes changes that activate transcription factors such as nuclear factor-kappa beta (NF-κB), resulting in pathologic gene expression. This sensitizes endothelial cells to proinflammatory cytokines, increases adhesion of inflammatory white blood cells to the endothelial surface, and induces procoagulant changes in the endothelium. Activation of protein kinase C (PKC) results in decreased nitric oxide production, increased vascular endothelial growth factor

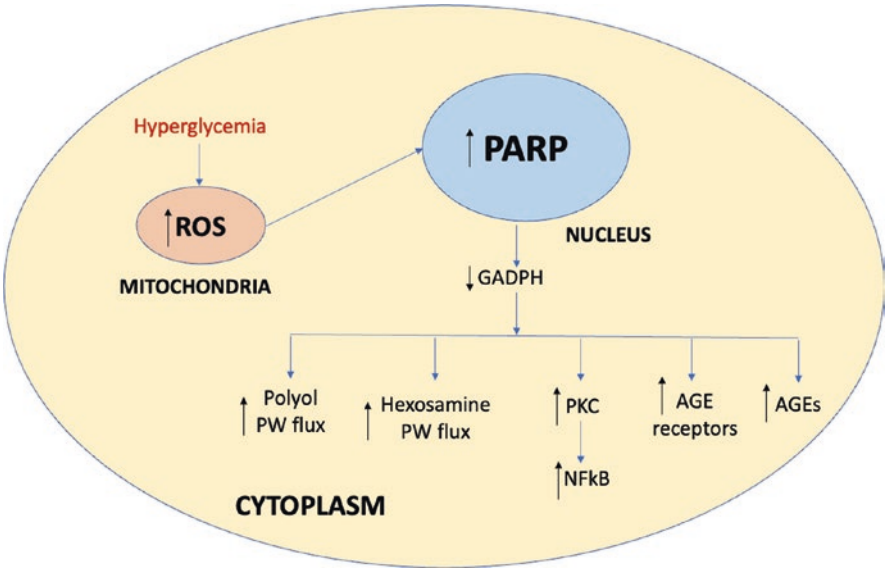

Fig. 19.2 Mechanisms of hyperglycemia-induced cellular damage. *ROS* reactive oxygen species, *AGE* advanced glycation end products, *PKC* protein kinase C, *PW* pathway. (Reproduced with permission from Quispe, Renato; Martin, Seth S.; Jones, Steven R. Triglycerides to high-density lipoprotein–cholesterol ratio, glycemic control and cardiovascular risk in obese patients with type 2 diabetes. Current Opinion in Endocrinology & Diabetes and Obesity: April 2016—Volume 23—Issue 2—p 150–156)

(VEGF), increased expression of transforming growth factor beta (TGF-β), increased fibronectin and type IV collagen, and overexpression of plasminogen activator inhibitor (PAI)-1 in endothelial and vascular smooth muscle cells. All these molecular changes promote endothelial dysfunction, increased permeability, overproduction of collagen and extracellular matrix, and smooth muscle hypertrophy that contribute to atherogenesis. And finally, diversion of intracellular metabolic intermediates into the hexosamine pathway results in increased gene transcription of TGF-α, TGF-β, and PAI-1. All five of the mechanisms described are set in motion by hyperglycemia-induced mitochondrial dysfunction and excess ROS [6]. All five converge in a final common pathway of endothelial dysfunction and cellular injury, accumulation of lipoproteins in vessel walls, recruitment of T cells and macrophages, and, over time, development of atherosclerotic plaques that predispose to ischemic events such as stroke [4–6].

An important consequence of the epigenetic changes described above is "glycemic memory" [6]. The hyperglycemia, mitochondrial dysfunction, and overabundance of intracellular ROS result in the activation of transcription factors, changes in methylation patterns of histones, and pathologic gene expression that contribute to atherogenesis. These epigenetic changes can be long-lasting and become independent of subsequent glycemic control, creating a legacy effect of early hyperglycemia [6]. This concept has been borne out in large outcome trials in which benefits of early

glycemic control in DM persist over decades despite subsequent elimination of glycemic differences between treatment groups [46]. Correspondingly, early or long-standing hyperglycemia can produce permanent increases in macrovascular risk that are not mitigated by glycemic control later on. This emphasizes the importance of early diagnosis and treatment for DM to prevent complications such as stroke.

The cellular damage caused by mitochondrial dysfunction and excess ROS as well as the dyslipidemia and cholesterol deposition within endothelium induce the release of local and systemic proinflammatory cytokines. These include C-reactive protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interferon-γ, adipokines, and local leukocyte adhesion molecules. These factors facilitate platelet activation and adhesion to the endothelium and recruitment of circulating monocytes and T cells into the arterial wall that further propagate the inflammatory response. Monocytes mature into macrophages and accumulate oxidized lipoprotein particles and cholesterol esters, becoming foam cells which eventually die and contribute to the necrotic lipid-rich core of plaques. This final common pathway of inflammation and atherogenesis results in the development of plaque throughout the arteries, and ultimately the end-organ complication of tissue ischemia and infarction when plaques rupture and cause thrombosis and embolism [4, 5].

In addition to the risks for thrombotic stroke, DM is also an independent risk factor for the development of atrial fibrillation, as shown in the Framingham Study [47], which predisposes to cardioembolic strokes. The mechanism for this association has not been fully elucidated, but it is possibly related to the effects of systemic inflammation on the myocardium, changes in the autonomic nervous system, left ventricular remodeling and hypertrophy, as well as increased arterial stiffness [48].

Pathophysiologic Role of Lipids in Ischemic Stroke in DM

Individuals with DM have increased risk of dyslipidemia, regardless of the presence of obesity, predisposing this population to developing atherosclerosis, and thereby ischemic stroke. The insulin resistance in individuals with DM has multiple effects on lipid metabolism, including increased lipolysis in adipose tissue by enhanced lipoprotein lipase activity, which results in reduced breakdown of chylomicrons and VLDLs, increased synthesis and release of fatty acids, and increase in VLDL production [4]. One of the major physiologic impairments in DM, endothelial dysfunction driven by dyslipidemia, has been shown to be the foundation of atherogenesis [49]. The vascular deposition of excess lipoproteins in the presence of hyperglycemia results in extracellular glycation and oxidative modification of cholesterol and matrix proteins that induce the release of inflammatory cytokines and leukocyte adhesion molecules. These changes promote recruitment and retention of leukocytes within the endothelium and vessel wall, another important step in the development of atherosclerotic plaque [4, 5].

Insulin resistance increases lipolysis in adipose tissue leading to a rise in circulating VLDL, TG, and free fatty acids. Increased levels of non-fasting TG indicate the presence of increased levels of chylomicrons and VLDL [29]. Of these two, VLDL and their remnants are able to penetrate the arterial endothelium. Since cholesterol in remnant particles cannot be degraded when scavenged by intimal macrophages, these cells are transformed into foam cells, which lead to fatty streak formation and eventually development of atherosclerosis [50].

Patients with DM will frequently have relatively normal levels of LDL-C, but with a change in the distribution of particles and an increased shift toward the small, dense subtype of LDL particles. These smaller, more dense LDL particles are toxic to the endothelium and more atherogenic [51]. Diabetes also alters metabolism of HDL-C and reduces plasma levels of HDL. In the setting of insulin resistance, the activity of lipoprotein lipase is reduced by an increase in the ratio of apo CIII/apo CII. This leads to increased off-loading of TG from TG-rich lipoproteins such as VLDL to HDL particles, rendering them better substrates for lipolysis by hepatic lipase, leading to increased catabolism and lower serum levels of HDL. High serum TG levels can also induce endothelial dysfunction. The net effect of these pathologic lipid profile changes is to increase the deposition of lipoproteins and cholesterol esters within the endothelium and reduce the efflux of cholesterol from the vessel wall, contributing to atherogenesis.

Chronic dyslipidemia increases the production of local ROS, directly impairing endothelial cell function [52]. Oxygen free radicals cause membrane and mitochondrial damage and accelerate nitric oxide decay, which reduces its vasodilator activity. Lipoproteins, particularly LDL, tend to accumulate within the intimal layer of blood vessels in the coronary and cerebral circulation, where they may be oxidized by free radicals. These modified LDL particles can then induce smooth muscle cell activation and secrete inflammatory mediators, which in turn enhance further oxidation of LDL particles. Monocytes are recruited into the intima and become tissue macrophages, which internalize oxidized LDL particles via scavenger receptors. This process leads to the creation of lipid-laden foam cells, which compose fatty streaks, the foundation of atherosclerotic plaque.

Not only oxidized LDL particles are directly toxic to endothelial cells, macrophages, and smooth muscle cells, but also their internalization by macrophages promotes the release of inflammatory growth factors, cytokines, and chemokines [53] that propagate the cycle of monocyte recruitment and activation [52]. Activated macrophages then release ROS that also further promotes further LDL oxidation. The released growth factors stimulate smooth muscle cell proliferation and synthesize extracellular matrix proteins, both of which convert the fatty streak into a mature atheroma. Smooth muscle cells, which produce the extracellular matrix protein collagen, tend to stabilize atheromas, whereas inflammatory cells may increase extracellular matrix breakdown and result in unstable plaques [54]. Markers of inflammation, such as interleukin-6 (IL-6) and C-reactive protein (CRP), are often elevated in DM, whereas there are usually fewer smooth muscle cells, both physiologic phenomena that may explain why those with DM are at higher risk of unstable plaques [49]. The central core of lipid-laden cells and fatty debris in the

atherosclerotic plaque may also become calcified. This plaque in turn progressively invades the intimal lumen, or even compresses the underlying media, leading to its deterioration. This may then expose tissue factor and other thrombogenic factors that promote thrombus formation and acute vascular occlusion. Acute plaque rupture tends to occur in plaques that contain large lipid cores, few smooth muscle cells, dense inflammatory infiltrates, and thin fibrous caps [52]. Fibrous caps are typically stabilized by collagen and are continuously undergoing remodeling, which may promote either stabilization or rupture.

Evidence suggests an association between plasma concentration of LDL particles (LDL-P), measured by nuclear magnetic resonance (NMR), and ischemic stroke. An analysis from the Framingham Offspring Study showed that among 3066 middle-aged White participants who were followed for ~15 years, LDL-P was more strongly associated with incident ASCVD (which included MI, CHD death, congestive heart failure, ischemic stroke, and TIA) than LDL-C or non-HDL-C [55]. A recent nested case-control study from the China Kadoorie Biobank showed a significant direct association between LDL-P and risk of ischemic stroke [56]. Another study of South-European population showed that increasing levels of LDL-P, particularly medium size, were associated with the incidence of stroke independent of several covariates, including DM [57]. A discordance analysis from MESA showed that greater magnitude of LDL-P to LDL-C discordance (particularly prevalent in metabolic syndrome or DM) predicts the presence of ASCVD, which includes both CHD and ischemic stroke [58]. Similar findings were seen for discordance between LDL-P and LDL-C and carotid intima-media thickness [59]. A prospective nested case-control study of postmenopausal women from the Women's Health Initiative Observational study showed that baseline LDL-P levels were significantly higher among women with incident ischemic stroke, although the association was attenuated when adjusting for other covariates including DM [60].

Lp(a) has many pathophysiologic properties that could potentially explain its role in ischemic stroke. First, it consists of an apolipoprotein B (apoB) containing lipoprotein, which could directly promote atherogenesis through direct deposition in the arterial wall [61]. Second, Lp(a) has a covalently bound apolipoprotein a molecule, which shares strong sequence homology with plasminogen. Therefore, Lp(a) has a high affinity for immobilized fibrinogen and fibrin, preventing plasminogen binding and promoting antithrombolysis [62]. By competing with plasminogen for endothelial cell receptors, Lp(a) therefore delays plasmin formation and clot lysis. Indeed, patients with elevated Lp(a) have been shown to have decreased propensity toward a bleeding diathesis [61]. This process similarly leads to foam cell formation and atherosclerotic plaque formation [63]. Lp(a) has also been shown to displace plasminogen from the surfaces of activated macrophages in atherosclerotic plaques, minimizing the activation of latent TGF-β, causing smooth muscle cell proliferation and transformation of these cells into more atherogenic phenotypes [64]. The data on Lp(a) in DM has been mixed due to small sample sizes. However, there is evidence that those with DM who have atherosclerotic complications or nephropathy tend to have higher levels of Lp(a) than their counterparts without DM [65].

As discussed earlier, the association of HDL-C with ischemic stroke is overall not as clear, and the relationship between HDL subclasses and stroke becomes even more complex as some studies have found that the association varies by size and subclass of HDL. An analysis from the MESA population showed that higher concentration of large HDL particles was associated with lower risk of stroke in Black individuals [25]. Other studies have reported that individuals with ischemic strokes were found to have significantly smaller HDL size with higher levels of HDL_3 and lower levels of HDL_2 subclasses [66]. The mechanism behind these findings has not been fully elucidated, but it is possible that larger HDL particles contain a higher number of copies of apolipoprotein A1, the major protein in HDL that has been shown to be protective against atherosclerosis in animal models [67]. The protective properties of HDL are thought to be related to its central role in reverse cholesterol transport, a process whereby cholesterol from macrophages in atherosclerotic plaque is externalized to HDL, which then returns it to the liver for metabolism or disposal [68]. HDL also inhibits some of the key inflammatory and oxidative pathways that contribute to atherosclerosis as mentioned above. There is a paucity of data with regard to HDL subclasses and ischemic stroke in patients with DM; however, HDL has been shown to promote islet cell function and improve glycemic control in patients with diabetes, although it is overall less protective against ischemic stroke in this population [69].

Evidence has shown that there is an association between remnant cholesterol (RC), estimated as non-HDL-C minus LDL-C, and ischemic stroke. Among 102,924 individuals from the Copenhagen General Population Study that were followed for up to 14 years, those who had RC ≥58 mg/dL had ~2 times independent higher risk of ischemic stroke compared to those with RC <19 mg/dL (HR 1.99; 95% CI 1.49–2.67). Of note, this association did not significantly change after adjusting for DM (HR 1.94; 95% CI 1.45–2.59) and no evidence for effect modification by diabetes was found [70]. Similar results were obtained in the Copenhagen City Heart Study (HR 2.1, 95% CI 1.5–3.1), which included individuals who were followed for up to 43 years [71].

Evidence of Lipid-Lowering Therapy and Ischemic Stroke in Diabetes

Pharmacologic LDL-C reduction with statin therapy has been proven to be beneficial by multiple studies for stroke prevention. Intensive therapy to target serum lipids and particularly to lower LDL-C remains the hallmark of treatment after a TIA or a stroke. The current guidelines of the American Heart Association (AHA) and the American Stroke Association (ASA) stress the importance of intense statin therapy after an ischemic stroke of atherosclerotic origin [72]. A summary of randomized clinical trials (RCTs) to date examining the effect of different lipid-lowering approaches on ischemic stroke is shown in Table 19.1.

Table 19.1 Summary of RCTs for lipid-lowering therapies in ischemic stroke and diabetes

RCT	Study population	Arms	Outcome (type)	Specific findings for ischemic stroke
Statins				
Heart Protection Study [73]	Adults with DM	Simvastatin vs. placebo	Nonfatal and fatal ischemic stroke (secondary outcome)	RR 0.75; 95% CI 0.66–0.85; $p <$ **0.000**
CARDS [74]	Adults with DM	Atorvastatin vs. placebo	ACS, coronary revascularization, or stroke	HR 0.52; 95% CI 0.31–0.89 $p =$ **0.001**
SPARCL [75]	Adults with previous stroke/TIA	Atorvastatin vs. placebo	Recurrent ischemic stroke	HR 0.84; 95% CI 0.71–0.99; $p =$ **0.03**
Treat Stroke to Target [76]	Adults with previous stroke/TIA	LDL-C 90–110 vs. <70 mg/dL	Composite outcome (ischemic stroke, MI, urgent coronary or carotid revascularization, or CV death)	Adjusted HR 0.78; 95% CI 0.61–0.98; $p =$ **0.04**
Ezetimibe				
IMPROVE-IT [77]	High-risk individuals and those with DM	Ezetimibe vs. placebo	Ischemic stroke (tertiary outcome)	HR 0.79; 95% CI 0.67–0.94; $p =$ **0.008**
EWTOPIA 75 [78]	ASCVD-free individuals aged ≥75 years	Ezetimibe vs. placebo	Composite outcome (SCD, fatal/nonfatal MI, coronary revascularization, or fatal/nonfatal ischemic stroke)	HR 0.78; 95% CI 0.55–1.11; $p = 0.17$
PCSK9 inhibitors				
FOURIER [79]	Patients with ASCVD	Evolocumab vs. placebo	Ischemic stroke (secondary outcome)	HR 0.79, 95% CI 0.66–0.95, $p =$ **0.01**
ODYSSEY OUTCOMES [80]	Patients with recent ACS	Alirocumab vs. placebo	Composite outcome (CHD death, nonfatal MI, fatal/nonfatal ischemic stroke, unstable angina requiring hospitalization)	HR 2.89, 95% CI 1.84–4.56, $p <$ **0.0001** (DM vs. normoglycemic)

(continued)

Table 19.1 (continued)

RCT	Study population	Arms	Outcome (type)	Specific findings for ischemic stroke
Omega-3 fatty acids				
JELIS [81]	Patients with hypercholesterolemia	Eicosapentaenoic acid/statin vs. statin alone	Fatal/nonfatal stroke (secondary outcome)	HR 1.08; 95% CI 0.95–1.22, $p = 0.244$ (incident stroke) HR 0.80; 95% CI 0.64–0.997, $p =$ **0.047** (recurrent stroke)
REDUCE-IT [82]	High-risk individuals and/or DM	Icosapent ethyl vs. placebo	Fatal/nonfatal stroke (secondary outcome)	HR 0.72, 95% CI 0.55–0.93, $p =$ **0.01**
Fibrates				
VA-HIT [83]	Male patients with CHD	Gemfibrozil vs. placebo	Fatal/nonfatal stroke (secondary outcome)	RR 31%, 95% CI, 2–52%, $p =$ **0.036**
BIP [84]	Patients with CHD	Bezafibrate vs. placebo	Ischemic stroke (secondary outcome)	RR 8%, $p = 0.66$
FIELD [85]	Patients with DM	Fenofibrate vs. placebo	Fatal/nonfatal stroke (secondary outcome)	HR 0.90, 95% CI 0.73–1.12, $p = 0.36$
ACCORD [86]	Patients with DM	Fenofibrate vs. placebo	Nonfatal ischemic stroke (secondary outcome)	HR 1.06; 95% CI 0.75–1.50; $p = 0.74$
Extended-release niacin				
AIM-HIGH [87]	Patients with ASCVD	Extended-release niacin vs. placebo	Ischemic stroke (tertiary outcome)	HR 1.61, 95% CI 0.89–2.90, $p = 0.11$
HPS2-THRIVE [88]	Patients with vascular disease	Extended-release niacin vs. placebo	Ischemic stroke (secondary outcome)	RR 0.94, 95% CI 0.82–1.08 $p = 0.3499$

DM diabetes mellitus, *ASCVD* atherosclerotic cardiovascular disease, *CHD* coronary heart disease, *MI* myocardial infarction, *TIA* transient ischemic attack, *HR* hazard ratio, *RR* risk reduction

The Heart Protection Study was a first-of-its-kind large RCT in 2002 that randomized 5963 adults with DM to simvastatin 40 mg vs. placebo and showed that simvastatin had a 25% relative risk reduction in nonfatal and fatal stroke and a 24% relative risk reduction in nonfatal MI, coronary death, stroke, or revascularization. This effect remained significant even in patients who did not have high LDL-C levels at baseline [73].

The Collaborative Atorvastatin Diabetes Study (CARDS) trial in 2004 was a multicenter RCT that randomized 2838 patients from the UK and Ireland with non-insulin-dependent DM, LDL-C <160 mg/dL, TG <600 mg/dL, and one or more

other risk factors (retinopathy, albuminuria, current smoking, hypertension) to low-dose atorvastatin vs. placebo. CARDS was terminated 2 years earlier than expected because it showed significantly lower rates of coronary events, stroke, and coronary revascularization in the atorvastatin arm. More specifically, atorvastatin reduced the rate of stroke by 48% [74].

The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial in 2006 enrolled 4731 patients with previous stroke or TIA, and a moderately elevated LDL-C, and randomized them to high-intensity atorvastatin vs. placebo. SPARCL showed a 16% lower incidence in recurrent strokes in patients receiving atorvastatin in comparison to placebo [75]. In a post hoc analysis, achieving LDL-C lowering of 50% or greater from baseline with atorvastatin 80 mg was associated with 31% risk reduction for stroke [89]. However, another secondary analysis looked specifically at SPARCL patients with type 2 DM and showed that although this group had a higher risk for recurrent stroke, statin treatment did not have a significant effect on the rate of stroke [90].

More recently, the Treat Stroke to Target trial was the first trial to look at different LDL-C target levels for stroke prevention. The investigators randomly assigned patients with ischemic stroke or TIA to a target LDL-C of 90–110 mg/dL vs. <70 mg/dL. The trial demonstrated that a lower LDL-C target was associated with a lower risk of cardiovascular events including stroke, MI, new symptoms leading to urgent coronary or carotid revascularization, or death from cardiovascular causes [76].

The Cholesterol Treatment Trialists' (CTT) Collaborators performed a large meta-analysis of 14 RCTs of statin therapy in 1455 individuals with type 1 DM and 17,220 with type 2 DM and showed that statin use conferred a 21% reduction in stroke risk over a mean follow-up of 4.3 years [91].

Although most evidence for LDL-C reduction in prevention of stroke has been obtained from statin trials, other studies support additional benefit from non-statin agents. In a subgroup analysis of the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) trial, the use of ezetimibe on top of statin therapy was found to confer a 39% risk reduction in ischemic stroke among 4933 patients with DM [77]. On the other hand, the Ezetimibe Lipid-Lowering Trial on Prevention of Atherosclerotic Cardiovascular Disease in 75 or Older (EWTOPIA 75) randomized ASCVD-free Japanese patients aged ≥75 years to ezetimibe vs. usual care and showed reduction of the composite outcome; however, no difference in the incidence of stroke was seen between the two groups [78].

Other more potent LDL-C-lowering agents such as PCSK9 inhibitors have been shown to further reduce the risk of ischemic stroke in patients with DM who do not achieve LDL-C targets despite maximally tolerated statin therapy and ezetimibe. The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial randomized 27,564 patients with ASCVD and LDL-C levels ≥70 mg/dL who were already on a statin to receive evolocumab 140 mg every 2 weeks or 420 mg every 4 weeks or placebo. FOURIER showed that evolocumab reduced the risk of ischemic stroke by 27% after a median follow-up of 2.2 years [92], which was observed regardless of the presence of diabetes [79].

The Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trial randomized 18,924 patients with recent acute coronary syndrome hospitalization with LDL-C levels ≥70 mg/dL on high-intensity statin therapy to alirocumab or placebo. Alirocumab was shown to reduce the risk of ischemic stroke by 27% without increasing the incidence of hemorrhagic stroke and had a similar effect among patients with or without a history of cerebrovascular disease [80]. Interestingly, alirocumab produced twice the absolute reduction in cardiovascular events, including stroke, in patients with compared to those without DM [93].

Other agents without LDL-C-lowering effect have been studied. In a subgroup analysis of Japan EPA Lipid Intervention Study (JELIS), which randomized 18,645 Japanese patients with hypercholesterolemia randomized to 1800 mg per day of eicosapentaenoic acid (EPA) plus a low-intensity statin or statin alone (control group), EPA administration in patients with a history of stroke reduced the risk of stroke by 20% [81]. The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) showed that icosapent ethyl reduced the risk of fatal or nonfatal stroke by 28% after a median follow-up of 4.8 years. In the subpopulation of patients with DM ($n = 4787$), the rate of cardiovascular death, stroke, or myocardial infarction was reduced from 22.2% to 21.2% ($p < 0.00001$) [82].

The Veterans Affairs HDL Intervention Trial (VA-HIT) randomized male patients with CHD to gemfibrozil vs. placebo and showed a significant risk reduction in stroke (of which 90% were ischemic) in the gemfibrozil arm after 5 years of follow-up [83]. The Bezafibrate Infarction Prevention (BIP) study randomized patients with a history of CHD to bezafibrate vs. placebo and did not find risk reduction in the primary outcome (MI or sudden death) nor in ischemic stroke [84]. Interestingly, a substudy of BIP participants with no history of stroke or TIA at baseline showed that the risk of incident ischemic stroke or TIA was associated with TG >200 mg/dL (OR 1.27; 95% CI 1.01–1.60) [31]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study randomized patients with type 2 DM to fenofibrate vs. placebo and did not find a difference in the primary outcome (defined as coronary events only). One of the secondary outcomes was total stroke, and no difference was observed in FIELD between the two arms (HR 0.90; 95% CI 0.73–1.12) [85]. However, it is important to note that the trial was not powered for this outcome. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) LIPID study randomized statin-treated patients with type 2 DM to fenofibrate vs. placebo and did not find a significant reduction in the primary outcome nor ischemic stroke (HR 1.05; 95% CI 0.75–1.10) [86]. A population-based cohort in patients with DM from South Korea showed reduced risk of ischemic stroke (HR 0.621; 95% CI 0.463–0.833) in fenofibrate users compared to nonusers in a median follow-up of 3 years [94]. However, a propensity-matched cohort study of patients with metabolic syndrome from Korea found no difference between fibrate/statin vs. statin monotherapy (HR 0.48; 95% CI 0.18–1.23) [95].

The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial randomized patients with established ASCVD to extended-release niacin vs. placebo and

did not find a significant risk reduction in the primary composite outcome nor in ischemic stroke (HR 1.61; 95% CI 0.89–2.90) [87]. The Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trial randomized adults with vascular disease to extended-release niacin vs. placebo and did not find a significant risk reduction for ischemic stroke (HR 0.94; 95% CI 0.82–1.08) [88].

Conclusions

Ischemic stroke is highly prevalent in the adult population, particularly among patients with DM. The pathogenesis of ischemic stroke in DM does not appear to be related to hyperglycemia, but rather to other comorbidities strongly associated with DM, in particular disturbances in lipoproteins, including LDL, TG-rich lipoproteins, and Lp(a), which may have a strong role in the development of atherosclerosis in the presence of DM. Evidence supports the important role of aggressive LDL-C-lowering therapies (statins, ezetimibe, PCSK9 inhibitors), and more recently of TG-lowering agents (i.e., omega-3 fatty acids) in the prevention of ischemic stroke, which has remained consistent among patients with DM. Additional studies are needed to better characterize the role of other lipid parameters such as HDL—and lipoprotein subclasses—as well as Lp(a).

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Part III
Lipoprotein Treatment in Diabetes

Chapter 20
About Randomized Clinical Trials Related to Lipoproteins in Diabetes Mellitus

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Introduction

Many wise people have made statements relevant to the practice of medicine, including Hippocrates (c. 460 BC–370 BC) who commented that "Life is short, the art of medicine long; the opportunity is fleeting, experience perilous, and decisions difficult." More recently, in 1982, Richard Doll stated that "Every time a doctor treats a patient … he is performing an experiment." Fortunately, in this era of evidence-based medicine, we have many studies and, specifically, randomized controlled trials (RCTs) to guide clinical practice, including individual patient care and development of treatment algorithms and guidelines, and to inform public health policy.

As diabetes mellitus, dyslipidemia, and their vascular complications are increasingly common and costly, both in personal and economic terms, many research studies related to the management of lipoproteins in people with diabetes have been conducted, are in progress, and are in development. The most directly relevant to clinical practice is the RCT. Most RCTs in the field of lipoproteins in diabetes relate to adults with type 2 diabetes mellitus or to an admixture of people with type 1 diabetes and type 2 diabetes. Lipid drug studies are sometimes conducted specifically in youth with type 1 diabetes, such as part of the Adolescent type 1 Diabetes cardio-renal Intervention Trial (AdDIT) study, which evaluated the effects of a

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statin and an angiotensin-converting enzyme (ACE) inhibitor (or their combination) on surrogate vascular end points [1]. The conduct of such studies and their translation from the research setting to clinical practice, if merited, have undoubtedly contributed to the improving outcomes of diabetes and its risk of cardiovascular disease and microvascular complications. "Negative" study results can also be helpful in guiding clinical practice and future research, provided that the trial was well designed and conducted. Furthermore, the largest clinical trials often include sufficient participants with diabetes that robust subgroup analyses are possible. In this chapter, we describe the elements of a good RCT, challenges to its conduct, and aspects to consider when reporting or reading and assessing a clinical trial, including its generalizability to clinical practice and the future of RCTs.

Definition

An RCT is a prospective scientific experiment comparing the effects of a specific treatment strategy in an experimental group with an alternate strategy in a similar (control) group, in which chance (randomization) determines to which group each subject is allocated, so as to reduce bias [2].

Most RCTs related to lipoproteins in diabetes evaluate the clinical effects of a single lipid drug vs. a placebo, as in the Scandinavian Simvastatin Survival Study (4S) Study [3], the Collaborative Atorvastatin Diabetes Study (CARDS) [4], and the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) [5] trial, or test a combination of treatments vs. a single treatment, such as in the Action to Control Cardiovascular Risk in Diabetes (ACCORD)-LIPID [6] study, which tested fenofibrate and simvastatin vs. placebo and simvastatin in adults with type 2 diabetes. In the Heart Protection Study (HPS) [7], simvastatin vs. placebo and combination antioxidant vitamins E, C, and beta-carotene versus placebo were evaluated in a 2 × 2 factorial designed trial. More recently, two trials evaluating the effects of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors versus placebo, in combination with optimal statin therapy, have completed, with substantial numbers of subjects with diabetes included [8–11].

Common RCT end points are usually hard (often centrally adjudicated) clinical events such as mortality, myocardial infarction, leg amputation, kidney disease, retinopathy, neuropathy, or a combination thereof. Some alternate RCT end points are intermediate measures of vascular damage such as carotid intima-medial thickness and results of pulse-wave analysis or of lipoprotein-related measures such as LDL cholesterol levels. Rather than testing a drug, some RCTs related to lipoproteins in diabetes will test the effects of a diet or lifestyle, as discussed elsewhere in this book.

Precursors to and Phases of an RCT

Preclinical Research An RCT usually stems from many years of costly biochemical, cell-based, animal, and human preclinical research. Prior to and after the conduct of an RCT testing a pharmaceutical agent, there are several general "phases" of trials. A drug may be tested in more than one phase simultaneously, in different trials, and some trials may overlap two phases. Many regulatory and ethics committee approvals are also required, ideally including trial registration, such as at the National Institutes of Health (NIH) trial registry https://clinicaltrials.gov or other registries. Such registries can help investigators and the public find out about trials and are a repository of the original study design.

There are five phases of trials, as described below [12]:

Phase 0. A phase 0 trial is an exploratory study usually conducted in a small number of subjects (often less than 20) using subtherapeutic drug doses.

Phase 1. Phase 1 studies are early-stage testing in human subjects, predominantly to evaluate the safety and pharmacologic aspects such as drug absorption, distribution, excretion, and half-life. Subjects are usually healthy volunteers and often young adults. Usually, small numbers of subjects (10–20) are tested, often in specialized facilities.

Phase 2. Phase 2 studies are usually conducted in larger groups (often 100–300) of people with the condition of interest, in this case diabetes, so as to demonstrate safety and efficacy. A phase 2 study of a lipid drug in people with diabetes may report side effects, effects on renal and liver function and on glycemia and lipid levels.

Phase 3. Phase 3 studies aim to provide conclusive evidence with regard to the safety and effectiveness of a test drug. A phase 3 RCT usually involves hundreds to thousands, even tens of thousands, of subjects, with the condition of interest (e.g., diabetes or its complications) in multiple centers in multiple countries.

Phase 4. After regulatory body approval (e.g., by the USA's Federal and Drug Administration (FDA)), phase 4 studies monitor long-term safety and efficacy and are essential, as often people given the drug differ clinically from those subjects in whom the earlier phase studies were conducted. If there are sufficient concerns, drugs may be removed from clinical use at this stage or warnings related to its clinical use in certain conditions mandated.

Elements of a Good RCT

The quality of RCTs and the evidence resulting from them can vary; hence, it is important not only that the clinicians, scientists, statisticians, and community representatives involved in their planning and conduct have a good understanding of

optimal RCT design and conduct, and also that people who may utilize RCT results in their clinical practice or in their own research can assess study quality [2].

Important elements of an RCT include subject selection, characterization, randomization and masking, study end-point choice and measurement, statistical power and data analysis, reporting and interpretation of study outcomes, recognition of potential confounders, relevance to clinical practice, and its generalizability.

General Study Considerations An RCT must be of sufficient scientific quality to be able to satisfactorily answer the questions of interest; to account for the potential confounders, such as arising from different responses to treatment between men and women or between younger and older subjects; and to control for statistical uncertainty (type 1 and type 2 errors, discussed below). Scientific quality would include ensuring that sufficient numbers of participants are recruited, accuracy in their characterization, measurement of the study outcomes, appropriate number of repeated measurements (where required), well-implemented methods of randomization and allocation concealment, and minimal attrition and low rates of missing/incomplete observations on participants. Study outcomes refer to measurements pertaining to an individual participant, such as success/failure, time to an event, or levels/scores in the case of continuous measurements. Study end points on the other hand generally refer to the summary measures of the benefit of the intervention over the control and are usually differences in mean levels (for continuous outcomes), odds ratios, or proportions (for binary outcomes) and hazard or risk ratios (for time to event studies).

Sample size calculations in an RCT are usually based on the minimum clinical difference that is deemed worthwhile between the control and intervention groups in the primary study outcome. This chosen difference to seek is usually based on the results of previous studies, if they exist, and on clinical judgement, from epidemiologic studies and from the likely cost of treatment, and reflects the potential importance and value of the benefit which could be provided by the intervention in clinical practice. If the study outcome is a clinical vascular event, larger studies for several years are usually required, given their slow development. Further, the phenomenon of metabolic memory may delay the clinical manifestations of modifying lipoprotein or glucose levels for years. Study size impacts the statistical power of a study (and vice versa). The statistical power of a study is the likelihood that the study will correctly identify a true advantage of a tested intervention compared with control; that is, it is the probability that a true effect of a certain (usually clinically useful) size will be detected, and a statistically significant result will be obtained from an RCT of a given size. In general, this likelihood is fixed in the study design and generally set at 80% or 90%, based on numerous assumptions. Thus, if a study on many thousands of patients (not likely to be repeated) is planned, the investigators would want the chance of the study "missing the targeted effect" (if it really is beneficial) to be small, usually 1 in 10 chance—i.e., 90% power, or a 9 in 10 chance of the study correctly declaring a significant difference. For other studies (e.g., cancer trials), a 4 in 5 chance is often deemed sufficient. The chance of missing the targeted

effect is referred to as the type 2 error (a statistical term) and is the complement of the power, i.e., power + type 2 error = 1. Fixing the power is required to determine the sample size of the study, and so the calculations underpinning the planned sample size (including assumed control group event rates, expected compliance losses, designed power, chosen level of significance [*p* value], and size of treatment effect being sought) are usually performed in the design stage before the RCT begins. This information needs to be included in the applications for RCT funding and also in the study reports.

The inclusion and exclusion criteria are important parts of an RCT and subsequently impact the translation of the trial results to clinical practice. Both should be carefully considered in study design and described in detail in all RCT reports. Ideally, the subjects included will represent those who are expected to benefit from the treatment being tested in the RCT and are representative of the majority with the condition of interest. The exclusion criteria are usually designed to avoid participation of those perceived to be at high risk of potential harm by either treatment or who may confound the study outcomes, such as those with limited life expectancy from other causes or with, for example, severe renal or liver disease.

Randomization It is the process used to allocate willing, eligible, and consented participants to one or other study treatment, hence into either the intervention or the control arm of an RCT, and it aims to ensure similarity between the two groups at baseline, such that any observed differences emerging from the trial are related to the intervention (or to the play of chance (*p* values and confidence intervals quantify the likelihood of chance differences of the magnitude observed)). The randomization process intends preferential assignment to any of the RCT arms. When the randomization is not equal (e.g., 2:1), the treatment assignment is still randomly allocated but weighted to the group receiving the higher number of patients. Subjects should generally only be randomized after written informed consent has been provided, and they (and usually also the investigators) should remain masked to which treatment group they have been allocated (discussed below). In most RCTs, randomization is done "centrally," away from the investigators and trial participants, and often involves telephone, the Internet, or interactive voice-activated programs, which must be available around the clock, particularly for international multicenter RCTs. If the randomization procedure has worked well, the major demographic groups of the treatment arms at baseline should not differ significantly regarding such variables as age, sex, diabetes duration, baseline lipids, blood pressure, smoking status, and glycemic control (e.g., HbA1c levels), except occasionally by chance alone.

There are several types of randomization in common use [13]. In a *simple randomization* process, each trial subject has an equal chance of being assigned to the intervention arm or the control group. This type of randomization can be achieved using random numbers from a statistical textbook or more commonly using a computer-generated algorithm.

Stratified randomization is sometimes used to achieve better balance between groups on factors which are known to influence study outcomes. For example,

separate random allocation sequences may be used for men and for women or for people with diabetes with, versus without, prior diabetic renal damage or cardiovascular disease to ensure better balance between groups on these factors.

Permuted block randomization is commonly used for small RCTs (of less than 1000 subjects), as simple randomization can sometimes result in large chance differences in factors (e.g., such as sex) between treatment groups. In block randomization, blocks having equal numbers of control and intervention allocations (e.g., two controls (C) and two intervention (I) subjects in a block of four) are used, with the order of treatments in the block being randomly permuted. For example, a block of four subjects has six possible treatment arrangements: CCII, CICI, CIIC, IICC, ICIC, and ICCI. A random number sequence is used to choose the particular block, which then sets the allocation order. A minor drawback of block randomization however is that if at the end of the study there are numerous incompleted blocks in use across many centers, there may still be a substantial overall imbalance in the number of participants allocated to each of the treatment(s). A further limitation is that, unless the block sizes are allowed to vary randomly in length (e.g., 4, 6, 4, 8, 6), the overall sequence can sometimes be identified, resulting in the potential ability to predict the next treatment allocation to be issued should you choose to randomize a particular patient.

Dynamic random allocation methods, also known as adaptive allocation, are an alternate randomization procedure. This process allocates trial participants to the RCT treatment arms by first checking the allocation tallies of similar participants who have already been randomized, so as to achieve the best balance between treatment groups across all nominated stratification variables. Stratified minimization and dynamic balanced randomization are two examples. Computer-based algorithms are able to facilitate this process.

Unsuitable randomization methods include alternate allocation to control or intervention groups, or allocation based on the day of clinic attendance or birth date, or sealed envelopes held at the site. With these methods, it cannot be guaranteed that the process has not been breached (e.g., by transilluminating envelopes or by rescheduling a subject's randomization day) and that each participant was truly randomly allocated to their originally assigned treatment.

The allocation status should of course be concealed from the site staff and the participant. This process is called allocation concealment.

Blinding or Masking RCTs in the field of lipoproteins in diabetes usually involve subjects being randomized to one of the two groups, one of which will receive a single active drug and the other of which will receive a matching placebo, as in CARDS [4]. In some RCTs, one active drug that is usually the best currently available treatment is given to all subjects, and one test drug or matching placebo is added (such as in the ACCORD-LIPID study [6]). Masking refers to the process by which the treatment allocation is hidden from the people involved in the study [14]. *Double-blind* refers to both the participant and the investigators being unaware of the treatment allocation. This process serves to minimize the potential for observer bias to occur and also for participants dropping out because of knowledge of treat-

ment arm or, if possible, through other means of acquiring one of the treatments, for example, if they were determined to receive a specific treatment. The masking of whether a treatment is active or placebo is more feasible in RCTs with tablet therapies, unlike surgical trials or device-related trials; however, when there are very common and specific drug effects, such as flushing with nicotinic acid, this can be difficult. For example, in the AIM-HIGH trial [15], low-dose nicotinic acid was added to the placebo to induce some flushing, but the dose used was sufficient to elevate HDL-C levels. With lipoprotein-related studies, a potential confounder is that some trial participants and their general practitioners, either inadvertently or in a desire to try to work out if the person is receiving the active drug, will order and discuss a lipid profile. In our experience, this has resulted in some trial participants choosing to cease participation in the trial as they determined (rightly or wrongly) that they were not allocated to the active treatment arm.

Outcomes There are usually multiple outcomes in RCTs, and what the primary, secondary, and sometimes even tertiary outcomes are should be pre-stated and the trial planned with appropriate study duration to be able to detect realistic changes, to provide adequate statistical power to avoid type 1 or type 2 errors, and to enable appropriate subgroup analyses. Outcomes, which are usually measured for each study participant, may range from hard clinical events, e.g., death, to intermediate measures such as carotid intima-media thickness (IMT) to lipid levels.

Statistical Power and Data Analysis Statistical analysis of RCT data provides an estimate of the magnitude of difference in outcome rates between the groups, and the probability that the trial results could have occurred by chance alone. A commonly used cutoff at which statistical significance is taken is at $p < 0.05$, meaning that the probability of the trial result (e.g., drug or benefit over placebo) occurring by chance alone is less than 5%. This value is referred to as the significance level of the trial, and the complement (95%) of the confidence level. It may be thought of as the level we are prepared to accept of a false-positive result. If there are multiple RCT end points, statistical significance may be taken at lower p values. There are two types of statistical errors that can occur in an RCT: type 1 error (chance of a false-positive result) and type 2 error (chance of missing a true benefit).

Type 1 error refers to concluding that there is a real difference between treatments (or groups) when none exists, i.e., rejecting the null hypothesis when it is correct.
Type 2 error refers to concluding that there is no effect of treatment when one does truly exist, i.e., accepting the null hypothesis when it is incorrect.

Clinical significance is a judgement that an effect is large enough to change the way a patient should be treated. Clinicians and those devising treatment algorithms and health policy can be assisted in these judgements by calculations of the number of patients needed to treat (NNT) to derive benefit (discussed below) and hazard or odds ratios. The hazard ratio is the proportion of subjects in the intervention arm of

the RCT (as the numerator) compared with the proportion of subjects in the control arm (as the denominator) having a (predefined) event during the RCT time period.

The number needed to treat is the number of patients who must be treated to prevent one specified event. It is the reciprocal of the absolute risk reduction. The NNT with a particular drug may vary according to the subject characteristics. For example, in the FIELD study, the NNT with fenofibrate for approximately 5 years to prevent one amputation in all FIELD subjects was 197, but the NNT to prevent one amputation in patients with a previous foot ulcer or amputation was only 25 [16].

Novel RCT Designs

In recent years, there has been an increase in the use of novel clinical trial designs that aim to achieve greater flexibility and efficiency in the way new treatments are assessed. The purpose of such designs is to make use of a common clinical trial infrastructure to answer many questions in parallel or sequentially, rather than relying on separate trials to address each new question of interest.

Adaptive Designs A key feature of novel clinical trial designs is the notion of an adaptive design [17]. An adaptive design is a design in which one or more features of the design change over the course of the trial. These design changes are called adaptations, hence the term adaptive design. Incorporating adaptive features in the design allows the study to evolve in response to the observed data, so that the design can be optimized in response to information that may not have been available at the beginning of the study. There are many design features that can be adapted in this manner, including the following:

- *Sample size*: The study may be stopped earlier than planned or may be extended to be larger than planned, on the basis of information obtained during the study.
- *Randomization*: The chance of being allocated to the available treatment arms may be changed over time based on participant responses or characteristics.
- *Treatments*: New experimental treatments may be added during the study as they become available or existing treatments may be dropped if they do not look promising.
- *Phases*: Seamless transition between the RCT phases discussed earlier in this chapter allows a promising treatment to graduate from early phase to later phase assessment within the same study.
- *Doses*: Treatment dosage may be escalated or de-escalated during the study on the basis of toxicities observed in earlier participants.
- *Population characteristics*: The study population may be enriched over time to selectively recruit participants having characteristics more likely to respond to treatment.

Typically, when we refer to an adaptive design, we are referring to a design in which one or more of these features are adapted based on participant outcomes or responses observed subsequent to randomization. However, it is also possible to adapt design features on the basis of pre-randomization baseline characteristics, also called covariates. Accordingly, sometimes the terms *response-adaptive* and *covariate-adaptive* are used to distinguish between designs that adapt based on response (post-randomization) information versus designs that adapt based on baseline (pre-randomization) information [18]. For example, a trial design that modifies the randomization proportions to each treatment arm on the basis of the primary outcomes observed in previous participants (with the intention of giving greater access to the more promising treatment) would be called response-adaptive, whereas a design that modifies the allocation proportions on the basis of imbalances in baseline characteristics (with the intention of achieving baseline balance) would be called covariate-adaptive. Trials with covariate-adaptive features provide a more traditional form of adaptivity. For example, the dynamic random allocation methods discussed earlier in this chapter, such as the minimization approach to achieving randomization covariate balance, have been in common use for decades [19]. In contrast, trials with response-adaptive features provide a more novel form of adaptivity that has only become common over the last decade or so.

Master Protocols Adaptive design features are typically embedded in one of a number of novel design frameworks governed by a master protocol that provides a common infrastructure for examining multiple questions simultaneously [20]. There are three primary master protocol designs that have become increasingly common over the past decade:

1. *Platform studies* provide an infrastructure for comparative evaluation of multiple treatments simultaneously, typically compared to a common control arm, including the ability to add and drop treatments during the trial without pre-specifying all treatments that may be studied [21, 22]. Platform studies typically involve a multistage structure that leads to an alternate name, multi-arm multistage studies (MAMS) [23]. A defining feature of a platform study is that it is a general framework for studying a disease or condition of interest that may have many potential existing or future interventions of interest.
2. *Basket studies* have a design in which a targeted therapy is evaluated on multiple tissue or disease types that express the same target. Basket studies are most popular in oncology, where they are particularly useful for studying multiple tumor types that have a common sub-type, such as a common genetic mutation [24]. This is often undertaken in earlier phase trials in which all participants receive the same treatment, but may also involve randomization to a control or experimental treatment [25]. A key motivation for using basket studies is the ability to borrow (or share) information between disease sub-types that may be too rare to provide adequate information on their own, thus providing increased statistical efficiency.

3. *Umbrella studies* contrast with basket studies in that they include a single disease type that expresses multiple targets. Thus, whereas basket studies involve a single sub-type such as a common genetic mutation, umbrella studies involve multiple sub-types such as different genetic mutations. For this reason, umbrella studies typically assess multiple experimental treatments for a given condition, each with different targets.

In each of these frameworks, an overarching master protocol governs the common infrastructure by which the study runs, with appendices to the master protocol providing the specifics of individual sub-studies, such as the different comparison domains of a platform study or the different sub-types of an umbrella study.

Complexities Despite their flexibility and efficiency, novel designs introduce a range of complexities that must be carefully managed to preserve trial integrity. An important implication of these complexities is that more extensive planning is required for trials with novel designs. In particular, although design characteristics are permitted to change over time, the manner and frequency of change should be anticipated and pre-specified. Unplanned changes to an RCT design have the potential to introduce significant bias into an RCT design [26]. Furthermore, the need to accommodate flexible design features typically introduces significant operational complexities in database and randomization systems, as well as in the final statistical analysis which will often require novel analytical techniques [27, 28]. For these reasons, novel designs require extensive input from clinical trial methodologists, particularly biostatisticians, and typically require statistical simulations of the design in order to understand the complex operating characteristics prior to finalizing key design features. Guidelines for adaptive trial designs have recently been developed as supplements to existing guidelines for more traditional designs [29, 30].

Novel Designs in Diabetes Although adaptive design features have occasionally been used in diabetes trials [31, 32], the uptake of the master protocol framework has been quite limited [20]. This may reflect fundamental differences with diseases where novel designs have been common, such as oncology, but also likely represents an opportunity for greater use of these novel design features in diabetes trials. RCT methodology is a rapidly evolving field with new types of RCT designs being constantly developed and existing but underutilized design features gaining greater prominence. In the past, diabetes trialists have used various nonstandard design features such as stepped wedge cluster designs [33, 34] and active run-in periods [5]. It is beyond our scope to review all of these design features here; however, given their successful implementation in past diabetes trials, increased utilization of RCT designs with adaptive and master protocol structures seems feasible and represents an important opportunity to increase the efficiency and flexibility with which new diabetes interventions are assessed.

Challenges of Conducting an RCT Related to Lipoproteins in Diabetes Mellitus

There are many challenges to conducting and interpreting the results of an RCT related to lipoproteins in diabetes. Some can be at least partially, if not fully, controlled by study design, but others cannot, but still should be addressed. Challenges include aspects related to diabetes and its complications and to lipoproteins, study outcome definitions and their measurement, and study reporting and generalizability. It is also important to recognize that people who participate in an RCT can enjoy lower adverse clinical outcome rates, even if allocated to a placebo arm, than those who do not. This phenomenon of people tending to perform better when in a study is called the Hawthorne effect and was first described in a Harvard-based study evaluating the relationship between productivity and work environment in an industrial setting, the Hawthorne Works Plant [35]. The day-to-day efforts of a person with diabetes, including attention to their diet, exercise, nonsmoking status, foot care, and adherence to often multiple recommended drug treatments, and to monitoring, such as of glucose levels, are substantial. These factors can substantially impact their weight, vascular risk factors, and risk of development of diabetes complications and potentially the magnitude of observed response, or lack thereof, to a lipoprotein-targeted intervention.

Different Types and Stages of Diabetes

The type of diabetes, be it type 1 or type 2 diabetes, the stage and duration of diabetes, and the level of glucose control can impact lipoprotein levels and potentially the response to treatment being tested in an RCT. The amount of endogenous insulin production and degree of insulin resistance can differ substantially in both types of diabetes, and this and the level of glycemic control, usually reflected by HbA1c levels, can impact lipoproteins. Hypertriglyceridemia and low HDL cholesterol levels are more common in type 2 diabetes than in type 1 diabetes, and this dyslipidemic profile is accentuated by poor glycemic control, obesity, or renal dysfunction [36, 37]. The number of people with type 1 or type 2 diabetes, their glucose control modality and level, and complication status should be considered and reported and subgroup analyses performed if there are enough subjects available to provide adequate statistical power.

Multiple Risk Factors for Complications Including Genetic and Epigenetic Effects

As mentioned above, lipoprotein levels are impacted by many variables, some of which are fixed, for example, genotypes affecting lipoprotein levels [38] and treatment response [39], and others may vary over time, such as diet, smoking, exercise, and medication adherence. All these things tend to balance out between treatment arms in larger studies but may still confound trial results in smaller trials of just a few hundred people. Epigenetic effects may enable environmental factors and even the lipoprotein-targeting drugs to modulate the effects of the inherited genotype [40].

Whilst abnormal lipid levels are major risk factors for both the macrovascular and microvascular complications of diabetes [36, 37], other factors such as age, diabetes duration, family history, poor glycemic control, hypertension, smoking, obesity, and periodontal disease [41] contribute to the development and progression of vascular disease in diabetes and hence may impact the rates of complication development.

Slow Vascular Disease Development

Atherosclerosis and its related clinical events of myocardial infarction (which is often silent in people with diabetes), stroke, claudication, gangrene and amputation, retinopathy, nephropathy, and neuropathy develop over years to decades. Atherosclerosis can begin in youth, even in the absence of diabetes, and the process of this inflammatory process is accelerated in diabetes [42]. Because of this, if vascular events or even some intermediate measures of vascular damage such as carotid intima-media thickness (IMT) are RCT outcomes, then the lipid-related study will need to last for years to modify these. Many lipid drug trials in diabetes with vascular event end points have a 5-year intervention period, and to increase the number of events that will accrue and statistical power, large numbers (thousands) of subjects are included. Intermediate end points, such as vascular function (e.g., flow-mediated dilation, pulse-wave analysis) and structural changes (such as assessed by coronary artery intravascular ultrasound (IVUS), carotid IMT, and CT-coronary angiograms), which may change over shorter time frames, are sometimes used in RCTs (or included as smaller substudies within larger RCTs). These studies usually still take several years to complete and may have lesser impact on clinical practice. An additional factor to consider is that of metabolic memory (also known as the legacy effect) of glucose control, which has been demonstrated in both type 1 and type 2 diabetes [43, 44].

Metabolic Memory or the Legacy Effect

These comparable terms were coined in relationship to the (type 1 diabetes) Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) and the 10-year follow-up of the United Kingdom Progression of Diabetes Study (UKPDS) type 2 diabetes studies. The legacy effect refers to the phenomenon by which the body's tissues, including arteries, retinae, kidneys, and nerves, continue to respond to poor or good glycemic control for years after the glucose control has improved or worsened. This memory can last for years, even decades. The UKPDS data demonstrates a legacy effect of glycemia for 10 years after 10 years with an HbA1c ≈ 7.0% [43]. This is in keeping with the time frame of metabolic memory in type 1 diabetes evidenced by the DCCT/EDIC study, in which ≈5.9 years of intensive vs. conventional diabetes management (HbA1c 9 vs. 7%) lowered vascular complication rates for 8–12 or more years [44]. It is not yet clear if there is a threshold level for metabolic memory and how long this effect is maintained for a given time at each HbA1c level across the full HbA1c spectrum. Potential modulators of metabolic memory are epigenetic effects and/or advanced glycation end products (AGEs), such as in the vascular wall matrix and basement membranes.

This legacy effect may also apply to other non-glucose vascular risk factors. The UKPDS also examined if there was a legacy effect for tight vs. less tight blood pressure control. Whilst the UKPDS showed clear vascular complication benefit for lower blood pressure levels, the UKPDS follow-up study did not find evidence for persistence of benefit beyond the randomized period [45]. Potential mitigating circumstances are the relatively high blood pressure targets during the UKPDS.

What About Lipid Memory? Some early lipid diet or drug trials with post-trial follow-up found persistent cardiovascular and mortality benefit in those with lower on-trial lipid levels, even though lipid level differences ceased soon after study ended [46–50]. As yet, there are no specific lipid-related studies exclusively in diabetes for which the legacy effect has been published, and given the major benefit of lipid-lowering drugs in type 2 diabetes, particularly statins, it could be ethically difficult to conduct such a study in the future.

Such metabolic memory for glucose, and potentially for lipid levels, means that the full impact of a lipoprotein-targeted intervention in diabetes may not be fully evident until many years after its commencement. The extremely high human and economic costs of running large and long-duration RCTs related to lipoproteins in people with diabetes usually require major pharmaceutical industry support and/or support from multiple funding agencies.

Variability in Some RCT End Points

When considering RCTs related to vascular damage in diabetes, one should consider the variability of the vascular end-point measure. Microaneurysms, a commonly used indicator of diabetic retinopathy, can regress [51]. Albuminuria, a commonly used measure of diabetic nephropathy, is highly variable within an individual, being affected by such factors as exercise, blood pressure, and glycemia. Even without a specific intervention, such as ACE inhibitor drugs, increased albuminuria levels can spontaneously regress in people with type 1 diabetes [52]. It is now recognized that in people with diabetes, renal function, reflected by glomerular filtration rate or creatinine clearance, may still decline even in the absence of increased urinary albumin loss [53]. Other renal function measures commonly used to characterize trial subjects and which may be an RCT end point include serum creatinine levels, time till doubling of serum creatinine levels, change in estimated glomerular filtration rate (eGFR slope), circulating cystatin C levels, end-stage renal disease, and commencement of peritoneal or renal dialysis or renal transplantation [54]. Renal function effects in RCTs may differ according to which renal function end point is chosen.

Measurement issues can also impact RCT results. Factors such as subject preparation (e.g., prandial status will greatly alter triglyceride levels), biological variation (e.g., circadian and seasonal effects), issues related to sample collection, storage and processing, quality of the assays chosen, operator-dependent factors, and any human or undetected instrument error may impact RCT biomarker levels.

In RCTs, traditional lipid levels (and other detailed lipoprotein-related characteristics such as lipoprotein composition, size, apolipoprotein content, related enzyme activities, lipoprotein modifications (discussed in other book chapters) such as nonenzymatic glycation and oxidation and lipoprotein function) can vary greatly over time. Whilst circulating lipoproteins exist only for hours to days, in most RCTs related to lipoproteins in diabetes, measures of lipoprotein quantity or quality are usually only evaluated at several time points, which may be months, sometimes years, apart. Whilst HbA1c levels reflect mean blood glucose levels over the previous 2–3 months, as yet there are no equivalents for lipid levels. Furthermore, for lipoprotein and vascular disease-related RCTs, it must be remembered that lipoprotein levels in blood are measured, yet this is not the site of disease. It is the amount of lipoprotein that has accumulated within the vasculature that is of major importance to clinical events. As an example, oxidized LDL is more atherogenic than unmodified (normal) LDL, and oxidized LDL levels are 70-fold higher in the arterial wall than in blood [55], yet intravascular oxidized levels cannot be readily measured in an RCT. Similarly, inflammatory markers, such as serum C-reactive protein (CRP) and soluble forms of the vascular cell adhesion molecules (CAMs), are often measured in serum samples from RCTs [56, 57], yet it is likely the level of inflammation within the arteries, retinae, and renal tissue that matters most. Indeed, many blood- and urine-based biomarkers that are measured in RCTs are not at the site of disease, though they still often correlate with the risk of event and/or treatment

benefit. In some studies, the study outcome is the circulating level or quality of a lipoprotein.

Pleiotropic Effects

Drug treatments used in RCTs related to lipoproteins in diabetes may also have pleiotropic effects, which can favorably or unfavorably affect study end points. Many pleiotropic effects of some lipid drugs, such as statins and fibrates, are relevant to vascular health and include anti-inflammatory, antioxidant, antiplatelet, anticlotting, vasodilation, angiogenesis-related, and genetic effects and alterations in cell signaling [58, 59]. Not all pleiotropic effects are potentially vasoprotective; for example, fenofibrate increases levels of the vascular risk marker homocysteine [60].

It is important to consider the potential contribution of pleiotropic drug effects in RCT reporting and assessment, though it cannot be readily quantified. Whilst lipid levels, including elevated triglycerides and low HDL cholesterol levels, are commonly associated with and predictive of the vascular complications of diabetes and of regression of increased albuminuria [36, 37], fenofibrate, which substantially lowers triglyceride levels and increases apoA1 and HDL levels, was associated with significant improvements in diabetic retinopathy [61], nephropathy [62], and amputations [16] in the FIELD trial. In the FIELD trial, most of these major microvascular benefits were reported not to clearly relate to changes in traditional lipid levels. The authors' preliminary data analyses of combined microvascular and combined macrovascular end points in the FIELD study support independent associations with factors related to oxidative stress, inflammation, and adipokines and effects of fenofibrate on circulating levels of many of these biomarkers.

Reporting and Interpreting RCT Results

In reporting or assessing and interpreting the results of an RCT, the underlying hypothesis, aims, methods, and results should be clearly presented, along with a balanced discussion of the study outcomes, strengths, and weaknesses of the study design, similarities and differences with previous studies, remaining or new questions, and clinical implications. The appropriateness and limitations of the study design (e.g., length of intervention), subject inclusion and exclusion criteria, clinical and laboratory tools used, and statistical power should be considered, and the authors, prompted if need be by their manuscript reviewers and editors, should provide sufficient detail to enable a thorough assessment [63].

Adverse Events

There must be sufficient detail collected and reported in an RCT to judge the severity and relationship of possible adverse events to the treatment(s) allocated. A definition of adverse events has been adopted by the International Conference on Harmonization [64], which is a collaboration between drug marketing regulatory bodies in the USA, the European Union, and Japan. "An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event therefore can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product." A subset of adverse events is that of adverse drug reactions, which are those unfavorable conditions that may reasonably be related to the drug, provided that it was used in the approved dose range in the target population for the treatment of the appropriate disease.

Adverse events or drug reactions can be classified as serious or nonserious. A serious adverse event is one that (a) causes death, (b) is life-threatening, (c) necessitates or prolongs an inpatient hospital stay, (d) causes persistent or significant disability, or (e) causes a birth defect. An example of a serious adverse event in a lipid drug RCT is that of rhabdomyolysis possibly due to statin treatment (sometimes aggravated by another concomitant medication), and nonserious adverse events may include headache, rash, or lethargy. Nonserious adverse events are usually divided into those that can confidently be regarded as not being drug related, whilst others are classified as those that are either definitely or possibly drug related.

So as to enable comparison between different RCTs, adverse events are usually listed by body system, e.g., cardiovascular, gastrointestinal, and renal. Commonly used schemes are those of the International Classification of Diseases (ICD) [65] and the Medical Dictionary for Regulatory Activities (MedRA) [66].

As stated by the Declaration of Helsinki World Medical Association, "it is the duty of the physician in medical research to protect the life, health, privacy and dignity of the human subject" [67]. To facilitate this in RCTs, the research subjects should be advised to report any changes in their well-being, and the trial nurses and doctors should document and report the details. A data safety and monitoring committee (DSMC) or board of independent, preappointed, and appropriately experienced people can review the events whilst unmasked to treatment allocation. They play a vital role in ensuring RCT subject safety and can mandate the early cessation of an RCT as soon as it is evident that the treatment being tested is harmful or beneficial overall. For example, the ILLUMINATE trial of a CETP inhibitor, torcetrapib, was ceased early due to an excess of harm (including death and severe hypertension) [68]. CARDS [4] was intended to be a 4-year primary prevention double-blind trial of atorvastatin 10 mg/day vs. matching placebo in patients with type 2 diabetes with an LDL cholesterol level $\leq$160 mg/dL, fasting triglycerides $\leq$600 mg/dL, and at least one additional cardiovascular risk factor. The study end

was to take place after 304 primary end points, but there was substantial benefit of atorvastatin seen on the planned interim analysis, after 149 primary end points, and the independent steering committee recommended that the study should be stopped early. This (CARDS) result and similar findings among people with diabetes from the Heart Protection Study [7] were some of the major RCT findings in this area to influence clinical practice, such that many people with type 2 diabetes are now offered statin treatment to reduce CVD risk.

Generalizability of RCT Results to Clinical Practice

In 1980, Bernard Fisher said, "I consider the prospective randomized trial mechanism one of the most important advances of this century and the most effective method available for transferring medical practice from an art to a science."

One of the main purposes for conducting RCTs is to guide clinical practice. In making clinical decisions for individual patients, preparing guidelines, or deciding public policy, major factors to be considered include the relevance of the RCT to the clinical question, the similarity of the RCT participants to those in question, the quality of the RCT, and whether other evidences, including the outcomes of other RCTs, agree.

RCTs often focus on one or two interventions, which are given to specially selected well-motivated and usually treatment-adherent subjects who will be monitored relatively closely compared to usual clinical practice. In the "real world," patients may differ from subjects in the RCT, and because of this need to compare applicability to the clinical care setting, subject inclusion and exclusion criteria and demographics should be described in detail. Potential differences relate to age—clinical care subjects may be older or younger or of different ethnic background to the RCT participants. They may have multiple comorbidities, which may include renal or liver dysfunction, which may impact drug handling. They may be taking many other medications, excess alcohol, or recreational drugs, which may increase the risk of drug interactions. As another example, two major RCTs of fenofibrate in people with type 2 diabetes demonstrated protective effects against diabetic retinopathy [61, 69], but this evidence may not apply directly to people with type 1 diabetes. Such a trial is in progress (see FAME 1 Eye study at trials.gov). Whilst RCTs of statins have shown vascular benefit in tens of thousands of subjects, those with advanced renal or liver disease, or moderate degrees or both, which are not uncommon in clinical practice, have usually been excluded. The SHARP trial has now demonstrated benefits of simvastatin and ezetimibe in combination to reduce cardiovascular events in people with advanced renal disease [70]. It is because of such factors that the (phase 4) marketing surveillance and reporting of major adverse events and drug interactions are very important. Regulatory bodies can add safety warnings or even withdraw a therapy after its approval for use in clinical practice. For example, the FDA has issued safety warnings against the use of full-dose (80 mg) simvastatin, due to higher rates of myositis [71]. Post-marketing

Table 20.1 Baseline characteristics and eligibility criteria of participating trials

	Number of patients	Treatment comparison (mg/day)	Median follow-up in survivors (years)[a]	Mean age (years)	Baseline LDL-C (mmol/L)	Prior CHD[b]	Other vascular disease[c] *n* (%)	No prior vascular disease *N* (%)	Women *n* (%)	LDL-C difference at 1 year (mmol/L)
Statin vs. control										
SSSS	4444	S20-40 vs. placebo	5.4	59	4.88	4444 (100)	126 (3)	0 (0)	827 (19)	−1.77
WOSCOPS	6595	P40 vs. placebo	4.8	55	4.96	338 (5)	193 (3)	6096 (92)	0 (0)	−1.07
CARE	4159	P40 vs. placebo	5.0	59	3.58	4159 (100)	0 (0)	0 (0)	576 (14)	−1.03
Post-CABG	1351	L40-80 vs. L2.5-5	4.3	61	4.02	1351 (100)	37 (3)	0 (0)	102 (8)	−1.07
AFCAPS/ TexCaps	6605	L20-40 vs. placebo	5.2	58	3.89	10 (1)	9 (0)	6586 (0.99)	997 (15)	−0.94
LIPID	9014	P40 vs. placebo	6.0	61	3.88	9014 (100)	905 (10)	0 (0)	1516 (17)	−1.03
GISSI–P	4271	P20 vs. no treatment	2.0	59	3.92	4271 (100)	179 (4)	0 (0)	587 (14)	−0.35
LIPS	1677	F80 vs. placebo	3.9	60	3.42	1677 (100)	142 (8)	0 (0)	271 (16)	−0.92
HPS	20,536	S40 vs. placebo	5.4	63	3.38	13,386 (65)	8865 (43)	3161 (15)	5082 (25)	−1.29
PROSPER	5804	P40 vs. placebo	3.3	75	3.79	1881 (32)	1026 (18)	3254 (56)	3000 (52)	−1.04
ALLHAT-LLT	10,355	P40 vs. usual care	4.9	67	3.76	1188 (11)	1788 (17)	8037 (78)	5051 (49)	−0.54
ASCOT-LLA	10,305	A10 vs. placebo	3.3	63	3.44	15 (1)	1435 (14)	8860 (86)	1942 (19)	−1.07
ALERT	2102	F40 vs. placebo	5.5	50	4.14	400 (19)	241 (11)	1702 (81)	715 (34)	−0.84

CARDS	2838	A10 vs. placebo	4.1	62	3.03	9 (1)	97 (3)	2738 (96)	909 (32)	−1.14
ALLIANCE	2442	A10-80 vs. usual care	4.7	61	3.80	2442 (100)	162 (7)	0 (0)	434 (18)	−1.16
4D	1255	A20 vs. placebo	4.0	66	3.25	630 (50)	666 (53)	344 (27)	578 (46)	−0.89
ASPEN	2410	A10 vs. placebo	4.0	61	2.93	578 (24)	302 (13)	1663 (69)	811 (34)	−0.99
MEGA[d]	8214	P10-20 vs. usual care	5.0	58	4.05	42 (1)	53 (1)	8119 (99)	5547 (68)	−0.67
JUPITER	17,802	R20 vs. placebo	2.0	66	2.70	0 (0)	0 (0)	17,802 (100)	6801 (38)	−1.09
GISSI-HF	4574	R10 vs. placebo	4.2	67	3.06	1797 (39)	4574 (100)	0 (0)	1032 (23)	−0.92
AURORA	2773	R10 vs. placebo	4.6	64	2.58	659 (24)	743 (27)	1663 (60)	1050 (38)	−0.99
CORONA	5011	R10 vs. placebo	3.0	73	3.55	4377 (87)	5011 (100)	0 (0)	1180 (24)	−1.19
Subtotal (22 trials)	134,537	–	4.8[e]	63[e]	3.70[e]	52,668 (39)	26,554 (20)	70,025 (52)	39,008 (29)	−1.08
More vs. less statin										
PROVE-IT	4162	A80 vs. P40	2.1	58	2.62[f]	4162 (100)	328 (8)	0 (0)	911 (22)	−0.65
A to Z	4497	S40 then S80 vs. placebo then S20	2.0	60	2.09[f]	4497 (100)	479 (11)	0 (0)	1100 (24)	−0.30
TNT	10,001	A80 vs. A10	5.0	61	2.52	10,001 (100)	1537 (15)	0 (0)	1902 (19)	−0.62
IDEAL	8888	A40-80 vs. S20-40	4.8	62	2.64[f]	8888 (100)	971 (11)	0 (0)	1702 (19)	−0.55

(continued)

Table 20.1 (continued)

	Number of patients	Treatment comparison (mg/day)	Median follow-up in survivors (years)[a]	Mean age (years)	Baseline LDL-C (mmol/L)	Prior CHD[b]	Other vascular disease[c] *n* (%)	No prior vascular disease *N* (%)	Women *n* (%)	LDL-C difference at 1 year (mmol/L)
SEARCH	12,064	S80 vs. S20	7.0	64	2.50	12,064 (100)	1062 (9)	0 (0)	2052 (17)	−0.39
Subtotal (5 trials)	39,612	–	5.1[e]	62[e]	2.53[e]	39,612 (100)	4377 (11)	0 (0)	7667 (19)	−0.51
Total (27 trials)	174,149	–	4.9[e]	63[e]	–	92,280 (53)	30,931 (18)	70,025 (40)	46,675 (27)	–

Trials are ordered by their date of publication

A atorvastatin, *F* fluvastatin, *L* lovastatin, *P* pravastatin, *R* rosuvastatin, *S* simvastatin, *LDL-C* LDL cholesterol, *CHD* coronary heart disease, *4D* Die Deutsche Diabetes Dialyse Studie, *A to Z* Aggrastat to Zocor, *AFCAPS/TexCAPS* Air Force/Texas Coronary Atherosclerosis Prevention Study, *ALERT* Assessment of Lescol in Renal Transplantation, *ALLHAT-LLT* Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial, *ALLIANCE* Aggressive Lipid-Lowering Initiation Abates New Cardiac Events, *ASCOT-LLA* Anglo-Scandinavian Cardiac Outcomes Trial Lipid Lowering Arm, *ASPEN* Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in Non-Insulin-Dependent Diabetes Mellitus, *AURORA* A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events, *CARDS* Collaborative Atorvastatin Diabetes Study, *CARE* Cholesterol And Recurrent Events, *GISSI-HF* Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza cardiac, *GISSI-P* Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico, *HPS* Heart Protection Study, *IDEAL* Incremental Decrease in End-Points Through Aggressive Lipid Lowering Study Group, *JUPITER* Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin study group, *LIPID* Long-term Intervention with Pravastatin in Ischaemic Disease, *LIPS* Lescol Intervention Prevention Study, *MEGA* Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese Study Group, *Post-CABG* Post-Coronary Artery Bypass Graft, *PROSPER* PROspective Study of Pravastatin in the Elderly at Risk, *PROVE-IT* Pravastatin or Atorvastatin Evaluation and Infection Therapy, *SEARCH* Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine, *SSSS* Scandinavian Simvastatin Survival Study, *TNT* Treating to New Targets, *WOSCOPS* West of Scotland Coronary Prevention Study

[a] Estimated with standard Kaplan–Meier methods, with patients censored at their date of death

[b] History of MI or other symptomatic CHD

[c] History of intracerebral bleed, transient ischemic attack, ischemic stroke, unknown stroke, peripheral artery disease, or heart failure (if known)

[d] Includes 382 randomized patients who were excluded from the trialists' primary publication

[e] Median follow-up, and mean age, baseline LDL-C, and LDL-C difference at 1 year are weighted by the trial-specific variances of the observed logrank (o-e) statistic for major vascular events

[f] These three trials did not have active run-in periods; the values shown are the estimated on-treatment LDL cholesterol levels in the standard statin group

Table 20.2 Number of participants with diabetes by trial

	Diabetes mellitus			
	Type 1, *n* (%)	Type 2[a], *n* (%)	Any type, *n* (%)	No diabetes, *n* (%)
4S	24 (0.5)	178 (4.0)	202 (4.5)	4242 (95.5)
WOSCOPS	8 (0.1)	68 (1.0)	76 (1.2)	6519 (98.8)
CARE	193 (4.6)	393 (9.4)	586 (14.1)	3573 (85.9)
Post-CABG	27 (2.0)	89 (6.6)	116 (8.6)	1235 (91.4)
AFCAPS/TexCAPS	0	155 (2.3)	155 (2.3)	6450 (97.7)
LIPID	106 (1.2)	676 (7.5)	782 (8.7)	8232 (91.3)
GISSI–P	120 (2.8)	462 (10.8)	582 (13.6)	3689 (86.4)
LIPS	39 (2.3)	163 (9.7)	202 (12.0)	1475 (88.0)
HPS	615 (3.0)	5348 (26.0)	5963 (29.0)	14,573 (71.0)
PROSPER	51 (0.9)	572 (9.9)	623 (10.7)	5181 (89.3)
ALLHAT-LLT	0	3638 (35.1)	3638 (35.1)	6717 (64.9)
ASCOT-LLA	0	2527 (24.5)	2527 (24.5)	7778 (75.5)
ALERT	280 (13.3)	116 (5.5)	396 (18.8)	1706 (81.2)
CARDS	3 (0.1)	2835 (99.9)	2838 (100)	0
Total	1466 (1.6)	17,220 (19.1)	18,686 (20.7)	71,370 (79.3)

Reprinted from The Lancet, 371 (9607), Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis., pp.:117–25. Copyright (2008), with permission from Elsevier

[a] Includes 13 participants with diabetes of unknown type

surveillance also led to warnings against the combination of a statin with a fibrate, due to the higher risk of myositis [72].

Apart from the efficacy of a therapy in treating lipoprotein disorders in people with diabetes, other aspects should be considered. This includes the rates and types of adverse effects of the treatment and the acceptability of the treatment regimen and any required monitoring. As dyslipidemia per se is not associated with symptoms (unless there is severe hypertriglyceridemia, which can cause acute pancreatitis and eruptive xanthomata [73]) and long-term treatment is needed, the ease of adherence should be high and side effects minimal to achieve good long-term compliance in practice. Another important aspect to consider in the translation of RCT results to clinical practice is the economic costs to the individual and others who cover the treatment costs. Health economics analyses and quality of life data related to an RCT can be helpful in this regard.

Landmark Trials of Lipoprotein Treatments in Diabetes

In recent decades, several RCTs related to lipoprotein treatments in diabetes have resulted in changes to clinical practice. Effects on clinical practice are usually greater after two or more large RCTs are supportive. As summarized in Table 20.1, the major statin trials addressing the value of lowering LDL cholesterol in diabetes have been the HPS, ALLHAT-LLT, ASCOT-LLA, CARDS, 4D, ASPEN, MEGA,

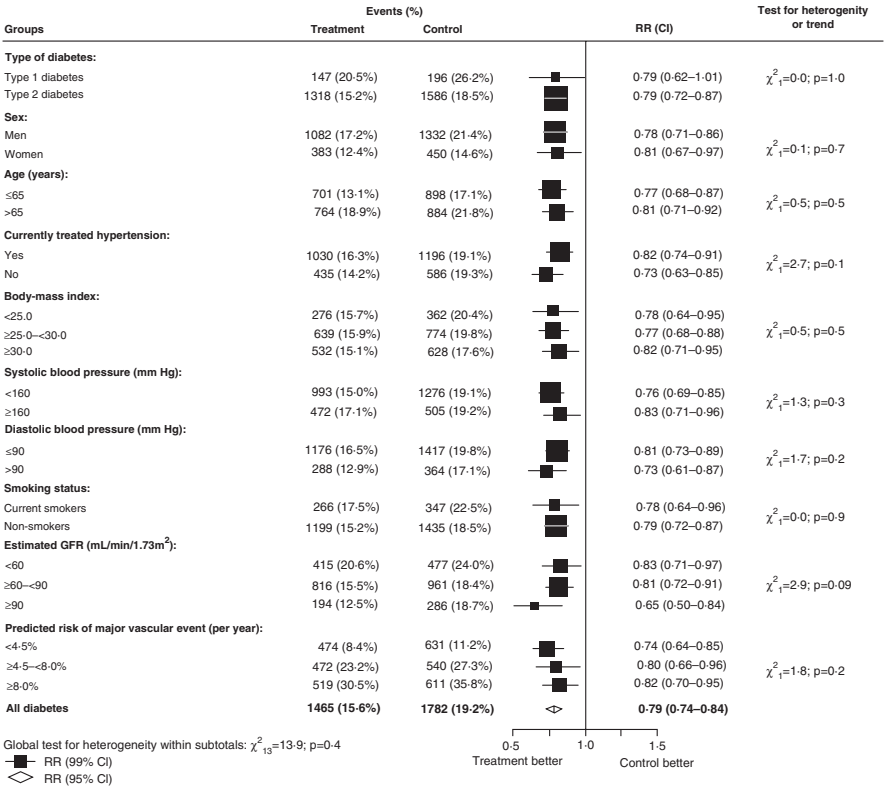

Fig. 20.1 Proportional effects on major vascular events per mmol/L reduction in LDL cholesterol by baseline prognostic factors in participants with diabetes rate ratios (RRs) are plotted comparing the outcome in participants who were allocated statin treatment to that in those allocated control, along with their CIs. The area of each square is proportional to the amount of statistical information in that particular category. Diamonds or squares to the left of the solid line indicate benefit with treatment, but this is significant (i.e., $p < 0.05$ and $p < 0.01$, respectively) only if the diamond or horizontal line does not overlap the solid line. The RRs are weighted to represent the reduction in the rate per 1 mmol/L LDL cholesterol reduction achieved by treatment at 1 year after randomization. Tests for trend are shown for subgroups involving three categories, and heterogeneity tests for those involving two. GFR glomerular filtration rate. (Reprinted from The Lancet, 371(9607), Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis., pp: 117–25. Copyright (2008), with permission from Elsevier)

A-Z, TNT, IDEAL, and SEARCH trials, all of which have included more than 1000 individuals with diabetes in their trials, though of these, only the CARDS, 4D, and ASPEN trials were conducted solely among people with diabetes [74]. Furthermore, only the HPS and ALERT trials have included more than 200 individuals each with type 1 diabetes (Table 20.2) [75]. Both completed trials of PCSK9 inhibitors, FOURIER and ODYSSEY, included larger numbers of participants with diabetes and have reported the benefits in those groups [8–11].

Collectively, however, they provide in meta-analysis (see below) strong evidence of the value of statin therapy in reducing vascular risk among people with both type 1 and type 2 diabetes (Fig. 20.1) [75].

The trials of fibrate therapy have also been of great interest for the treatment of people with diabetes. Both the FIELD study and then the ACCORD-LIPID study were conducted exclusively among people with type 2 diabetes, and both demonstrated large benefits to lower CVD risk among those individuals with dyslipidemia (low HDL-c and high TG), despite negative overall primary end points among all subjects [5, 6, 76]. This has been a highly consistent finding in all of the large fibrate trials. Further, both studies demonstrated highly significant and large reductions in measures of retinopathy [61, 69], in both cases a pre-specified other end point. FIELD in addition demonstrated reduced amputations with fenofibrate [16], and in both trials, new or progression of albuminuria was reduced with treatment [6, 62]. Major CVD benefits in individuals with diabetes have been demonstrated since publication of the first edition, by large-scale trials of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, which demonstrate a profound lowering of LDL cholesterol levels, even when added to high-dose statin therapy [8, 9].

Combining Results from RCTs

Systematic Reviews: A systematic review is a synthesis of published results and conclusions of previous relevant investigations. There are explicit methods for the literature search, study appraisals, and data analysis to answer a clearly stated a priori research question. The related term "meta-analysis" refers to the statistical techniques used in a systematic review, which pools the results of several RCTs. Both RCTs and systematic reviews of RCTs can provide valuable healthcare decision support. RCTs are often helpful to address a specific question such as whether low- or high-dose statin therapy is more effective at reducing cardiovascular events, and sometimes a single RCT will provide clear-cut evidence, such as in the CARDS [4]. However, when the effect sizes in the RCT results are contrasting or modest, a systematic review can be helpful. An example of a helpful systematic review relates to the use of statins in people with diabetes (discussed above). The Cholesterol Trialists Treatment Collaboration was able to demonstrate the benefit of statin therapy for people with type 1 diabetes [75], among other findings.

The Art of Medicine: There are not always clear-cut results of RCTs available to answer all clinical questions related to the treatment of lipoproteins in people with diabetes. This may relate to lack of resources to conduct the required studies, contrasting results of similar RCTs, or be due to a suitable RCT still being in progress. For example, there are no RCTs of statins in only people with type 1 diabetes for the primary prevention of cardiovascular disease, nor are there completed RCTs related to the use of fibrates or a fibrate and a statin to reduce microvascular events in type 1 diabetes. In these situations, expert opinion groups or the individual clinician must decide. Factors they may take into consideration are the results of RCTs in related groups (e.g., they may use the results of RCTs in type 2 diabetes patients or in nondiabetic subjects to decide treatment recom-

mendations for people with type 1 diabetes). Results of case series, pilot studies, and judgement based on knowledge of the disease process and effects of treatment may also be influential. The physician should discuss the reasons and risks behind their treatment recommendations, which may include not using a drug treatment, with the patient and regularly review the medical evidence and adjust their treatment recommendations accordingly. Such is the art of medicine.

Other Resources

The purpose of this chapter has been to provide an overview of relevant issues to the conduct and interpretation of RCTs related to lipoproteins in diabetes. This proper design, conduct, and reporting of an RCT is a large enough topic for several textbooks [77, 78] and courses. For readers who wish to learn more about RCTs, there are many courses, including short courses, papers, websites, and textbooks that may be of assistance. Discussions with trialists and studying or working as part of a multidisciplinary team experienced in RCTs are also valuable tools.

Conclusions and the Future of RCTs of Treatments Related to Lipoproteins in Diabetes

Diabetes mellitus and lipid problems are common and costly health conditions. Existent non-pharmacologic and drug therapies for the treatment of lipid disorders have already improved clinical outcomes for people with diabetes, but residual risk remains high, including some related to quantitative and qualitative changes in lipoproteins. Additional treatments, including more efficient drugs from existent drug classes, new classes of drugs, and gene-based therapeutic agents, are emerging. After rigorous preclinical testing and testing in phases 0, 1, and 2 clinical trials, some therapies will reach the (phase 3) RCT stage and, if successful, clinical practice and post-marketing (phase 4) surveillance. The size, workload, and cost of such RCTs are usually extremely high. To maximize the knowledge gained from RCTs and the cost-effectiveness of relevant research, it is desirable, and usual practice, to obtain consent for and store blood, including DNA, and urine for future analyses. Sometimes, the biomarkers subsequently quantified were not known of at the time, or the earlier available assays lacked sensitivity or specificity. With the evolution of biomedical science, increasingly sophisticated biomarkers are available. Such tools as MRIs, IVUS, PET scans, microRNAs, microparticles, circulating stem cells, epigenetics, lipidomics, proteomics, and metabolomics can help evaluate the disease process in living subjects, and assay results can provide mechanistic insights. Often, these studies require separate research funding, but existent data and a biorepository from a well-conducted RCT make this type of research extremely time-, labor-, and

cost-effective. Most importantly, RCTs related to lipoproteins in diabetes have made substantial contributions to the well-being of people with diabetes, and further RCTs in this area should continue to do so in the future.

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Chapter 21
Effects of Lifestyle (Diet, Plant Sterols, Exercise, and Smoking) and Glycemic Control on Lipoproteins in Diabetes

Peter Clifton

Abbreviations

ADA	American Diabetes Association
Apo	Apolipoprotein
BMI	Body mass index
CHD	Coronary heart disease
CHO	Carbohydrate
CVD	Cardiovascular disease
DSE	Diabetes support and education
EPA	Eicosapentaenoic acid
GI	Glycemic index
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment for insulin resistance
ILI	Intensive lifestyle intervention
LA	Linoleic acid
LCD	Low-calorie diet
LDL	Low-density lipoprotein
P:S	Polyunsaturated-to-saturated fat ratio
PVD	Peripheral vascular disease
RR	Relative risk
SFA	Saturated fatty acids
TG	Triglycerides
VLCK	Very-low-carbohydrate ketogenic
VLDL	Very-low-density lipoprotein
WMD	Weighted mean difference

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Lipid Conversion Units

To convert mmol/L of cholesterol to mg/dL, multiply by 38.8, and to convert mmol/L of triglyceride to mg/dL, multiply by 88.5.

General Considerations

Lipid changes occur quickly in response to diet, and in 2 weeks, 80% of the maximal effect is seen, with no further change beyond 4 weeks. If diet is maintained, then the effect on circulating lipid levels is persistent. Regardless of the background diet, or if the study is parallel or crossover, then contrasting the effect of two diets on lipids at the end of 4 weeks is more than adequate to see a clear effect. Washout periods are not required. BMI, diabetes control, or type of diabetes does not appear to interact with responses to fat or fiber, but they do appear to be related to response to cholesterol and plant sterols.

Dietary Fat and Lipoproteins

Saturated, n6 Polyunsaturated, and Monounsaturated Fat

In nondiabetic subjects, the effects of dietary saturated, polyunsaturated, and monounsaturated fat are well described in a meta-analysis of 60 clinical trials published in 2003 by Mensink et al. [1]. In this paper, 1% of energy from saturated fat elevated LDL cholesterol by 0.03 mmol/L when it replaced carbohydrate, while n6 polyunsaturated fat lowered LDL cholesterol levels by 0.02 mmol/L when it replaced carbohydrate. The concentration of HDL cholesterol was elevated by about 0.01 mmol/L by saturated and unsaturated fat in comparison with carbohydrate. Thus, an absolute 10% energy reduction in saturated fat (a very large change) and replacement of this fat entirely with n6 polyunsaturated fat would lower LDL cholesterol levels by 0.5 mmol/L (or about 14–15%). If the 10% saturated fat was replaced entirely by carbohydrate, then LDL cholesterol would fall by 0.3 mmol/L, HDL cholesterol would fall by 0.1 mmol/L, and triglyceride levels would rise by 0.24 mmol/L. Is there any evidence that people with diabetes behave in a different way to changes in dietary fat composition? Somewhat surprisingly, it is difficult to answer this question as there have been a very limited number of studies in people with diabetes—either type 1 or type 2 diabetes—and much of the focus has been on glycemic rather than lipid control. All studies contained very small numbers of volunteers, except for the Oxford study.

The Oxford study was begun at a time (1973) when the standard dietary advice was a high-fat (40% of energy), low-carbohydrate diet (maximum of 40% of energy)

and little attention was paid to the type of fat in the diet, which was mostly saturated. The study contrasted the standard diet with a modified fat diet of 30% of energy with a polyunsaturated/saturated (P/S) fat ratio of 0.9 or above. Two hundred and fifty people with newly diagnosed type 2 diabetes were enrolled between 1973 and 1976 [2]. Total cholesterol levels were 0.7 mmol/L lower on the modified fat diet averaged over 1, 3, and 5 years, which is consistent with an estimated reduction in saturated fat of about 15% and an increase in polyunsaturated fat of 10% [3]. Dietary records were not collected.

In a controlled study by Storm et al. [4], a palmitic acid-rich diet (16% of energy) increased total cholesterol compared with a carbohydrate-rich diet or a stearic acid-rich diet (13% of energy) for 3 weeks each, but surprisingly LDL and HDL cholesterol were not different. However, only 15 volunteers with type 2 diabetes were included in this relatively short study, and the difference in LDL cholesterol levels (based on total cholesterol changes) may have been about 0.2–0.3 mmol/L, which is about half of that expected. A 6-week study in 16 patients with type 2 diabetes compared 20% of energy as saturated fat with 20% as monounsaturated fat and a 20% trans-monounsaturated fat diet [5]. Lipids, lipoprotein, and apoB levels were the same on the three diets, which would not have been expected. A very small study in Pima Indians ($n = 7$) by Abbott et al. [6] showed a fall in LDL cholesterol levels by 17% with a change in saturated fat of 14% of energy, and the changes were very similar to those seen in the nondiabetic subjects in the same study. Kinetic studies showed that these changes were due to slower conversion of VLDL to LDL. HDL cholesterol and fasting TG concentrations were not significantly altered. Heine et al. [7] performed a 30-week study of two diets, one with a low polyunsaturated-to-saturated fat ratio (P:S 0.3) and one with a P:S of 1.0, in 14 patients with type 2 diabetes in a crossover study. Total dietary fat was 37–38% with linoleic acid (LA) increasing from 4.2% to 10.9%. LDL cholesterol levels declined by 9.8% ($p < 0.01$) during the high P:S diet. The change in LDL cholesterol levels of 0.4 mmol/L is what would be expected based on the Mensink meta-analysis. A combination of weight loss and reduced dietary saturated fat lowered LDL cholesterol levels by 10–17% with a high-carbohydrate or high-monounsaturated-fat diet, respectively [8].

Overall, despite the small number of studies, the data suggest that people with type 2 diabetes respond to dietary lipid changes in the same way as nondiabetic subjects. However, a Cochrane review in 2007 [9] of dietary advice for adults with type 2 diabetes, which examined 18 trials of more than 6 months' duration with 1467 participants and a wide variety of dietary interventions, concluded that there was insufficient data to conclude anything other than that exercise lowered HbA1c. In a review of polyunsaturated fat interventions on glycemic control in people with type 2 diabetes, Telle-Hansen [10] found that 6 of 12 studies of vegetable oil found no effect compared with control while some changes were seen in the other 6. Despite these intervention studies, a recent pooled analysis from prospective cohort studies demonstrated that higher levels of LA in blood were associated with a 43% reduced relative risk for type 2 diabetes [11]. This is in line with the results from the ULSAM study. Men who developed type 2 diabetes had a lower

proportion of LA and a higher proportion of saturated fatty acids (SFAs) (C:14 and C:16) in serum cholesterol esters compared with those who did not develop type 2 diabetes [12].

Dietary Fat vs. Carbohydrate

Lipids

Much of the major disagreements in nutrition over the last 20 years for people with type 2 diabetes have been related to replacing saturated fat with carbohydrate as opposed to unsaturated fat. For many years (1970s–1990s), a very-high-carbohydrate (and high-fiber) diet was strongly advocated, although some researchers suggested that high-carbohydrate diets were theoretically not optimal because of the lowering of HDL cholesterol and elevation of fasting triglyceride levels (which is also seen to the same degree in nondiabetic subjects). The meta-analysis (n = 133 subjects, nine studies) of Garg in 1998 [13] focused on comparing high-carbohydrate diets (49–60% of energy) with high-monounsaturated-fat diets (24–33% monounsaturated fat, 37–50% total fat). A high-monounsaturated-fat diet was associated with a reduction in fasting triglyceride levels of 0.36 mmol/L (19%) and a reduction in VLDL cholesterol levels of 22.5%. HDL cholesterol levels increased by 0.05 mmol/L or 4%. The remainder of the fat in both diets was 7–21%, presumably about 5% polyunsaturated fat with the remainder being saturated fat, but surprisingly in the meta-analysis, neither of these two fats was mentioned. LDL cholesterol levels were not different between the two diets, but the data are uninterpretable in relation to the effect of saturated fat, and one can only assume that saturated fat was not different between diets. The lack of difference between carbohydrate and monounsaturated fat on LDL cholesterol levels is consistent with the Mensink data [1] in nondiabetic subjects.

A later meta-analysis by Kodama et al. [14] examined 19 studies with 306 patients and again showed no effect of a high-carbohydrate diet on LDL cholesterol levels with a rise in triglycerides of 13% and a lowering of HDL cholesterol levels of 8%. These changes are similar to those expected in nondiabetic subjects [15]. Whether these changes with a high-carbohydrate diet promote an increased risk of cardiovascular disease (CVD) is not clear, but there are no data available to refute this suggestion. Secondary intervention studies in nondiabetic subjects suggest that replacing saturated fat with carbohydrate is not beneficial, whereas replacing it with n6 polyunsaturated fat is beneficial [16]. A (pro-atherogenic) smaller LDL particle size in those following a high-carbohydrate diet may contribute to the adverse effect [15].

Replacing carbohydrate with protein and/or polyunsaturated fat would be expected to have similar effects to replacing it with monounsaturated fat. This was demonstrated by Thompson et al. [17] and a small (n = 11 participants) study of a high-protein, lower carbohydrate Paleolithic diet that showed a reduction in

triglyceride levels of 0.4 mmol/L and an increase in HDL cholesterol levels of 0.08 mmol/L [18]. Although the Paleolithic diet had a lower glycemic load than the standard American Diabetes Association (ADA) diet, it was also reduced in energy and the 3 kg weight loss may account for some or all of these effects.

Glycemic Control

Glycemic control is very important in the management of lipids in diabetes as poor control can markedly elevate triglycerides. People with a triglyceride of >3.4 mmol/L had a threefold increase in the risk of having an HbA1c of >7% compared with those with a triglyceride of <1.7 mmol/L [19].

The Garg meta-analysis [13] showed that the high-monounsaturated-fat diet lowered fasting glucose by 0.23 mmol/L with improvement in a variety of other glucose indices such as 24-h glucose and insulin profiles and urinary glucose excretion in the few studies in which they were done. HbA1c and fructosamine did not change, but the duration of the studies was short. The Kodama meta-analysis also showed no changes in HbA1c with a low-carbohydrate diet. A more recent meta-analysis by Qian et al. [20] of 24 studies with 1460 participants showed a reduction in fasting glucose of 0.56 mmol/L, expected decreases in TG, and increases in HDL cholesterol, but in addition a decrease in systolic blood pressure. In the 14 studies with HbA1c measured, there were no differences between the diets.

A very-low-carbohydrate ketogenic diet (VLCK or a ketogenic diet <50 g of carbohydrate or 10% of energy) is popular despite the difficulty of maintaining a very-low-carbohydrate diet for a long term. A meta-analysis of ketogenic diets versus control diets was performed of eight RCTS of greater than 6 months' duration with 648 participants by Rafiullah et al. [21].

Compared with control diets, the VLCK diet resulted in a greater decrease in HbA1c after 3 months (weighted mean difference [WMD]: −6.7 mmol/mol or −0.61%; $P < 0.001$; moderate-certainty evidence) and after 6 months (−0.58%; low-certainty evidence). There was a significantly greater weight loss with the VLCK diet after 3 months (WMD: −2.91 kg) and after 6 months (−2.84 kg; low-certainty evidence). The VLCK diet was not better than a control diet after 12 months. It was superior in decreasing triglyceride levels, increasing HDL cholesterol levels, and reducing the use of antidiabetic medications for up to 12 months.

Goldenburg et al. [22] examined the efficacy and safety of low- and very-low-carbohydrate diets for type 2 diabetes remission in a systematic review and meta-analysis of 23 trials and 1460 participants in published and unpublished randomized trial data. The selection of trials was a comparison of a low-CHO diet (<130 g/day or <26% of a 2000 kcal/day diet) vs. a control diet for at least 3 months in overweight or obese people with type 2 diabetes. The control diets were isocaloric in only ten trials. At 6 months, compared with control diets, LCDs achieved higher rates of diabetes remission (defined as HbA1c < 6.5%) (76/133 (57%) vs. 41/131 (31%); risk difference 0.32, 95% confidence interval 0.17–0.47; 8 studies, $n = 264$,

I^2 = 58%). Conversely, smaller, nonsignificant effect sizes occurred when a remission definition of HbA1c < 6.5% without medication was used. HbA1c was reduced by 0.5% at 6 months and 0.2% at 12 months. Not surprisingly, greater weight loss was seen on the LCD, which depended on adherence to the diet—3.4 kg at 6 months and zero at 12 months. Worsening quality of life and increases in LDL cholesterol were noted at 12 months. The authors rated the evidence as moderate to low certainty.

Relationship Between Diet and Coronary Events in People with Type 2 Diabetes

Although there is now considerable controversy about the role of dietary saturated fat and cholesterol in promoting CVD, the data in people with type 2 diabetes are relatively clear in the US Nurses' Health Study [23]. Between 1980 and 1998, 619 new cases of CVD (nonfatal myocardial infarction, fatal coronary heart disease, and stroke) occurred in 5672 women with type 2 diabetes. The relative risk (RR) of CVD for an increase of 200 mg cholesterol/1000 kcal was 1.37 (p = 0.003). Each 5% of energy intake from saturated fat, as compared with equivalent energy from carbohydrates, was associated with a 29% greater risk of CVD (RR: 1.29 p = 0.04). Key's score (1.26 × (2 × % saturated fat − % polyunsaturated fat) + 1.5 × square root dietary cholesterol in mg/1000 kcal) was the most powerful predictor after multivariate adjustment (p = 0.001). The ratio of polyunsaturated to saturated fat was inversely associated with the risk of *fatal* CVD (p = 0.007). Replacement of 5% of energy from saturated fat with equivalent energy from monounsaturated fat was associated with a 37% lower risk of CVD.

Fish Oil

A Cochrane meta-analysis in 2008 examined 23 randomized controlled trials (1075 participants with type 2 diabetes), with a mean treatment duration of 8.9 weeks [24]. The mean dose of omega-3 PUFA used in the trials was 3.5 g/day. Among those taking omega-3 PUFA, circulating triglyceride levels were significantly lowered by 0.45 mmol/L (p < 0.00001) and levels of VLDL cholesterol lowered by 0.07 mmol/L (p = 0.04). LDL cholesterol levels were raised by 0.11 mmol/L (p = 0.05). No significant changes in levels of total or HDL cholesterol, HbA1c, fasting glucose, and fasting insulin or in body weight were observed. The decrease in VLDL cholesterol levels was significant only in trials of longer duration and in hypertriglyceridemic patients.

A meta-analysis of 83 trials of at least 6-month duration examined the effects of polyunsaturated fat (mostly long-chain N3 fats) on diabetes control. Long-chain omega-3 had little or no effect on the likelihood of diagnosis of diabetes (*HbA1c*

plasma glucose fasting insulin, or insulin resistance (calculated by HOMA-IR score)). A suggestion of negative outcomes was observed when the dose of long-chain N3 was above 4.4 g/day [25].

In a meta-analysis of 16 studies of fish intake and diabetes with over 500,000 participants and 24,000 cases of type 2 diabetes, there was high heterogeneity overall with no association among European studies, a significant direct association among US studies, and a significant inverse association among Asian/Australian studies. There was considerable heterogeneity in the first two geographical groups but none within the last group, which was not prospectively designed [26].

In a meta-analysis of ten controlled trials of fish oil people with type 2 diabetes, fish oil supplementation was associated with a decrease of triglyceride (TG) level by −0.40 (95% CI: −0.53 to −0.28, $p < 0.05$) and an increase of HDL cholesterol level by 0.21 (95% CI: 0.05–0.37, $p < 0.05$) [27].

In a larger randomized study of over 12,000 people with type 2 diabetes or prediabetes, 900 mg of EPA lowered TG by 14 mg/dL (10%) compared with placebo in a population with a median TG of 142 mg/dL. Blood pressure and other lipids were not altered compared with placebo. Not surprisingly, in this group of people with existing disease or a high risk of disease, this dose of EPA had no effect on CVD outcomes [28].

Dietary Cholesterol

A meta-analysis of 17 studies of dietary cholesterol in nondiabetic subjects showed that the addition of 100 mg dietary cholesterol/day increased the ratio of total to HDL cholesterol by 0.020 U, total cholesterol concentrations by 0.056 mmol, LDL cholesterol by 0.05 mmol/L, and HDL cholesterol concentrations by 0.008 mmol/L [29].

Dietary cholesterol had little effect on either total or LDL cholesterol in 31 overweight, insulin-resistant postmenopausal women over 4 weeks, and the effect was no different to the 34 women who were insulin sensitive [30]. A further 4-week study in insulin-sensitive individuals consuming four eggs/day showed a significant increase in non-HDL cholesterol levels and in inflammatory markers in insulin-sensitive individuals, which was not observed in lean or obese insulin-resistant individuals, but the difference between the groups was not statistically different [31].

A systematic review of ten publications from six original trials of egg consumption in people with type 2 diabetes or at risk of type 2 diabetes (prediabetic, insulin-resistant, or metabolic syndrome) found that 6–12 eggs/week had no effect on total cholesterol or LDL cholesterol although HDL cholesterol increased in four of the six studies [32]. It has been suggested the epidemiological association between cholesterol intake and CVD risk in type 2 diabetes is due to confounding factors [33]. However, similar associations have been seen in the nondiabetic US population in which an extra 300 mg/day increased CVD and total mortality by 17–18% [34].

Cholesterol Synthesis and Absorption

Cholesterol synthesis can be assessed by the circulating level of lathosterol, an intermediate in the cholesterol synthetic pathway, while absorption can be assessed by measuring the level of plant sterols sitosterol and campesterol or the level of an endogenous bacterial cholesterol metabolite, cholestanol. All of these are transported in lipoproteins, and the higher the lipoprotein level, the higher the sterol level, so adjustment needs to be made for the level of the carrier.

Insulin-sensitive individuals had higher plant sterol levels and lower lathosterol levels, indicative of higher cholesterol absorption and lower cholesterol synthesis. In 761 men of varying degrees of glucose tolerance, including 76 with type 2 diabetes, cholesterol synthesis markers were lowest and absorption markers highest in normoglycemia. Sitosterol was lower in subjects with impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes compared with normoglycemic subjects (111–115 ± 7 vs. 136 ± 3 μmol × 100/mmol of cholesterol, $p < 0.05$). Campesterol levels were also significantly lower in these groups relative to the normoglycemic control subjects. Peripheral insulin sensitivity evaluated by the Matsuda index was associated with the lathosterol/sitosterol ratio in the entire population ($r = -0.457$, $p < 0.001$) and with that of lathosterol/cholestanol independently of obesity [35].

Clinical research on dietary cholesterol and diabetes and lipid management is very limited.

A small study in ten male volunteers with type 1 diabetes showed that 800 mg/day of cholesterol for 3 weeks increased LDL cholesterol levels by 12% with a 7% increase in control subjects. HDL cholesterol levels remained the same but tended to increase in control subjects [36]. High-cholesterol absorption markers, e.g., sitosterol or campesterol, and low-cholesterol synthesis markers, e.g., lathosterol, appear to characterize type 1 diabetes [37], and these differ from people with type 2 diabetes [38].

Obesity is inversely related to fractional cholesterol absorption both in diabetic and nondiabetic subjects [39], but absorption is lower in subjects with type 2 diabetes [40]. Cholesterol absorption efficiency was 29 ± 1% in obese subjects with diabetes vs. 42 ± 2% in the obese control subjects ($p < 0.01$). Cholesterol synthesis was higher (17 ± 1 vs. 14 ± 1 mg/kg/day; $p < 0.05$), and neutral sterol and bile acid excretion and cholesterol turnover tended to be higher in the group with diabetes than in the control group. Blood glucose (measured twice 1 week apart) was positively related to cholesterol synthesis in the diabetic group ($r = 0.663$, $p < 0.01$) and in the control group ($r = 0.590$, $p < 0.05$), suggesting that the higher the blood glucose level, the higher the cholesterol synthesis. In 16 obese patients with type 2 diabetes, baseline cholesterol absorption and synthesis were related to respective serum sex hormone-binding globulin, glucose, and insulin values. Weight reduction of 6 kg increased cholesterol absorption efficiency, and ratio of serum plant sterols to cholesterol—indicators of cholesterol absorption—increased by 28% ($p < 0.01$) and 20–31% ($p < 0.05$ for both) and reduced blood glucose by 14%. Serum cholesterol levels did not change, but serum triglyceride levels fell by 13% [40].

Plant Sterols

Plant sterols are the plant equivalent of cholesterol and are found in cell walls and membranes. They differ from cholesterol by small changes to the side chain. They can be found naturally in oilseeds and cooking oils and are a normal part of the diet—up to 400–800 mg/day. Stanols are the same except for the removal of a double bond in the cholesterol nucleus. Some foods such as milk, margarine, orange juice, cheese, and chocolate are sometimes supplemented with sterols or stanols and deliver 2–2.5 g/day when consumed as directed.

Type 2 diabetes. The data above would suggest that obese subjects with type 2 diabetes would be less sensitive to dietary cholesterol and in turn less sensitive to the effects of dietary plant sterols. However, plant sterols appear to be just as efficacious in people with type 2 diabetes as in nondiabetic subjects. Plant sterols (1.8 g/day) for 21 days significantly reduced ($p < 0.05$) LDL cholesterol concentrations from baseline levels in 15 nondiabetic and 14 type 2 diabetic subjects by 15.1 and 26.8%, respectively, and these were not statistically different from each other [41]. A meta-analysis of five clinical trials, involving seven groups ($n = 148$ subjects with type 2 diabetes, with follow-up range of 3–12 weeks), found that the use of sterols/stanols significantly reduced LDL cholesterol levels by 0.30 mmol/L (9%, $p < 0.01$), with no apparent effect on triglycerides and a trend towards raising HDL cholesterol levels. These results are exactly the same as those seen in a meta-analysis of nondiabetic subjects [42].

Type 1 diabetes. Excellent efficacy of plant sterols is also seen in patients with type 1 diabetes with [43] or without [44] the concomitant use of statins.

Epidemiology of Cholesterol Intake and CVD

Despite the limited effect of dietary cholesterol on fasting lipids, egg consumption of one per day doubles the risk of coronary heart disease in women and all-cause mortality in men with type 2 diabetes compared with an intake of one egg per week [45, 46]. The incidence of type 2 diabetes is also increased with higher egg intake [47, 48].

Fiber

Very-high-fiber diets were actively promoted and studied in the 1980s both for glycemic and lipid control [49–52], but interest faded as patients found the diets too difficult or they were found in some studies to be ineffective [53–55].

A more recent small intervention study, published in the *New England Journal of Medicine* [56], in 13 patients with type 2 diabetes compared a high-fiber diet, which

provided 50 g of total fiber per day (as soluble and insoluble fiber 25 g each), with the standard ADA diet containing 24 g of total fiber per day, with 8 g as soluble fiber and 16 g as insoluble fiber. No fiber supplements were used. As compared with the ADA diet, the high-fiber diet resulted in a lower fasting plasma total cholesterol concentration (by 6.7%, $p = 0.02$), a lower plasma triglyceride concentration (by 10.2%, $p = 0.02$), and a lower plasma VLDL cholesterol concentration (by 12.5%, $p = 0.01$). The fasting plasma LDL cholesterol concentration was 6.3% lower with the high-fiber diet, but this was not statistically significant ($p = 0.11$), almost certainly due to the small size of the study. There were no significant differences between the two diets in fasting plasma HDL cholesterol concentrations.

A 6-month Canadian study [57] compared a low-glycemic-index (GI) diet with a high-fiber diet in 210 participants with type 2 diabetes. The high-cereal-fiber diet included 35 g of fiber, GI of 86, and glycemic load of 201. The low-GI diet included 42 g of fiber, GI of 62, and glycemic load of 141. There was an increase of HDL cholesterol levels in the low-GI diet by 1.7 mg/dL compared with a decrease of HDL cholesterol by −0.2 mg/dL in the high-cereal-fiber diet ($p = 0.005$), but this occurred only after about 16 weeks and was not associated with a change in triglyceride levels, so it is hard to conceive of a mechanism and may just be noise, although HbA1c improved modestly in the low-GI diet. LDL cholesterol levels did not change.

The effects of specific types of dietary fiber are now summarized. *Wheat bran* has no effect on lipid levels in type 2 diabetes [58] nor does adherence to a high-fiber, high-vegetable Mediterranean diet [59], admittedly in a small study. *Psyllium* in a low dose (3.5 g three times/day) in 40 participants for 2 months does not appear to significantly lower LDL cholesterol or triglyceride levels compared to a control group [60]. However, higher doses of psyllium (15 g/day) can significantly lower triglyceride levels compared with control when enough participants are studied ($n = 125$) [61]. Psyllium has also been demonstrated to lower LDL cholesterol levels in some studies [62, 63]. *Oat bran* can lower LDL cholesterol—an extra 15 g of fiber from oat bran lowered LDL cholesterol levels by 0.77 mol/L, but this study [64] was very small ($n = 8$). *Stabilized rice bran* (20 g/day for 12 weeks) lowered LDL cholesterol levels by 13.7% compared with the control group in a parallel study in 28 subjects with type 2 diabetes [65]. Triglyceride levels were also lowered by 0.5 mmol/L. *Guar gum* is well established as being able to lower LDL cholesterol levels [66–68], but is not widely used. It would appear from the limited number of studies (except for guar) that soluble fiber can reduce LDL cholesterol and triglyceride levels to the same degree as in nondiabetic subjects [69].

Low-Glycemic-Index Carbohydrate

As noted above, replacing fat with carbohydrate lowers HDL cholesterol and increases triglyceride levels. In most of these studies, the GI of the carbohydrate was not assessed. Low-GI carbohydrate may have lesser effects on these lipid levels compared with high-GI carbohydrates. A meta-analysis was performed by

Opperman et al. [70] in 2004 who examined lipid changes in 13 studies (eight in people with type 2 diabetes). Seven of the 10 studies found an improvement in mean LDL cholesterol concentrations on a low-GI diet. Overall, low-GI diets tended to decrease LDL cholesterol concentrations; however, it was not statistically significant (change 0.15 (95% CI 0.31, 0.00) mmol/L; $p = 0.06$). The GI of the diets was decreased by 21 (SD 10) units. In type 2 diabetes subjects, it appeared that LDL cholesterol concentrations were decreased to a greater extent (0.18 mmol/L, $p = 0.06$) than in healthy subjects. Only 6 of the 13 studies showed an improvement in triglyceride concentrations with a low-GI diet, and the overall change was not statistically significant (change 0.03 mmol/L, $p = 0.73$). When divided into subgroups, no significant difference was found within type 2 diabetes, coronary heart disease, or healthy subjects. No effect was observed when only subjects with elevated triglyceride concentrations were included. Lowering the GI of food did not cause an overall significant change in mean HDL cholesterol levels.

In a 1-year Canadian study [71], subjects with type 2 diabetes managed by diet alone ($n = 162$) were randomly assigned to receive high-carbohydrate, high-glycemic-index (high-GI), high-carbohydrate, low-glycemic-index (low-GI), or low-carbohydrate, high-monounsaturated-fat (low-CHO) diets. With the low-GI diet, overall mean triglyceride levels were 12% higher and HDL cholesterol levels were 4% lower than with the low-CHO diet ($p < 0.05$), despite a 26% lower glycemic load. The lack of benefit of a low-GI/low-GL diet on triglyceride and HDL cholesterol levels confirms the short-term meta-analytic results, but it is not clear why there were adverse changes. LDL cholesterol responses were not different between the diets.

Epidemiological studies, such as the Zutphen Elderly Study [72] and the EURODIAB Complications Study [73], failed to show a relationship between LDL cholesterol concentrations and low-GI diets, while other cross-sectional studies, such as the Survey of British Adults (1986–1987) [74] and the Third National Health and Nutrition Examination Survey (1988–1994) [75], found an increase in HDL cholesterol concentrations with long-term low-GI diets. No relationship was found between low-GI diets and triglyceride concentrations [72, 73].

In a Cochrane meta-analysis of adults and children with type 1 and type 2 diabetes and a low-GI diet of any duration (studies up to 2009), a positive effect on glycemic control was seen. In the six studies that examined HbA1c, low-GI diets reduced HbA1c by 0.5% [76].

A recent review of glycemic index concluded that there appears to be little benefit from low-GI diets in interventions or in epidemiological studies [77]. Interventions confined to people with type 2 diabetes in studies after 2009 showed a positive effect in two studies and no effect in five studies [78].

A meta-analysis of low-GI/low-GL diets with 29 trial comparisons in 1617 people with type 1 or type 2 DM showed a reduction in mean HbA1c of −0.31% (95% confidence interval −0.42 to −0.19%, $P < 0.001$; substantial heterogeneity, $I^2 = 75\%$, $P < 0.001$). A positive dose-response gradient was seen for the difference in GL and HbA1c. Reductions occurred also in fasting glucose, LDL-C, non-HDL-C, apoB, triglycerides, body weight, BMI, and CRP ($P < 0.05$), but not in blood insulin, HDL-C, waist circumference, or blood pressure [79].

Fructose

Fructose for many years was promoted as very suitable for people with diabetes because it lowered plasma glucose and insulin levels and improved HbA1c levels when it replaced starch, glucose, or sucrose [80, 81]. A recent acute study showed however that 10 g of fructose had no effect on the glucose profile after an OGTT in 24 patients with type 2 diabetes [82]. Gannon [83] showed that a high-fruit and high-vegetable diet with little starch lowered 24-h blood glucose levels without adverse effects on triglyceride levels compared with a high-starch diet or a usual American diet. 30–60 g/day of pure fructose supplementation (6–12% of energy) for 3–12 months had no adverse effects on lipids [84–88] or lipid metabolism [89]. A very high intake of fructose (>20% of energy) has been found to elevate lipids in some studies [80–93], but not in others [94, 95]. The threshold for adverse effects would appear to be about 60 g/day in comparison with starch in one meta-analysis of people with type 2 diabetes [96], or in another meta-analysis of 14 isocaloric trials (half in people with diabetes) and two hypercaloric trials, only the latter showed an effect with 25% excess energy and >175 g/day of fructose [97]. A low-fructose diet produced mainly by lowering of sucrose-sweetened drinks reduced TG significantly (by 20%) compared with the control diet but had no effect on glycemic control, weight, or blood pressure. Fructose was reduced from 25 to 9 g/day [98].

Weight Loss

Nondiabetic Subjects

Aucott [99] conducted a systematic review of studies that included lifestyle interventions for adults (18–65 years), with a mean baseline BMI <35 kg/m^2, with weight and lipid differences over 2 years. Between 1990 and 2010, 14 studies were identified. From meta-regression, they found that a 1 kg maintained weight loss in the long term (2–3 years) could be expected to result in reductions of 1.3% in total cholesterol, 1.6% for triglycerides, and 0.34% for LDL cholesterol levels with a 4% increase of HDL cholesterol levels.

An earlier meta-analysis by Poobalan [100] of 13 long-term studies (both cohort and surgical and nonsurgical and drug-based weight loss interventions) with a follow-up of more than 2 years found that total cholesterol concentrations had a significant positive linear relationship with weight change (r = 0.89), where change in weight explained about 80% of the cholesterol difference variation. For every 10 kg weight loss, a drop of 0.23 mmol/L in total cholesterol levels may be expected (about 5%). Triglycerides and LDL cholesterol concentrations were similarly related to weight loss, with a 10 kg change producing a 0.25 mmol/L and a 0.20 mmol/L change, respectively. HDL cholesterol changes were not related to weight loss. Participants in the two long-term meta-analyses could be on lipid-lowering medication.

In a meta-analysis of 70 short-term dietary weight loss studies in nondiabetic subjects, Dattilo and Kris Etherton [101] found that for every kilogram decrease in body weight, there was a 0.05 mmol/L decrease in total cholesterol levels (about 8–10%, $p < 0.01$), a 0.02 mmol/L decrease in LDL cholesterol levels ($p < 0.001$), a 0.007 mmol/L decrease in HDL cholesterol for active weight loss ($p < 0.05$), a 0.009 mmol/L increase in HDL cholesterol for stabilized weight loss ($p < 0.01$), and a 0.015 mmol/L decrease in triglyceride levels ($p < 0.05$). Correlations between weight loss and lipid changes were of the order of 0.3–0.4 and were much lower than in the long-term studies.

In the LIFE study [102] of 212 participants without diabetes, BMI fell in women from 35 to 33.7 kg/m^2 over 30 months and from 35 to 33 kg/m^2 in men, with a nadir at 12 months in both. In women, multivariate-adjusted HDL cholesterol concentrations at 6-month follow-up were significantly lower than at baseline, and at subsequent time points, HDL cholesterol concentration was significantly higher than at 6-month follow-up, with no significant differences between the later time points, which however were not significantly different from baseline. In men, the small decrease at 6 months was not statistically significant but later rises in HDL cholesterol levels were, with a maximum change at 18 months of about 10%. Triglyceride levels were significantly lower than baseline at 6 months but rose back to and beyond baseline in women, but remained low in men.

Diabetic Subjects

For participants with diabetes, there are much fewer studies available. The Look Ahead study was a very large randomized study ($n = 5145$) of intensive lifestyle interventions (ILI) or standard diabetes support and education (DSE) treatment in overweight or obese individuals with type 2 diabetes [103]. After 4 years, ILI participants had a greater percentage of weight loss than DSE participants (−6.15 vs. −0.88%; $p < 0.001$), and superior improvements in HDL cholesterol levels (3.7 vs. 2.0 mg/dL; $p < 0.001$) and triglyceride levels (−25.6 vs. −19.75 mg/dL; $p < 0.001$) averaged across all 4 years. Reductions in LDL cholesterol levels were greater in DSE than ILI participants (−11.3 vs. 12.8 mg/dL; $p = 0$ 0.009) owing to greater use of medications to lower lipid levels in the DSE group. The effects on triglyceride levels were not statistically significant at 4 years, but the HDL cholesterol level difference was consistent across all 4 years. These effects on lipid levels were lower than those in the long-term meta-analyses quoted above, but not different from those of the 2-year studies in nondiabetic subjects from Shai and Sacks [104, 105]. However, HDL cholesterol changes were very similar to the meta-analysis of short-term studies by Dattilo and Kris Etherton [101].

A weight loss of 4.5 kg in 2906 patients in the UKPDS reduced triglyceride levels by 0.41 mmol/L in men and 0.23 mmol/L in women with an HDL cholesterol increase of 0.02 and 0.01 mmol/L, respectively. LDL cholesterol levels did not change [106].

Glycemic Control

In 2220 type 2 diabetic patients (aged 35–91 years; male/female ratio, 1.07), HbA1c levels showed direct and significant correlations with total cholesterol, triglyceride, and LDL cholesterol levels and inverse correlation with levels of HDL cholesterol [107]. In Italian diabetes outpatient clinics, abnormal lipids were associated with markedly higher HbA1c levels [108] in 12,222 patients. On multiple regression, triglyceride levels were associated with HbA1c after adjustment for age, BMI, and diabetes treatment, and a variety of other factors, while HDL cholesterol levels were related to HbA1c levels in men only.

Interventions to Improve Glycemic Control

A Dutch study [109] which targeted a strict fasting capillary glucose of <6.5 mmol/L vs. a less strict regimen of <8.5 mmol/L in 214 patients over 2 years looked at individual changes in HbA1c vs. lipid changes. Individuals in whom HbA1c levels decreased had significant favorable concurrent changes in triglycerides $r = 0.26$ with HbA1c changes ($p = 0.001$) with an absolute difference of 0.25 mmol/L between those whose HbA1c fell (−0.17 mmol/L) vs. those whose HbA1c rose (0.08 mmol/L). Changes in LDL and HDL cholesterol levels were not statistically significant. The difference in HbA1c between the two groups was 1.09%.

In the Veterans Affairs Cooperative study in 513 male type 2 diabetes patients over 2 years, triglyceride levels decreased in the intensive-treatment arm from 2.25 ± 0.27 to 1.54 ± 0.14 mmol/L at 1 year ($p = 0.004$) and to 1.74 ± 0.18 mmol/L at 2 years ($p = 0.03$); there was no change in the standard-treatment arm. Total cholesterol levels decreased in the intensive-treatment arm at 1 year from 5.4 ± 0.21 to 4.99 ± 0.13 mmol/L ($p = 0.02$); there was no change in the standard-treatment arm. Levels of LDL and HDL cholesterol decreased in the standard-treatment arm only after 2 years, from 3.44 ± 0.13 to 3.16 ± 0.10 mmol/L ($p = 0.02$) and from 1.10 ± 0.03 to 1.00 ± 0.03 mmol/L ($p < 0.001$), respectively. Levels of apolipoprotein B decreased in both treatment arms ($p < 0.001$), and apolipoprotein A1 levels decreased in the standard-treatment arm ($p < 0.01$). A 2.1% difference in HbA1c levels was achieved over the 2-year period [110].

The Diabetes Control and Complications Trial (DCCT) [111] and the study by Cusp et al. [112] have shown falls in LDL cholesterol with intensive diabetes treatment. The latter study was very small ($n = 12$), and the fall in HbA1c achieved with 80 IU of insulin was 3.7% over 16 weeks. In the DCCT with 1441 patients with type 1 diabetes, changes in LDL cholesterol levels were small, 0.1–0.2 mmol/L, but the risk of developing an LDL cholesterol level of >4 mmol/L was reduced by 40% in the intensive diabetes treatment group, although rates were about 1 per 100 patient

years or less. Mean HbA1c level in the intensive and conventional treatment groups differed by about 2% throughout the follow-up period (7.2 vs. 9.1%, respectively, $p < 0.001$).

Alcohol Intake

A moderate alcohol intake is associated with about a 30% lower incidence of type 2 diabetes, but high alcohol intake and binge drinking increase the risk of type 2 diabetes [113, 114]. Alcohol intake in people with type 2 diabetes in the EPIC study [115] did not reduce mortality, although a prospective cohort study in older people showed an 80% reduction in death due to coronary heart disease with 14 g or more of alcohol/day [116] before and after adjustment for levels of HDL cholesterol and total cholesterol. In Japanese men with type 2 diabetes, alcohol intake was directly related to HDL cholesterol levels and hypertension, but the lowest triglyceride level was in the 1–22 g alcohol/day intake group compared with the nondrinker group [117]. There appear to be no alcohol intervention studies in people with diabetes.

Exercise

In a Cochrane meta-analysis [118], 14 randomized controlled trials comparing supervised or well-documented (aerobic, resistance, or mixed) exercise against "no exercise" in type 2 diabetes were identified involving 377 participants. Most studies had three 30–60-min exercise sessions per week. Trial duration ranged from 8 weeks to 12 months. No specific exercise program was given to the control group, but there were no reports on their incidental activity. The exercise intervention significantly decreased plasma triglyceride levels (−0.25 mmol/L, 95% CI −0.48 to −0.02). No significant difference was found between groups in plasma cholesterol levels or LDL cholesterol or HDL cholesterol levels.

Smoking

A recent meta-analysis [119] of observational studies in 130,000 people with diabetes showed that the relative risk comparing smokers with nonsmokers was 1.48 for total mortality (27 studies), 1.36 for cardiovascular mortality (9 studies), 1.54 for CHD (13 studies), 1.44 for stroke (9 studies), and 1.52 for MI (7 studies). The increased risk of smoking is similar to people without diabetes. Smoking lowers HDL cholesterol levels. The major lipid-related effect of smoking cessation is an increase of about 0.1 mmol/L or 3.9 mg/dL [120].

New Research Areas

Given the association between cholesterol intake and CVD events in people with diabetes, a cholesterol-feeding trial in people with both type 1 and type 2 diabetes needs to be done, focused not just on LDL and HDL cholesterol levels, but also on vascular adhesion molecules and other inflammatory markers.

Long-term dietary intervention studies examining low salt, low saturated fat, high polyunsaturated fat, and high fruit, vegetable, and fiber over a 3-year period need to be done with surrogate cardiovascular measures such as carotid intima-medial thickness as an endpoint.

Conclusions

There is a very limited amount of data related to the lifestyle effects on lipoproteins specifically involving people with diabetes. What data are available suggest that they respond in a similar way to people without diabetes to lifestyle measures. The expected responses of LDL cholesterol levels to dietary changes are summarized in Table 21.1. The effect of dietary cholesterol needs further exploration.

Table 21.1 Effects of dietary changes on circulating LDL cholesterol levels

Dietary component	LDL cholesterol lowering (%)
Saturated fat reduction 15–10%	5
Polyunsaturated fat increase 5–10%	3
Plant sterols 2 g/day	10
Oat bran 15 g/day	5
Low-GI carbohydrate in place of high-GI	5
Total possible change	28

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Chapter 22
Statin Therapy: Impact on Dyslipidemia and Cardiovascular Events in Patients with Diabetes

Brent M. Gudenkauf, Steven R. Jones, and Seth S. Martin

Introduction

This chapter begins by briefly discussing the basic biologic impact of HMG-CoA reductase inhibitor or "statin" therapy on dyslipidemia in patients with diabetes, emphasizing the important distinction between cholesterol and lipoprotein particles. Next, the section "Impact of Statin Therapy on Cardiovascular Events in Patients with Diabetes" focuses on randomized clinical trials that have investigated the impact of statin therapy on cardiovascular events in patients with diabetes. This section selectively discusses the details of four large trials, which account for the majority of data supporting the use of statins in patients with diabetes mellitus. We then place the evidence in perspective and describe an example of implementing the evidence in practice. Finally, the section "Residual Risk of Cardiovascular Events in Patients with Diabetes on Statin Therapy" addresses the observation that despite the notable impact of statin therapy, there remain a significant number of patients in the treatment arms of clinical trials who continue to sustain cardiovascular events. This "residual risk," along with the biology of dyslipidemia, invokes the potential role of lipoprotein targets that may serve as measurements of atherosclerotic risk beyond cholesterol parameters and, therefore, help guide clinical decisions in patients with diabetes. In the section "Residual Risk of Cardiovascular Events in Patients with

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Diabetes on Statin Therapy," we also note the recent advances in "add-on" therapy using other medication classes in addition to statin therapy to decrease lipoprotein levels, such as Niemann-Pick C1-like 1 protein inhibitors, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, and omega-3 polyunsaturated fatty acids. These medications are covered in much more detail in Chaps. 28–30.

Impact of Statin Therapy on Dyslipidemia

Understanding the central impact of statin therapy on dyslipidemia requires attention to the key role of atherogenic lipoprotein particles in the basic biology of atherosclerosis itself. Atherosclerosis begins with lipoprotein deposition in the arterial wall, which propagates through further lipoprotein deposition and subsequent inflammatory responses [1, 2]. The lipoprotein is made up of a core of lipid elements including cholesteryl esters and triglycerides surrounded by surface phospholipids and apolipoproteins. Atherogenic lipoproteins include low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), very-low-density lipoproteins (VLDL) and their remnants, and lipoprotein (a) (Lp(a)). Each of these atherogenic lipoproteins contains one copy of apolipoprotein B (apoB) on their surface. Proteoglycans in the arterial wall contain binding sites that recognize apoB, leading to retention of the particles, and therefore the presence of more circulating atherogenic particles translates into an increased risk of initiation and propagation of atherosclerosis. Lipoprotein matrix interactions are discussed in detail in Chaps. 9 and 11.

Notably, in the presence of diabetic dyslipidemia, the atherogenic particle concentration in plasma is frequently underestimated by lipoprotein cholesterol measurements. Along with higher levels of triglycerides and smaller high-density lipoprotein (HDL) particles, there is a trend towards small dense LDL (sdLDL) [3], particularly in those with type 2 diabetes. Because of the predominance of sdLDL in patients with diabetes, atherogenic potential is better reflected by measurements of non-HDL-C and apoB than LDL-C, which frequently underestimates the concentration of LDL particles in this setting. This underestimation may be particularly pronounced when LDL-C is estimated by the Friedewald equation, which has been shown to underestimate LDL-C levels [4]. Patients with diabetes commonly have normal or average LDL-C, but elevated apoB, which may in part explain high diabetic vascular risk.

While statin therapy exerts some effect on all lipid parameters, the most important effect is on apoB-containing lipoproteins. Statin therapy lowers LDL-C and non-HDL-C levels to a larger degree than LDL particle concentration (LDL-P) and overall atherogenic particle concentration as measured by apoB [5]. For example, in the combined analysis of the Treating to New Targets (TNT) and the Incremental Decrease in End Points through Aggressive Lipid-Lowering (IDEAL) trials, LDL-C, non-HDL-C, and apoB levels were measured in nearly 19,000 patients with established coronary heart disease who were assigned to usual-dose or high-dose

statin treatment [6]. In the patients on moderate statin doses (either atorvastatin 10 mg, simvastatin 20 mg, or simvastatin 40 mg), LDL-C (101–102 mg/dL) and non-HDL-C (129–132 mg/dL) were reduced to the 30–35th percentile for the American population; however, the corresponding apoB levels (107–113 mg/dL) were still markedly high relative to the American population at the 56–64th percentile. In the high-statin-dose arms (atorvastatin 80 mg), LDL-C (75–80 mg/dL) and non-HDL-C (101–102 mg/dL) levels were reduced to the 10–14th percentile for the American population. The corresponding apoB levels (84–91 mg/dL) were at the 20–31st percentile for the American population. The discrepancy between cholesterol reduction and particle reduction was also evident in patients with type 2 diabetes treated with statin therapy in the Collaborative Atorvastatin Diabetes Study (CARDS); LDL-C and non-HDL-C were lowered by 40.9 and 38.1%, while apoB levels were reduced by only 24.3% [7]. While numerous studies have undoubtedly shown the effect of statin therapy in reducing cardiovascular events in patients with diabetes, the discordance between cholesterol and particle reduction may in part explain the high residual risk remaining after statin therapy.

Impact of Statin Therapy on Cardiovascular Events in Patients with Diabetes

Evidence from Key Randomized Clinical Trials

Heart Protection Study

The TIMRC/BHF Heart Protection Study (HPS) opened a new clinical era by providing the first clear justification for routine use of statin therapy in patients with diabetes at sufficiently high risk for major cardiovascular events [8, 9]. Prior to HPS, only ~1500 secondary prevention and ~200 primary prevention patients with diabetes had participated in randomized statin trials. The HPS enrolled 5963 patients with diabetes (2912 were free of occlusive arterial disease) and an additional 14,573 patients without diabetes in the United Kingdom between 1994 and 1997. HPS included 615 patients with type 1 diabetes and 5348 patients with type 2 diabetes. Patients aged 40–80 years with non-fasting total cholesterol concentrations ≥3.5 mmol/L (135 mg/dL) were randomized to 40 mg of simvastatin daily versus matching placebo. Average statin use was 85% in the statin-allocated group compared with 17% in the placebo-allocated group, yielding an average LDL-C difference of ~1 mmol/L (39 mg/dL).

In line with results from the total study population, statin-treated patients with diabetes had a 22% (95% CI 13–30) relative risk reduction (RRR, event rate 20.2% versus 25.1%, $p < 0.0001$) in the first occurrence of major coronary events, stroke, or revascularization compared with their placebo-allocated counterparts [9]. Similar reductions were seen in those without baseline occlusive arterial disease (RRR 33% [95% CI 17–46], $p = 0.0003$) and those with baseline LDL-C levels <3.0 mmol/L

(116 mg/dL) (RRR 27% [95% CI 13–40], $p = 0.0007$). The risk reduction due to statin therapy did not depend on diabetes type, duration, or intensity of glycemic control, age, or hypertension. Importantly, adverse events of simvastatin were uncommon; there was a persistent elevation of transaminases in 0.09% of treated patients versus 0.04% of untreated patients ($p = 0.30$), myopathy without rhabdomyolysis in 0.05% of treated patients versus 0.01% of untreated patients, and myopathy with rhabdomyolysis in 0.05% of treated patients versus 0.03% of untreated patients. These were not significant differences. In sum, HPS showed that statin therapy improves outcomes across a broad range of patients with diabetes.

Anglo-Scandinavian Cardiac Outcomes Trial

The Anglo-Scandinavian Cardiac Outcomes Trial Lipid-Lowering Arm (ASCOT-LLA) addressed lipoprotein lowering in hypertensive patients in a 2 × 2 factorial investigation [10, 11]. Recruitment occurred between 1998 and 2000 at family practices in the United Kingdom, Ireland, and Nordic countries. Patients aged 40–79 years without a history of coronary heart disease, with untreated blood pressure ≥160/100 mmHg or treated blood pressure ≥140/90 mmHg, and additional risk factors, including type 2 diabetes, were randomized to antihypertensive treatment. Of the 19,342 randomized patients, the 10,305 patients with non-fasting total cholesterol concentrations ≤6.5 mmol/L entered the lipid-lowering arm and were randomized to 10 mg of atorvastatin daily versus placebo. A baseline diagnosis of diabetes was present in 2532 of participants. After a median follow-up of 3.3 years, total and LDL-C concentrations among patients with diabetes treated with atorvastatin were ~1 mmol/L (39 mg/dL) lower than those allocated to placebo, and the study was stopped early for efficacy. Like HPS, the proportional risk reduction in patients with diabetes was similar to patients without diabetes [11]. There were 116 (9.2%) major cardiovascular events or procedures in atorvastatin-allocated patients with diabetes and 151 (11.9%) events in the placebo group (hazard ratio 0.77 [95% CI 0.61–0.98], $p = 0.04$). Adverse events in the atorvastatin arm included cough (19% of treated patients versus 8% of untreated patients, $p < 0.0001$), eczema (5% of treated patients versus 4% of untreated patients, $p = 0.0002$), joint swelling (14% of treated patients versus 3% of untreated patients, $p < 0.0001$), and peripheral edema (23% of treated patients versus 6% of untreated patients, $p < 0.0001$). For the individual components of the composite end point, analyses were underpowered.

Collaborative Atorvastatin Diabetes Study

Concentrating on patients with diabetes in a primary prevention context, the Collaborative Atorvastatin Diabetes Study (CARDS) enrolled patients from the United Kingdom and Ireland from 1997 to 2001 [12]. Participating patients were 40–75 years in age with type 2 diabetes plus at least one additional risk factor, including hypertension, retinopathy, proteinuria, or smoking. The CARDS trial

randomized 2838 patients to atorvastatin 10 mg daily versus placebo. CARDS met its pre-specified early stopping rule for efficacy 2 years early after accumulating an average follow-up of 3.9 years. An acute coronary event, coronary revascularization, or stroke occurred in 127 patients allocated placebo and 83 allocated atorvastatin (RRR 37% [95% CI 17–52], $p = 0.001$). Relative risk reductions by individual outcomes were 36% for acute coronary events, 31% for coronary revascularizations, and 48% for stroke. A nonsignificant 27% reduction in mortality was also noted in favor of atorvastatin. Adverse events that were noted included elevation of transaminases and creatinine kinase, although *p*-values were not given. Discontinuation due to treatment-related adverse effects was not significantly different between groups.

Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial

The lipid-lowering trial (LLT) component of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) [13] was conducted from 1994 to 1998 primarily in community-based North American centers, included 3638 participants with diabetes aged ≥55 years, and had a similar design to ASCOT-LLT. The ALLHAT investigators demonstrated a neutral effect of pravastatin 20–40 mg daily versus usual care on cardiovascular events over a mean follow-up of 4.8 years. The findings of this trial do not contradict the aforementioned trials because there was a statin drop-in effect in the usual care arm. Nearly a third of usual care patients started lipid-lowering therapy during the trial. As a result, there was only a modest differential in total cholesterol (9.6%) between groups. This difference between treatment groups would not be expected to yield meaningful differences in risk for cardiovascular events. Combined with other major limitations of the trial, including its non-blinded design, the trial's neutrality is not unanticipated. It is important to note that 42 patients (16.6%) in the pravastatin arm were not taking any statin at year 6, and that half of these patients cited adverse reactions, but specific information about reactions was not recorded by investigators.

Meta-Analysis

The evidence supporting statin therapy for patients with diabetes was summarized in a prospective meta-analysis from the Cholesterol Treatment Trialists' (CTT) Collaborators [14]. The four trials discussed above, HPS, ASCOT-LLA, CARDS, and ALLHAT-LLT, accounted for 14,996 of the 18,686 patients (83%) included in the CTT meta-analysis. Of the 10,355 patients with diabetes enrolled in the trial, 35% had type 2 diabetes. The 14 trials included in the analysis (see Table 22.1 for details) were agreed upon before the results of trials were known and analyses were pre-specified. The pooled dataset provided greater power to assess the impact of statin therapy on individual outcomes in patients with diabetes and perform subgroup analyses.

Table 22.1 Randomized clinical trials of statin therapy in patients with diabetes

Randomized clinical trial	Original publication year	Trial participants with diabetes (*n*)	Trial focus group	Statin type (mg/day)	Average follow-up (years)	Primary enrollment locations
4S	1994	202	CHD	Simva 20–40	5.4	Scandinavia
WOSCOPS	1995	76	PP (men)	Prava 40	4.9	Scotland
CARE	1996	586	Post-MI	Prava 40	5	USA and Canada
Post-CABG	1997	116	CABG	Lova 2.5–80	4.3	USA
AFCAPS	1998	155	PP	Lova 20–40	5.2	USA
LIPID	1998	782	CHD	Prava 40	6.1	Australia and New Zealand
GISSI	2000	582	Post-MI	Prava 20	2	Italy
HPS	2002	5963	High-risk	Simva 40	5.3	UK
PROSPER	2002	623	Elderly	Prava 40	3.2	Scotland, Ireland, Netherlands
ALLHAT	2002	3638	HTN	Prava 20–40	4.8	USA and Canada
LIPS	2002	202	Post-PCI	Fluva 80	3.9	Europe, Canada, Brazil
ASCOT	2003	2527	HTN	Atorva 10	3.3	UK, Ireland, Nordic countries
ALERT	2003	396	Renal Txp	Fluva 40	5.1	Europe, Canada
CARDS	2004	2838	DM	Atorva 10	4	UK and Ireland

CHD coronary heart disease, *PP* primary prevention, *HTN* hypertension, *PCI* percutaneous coronary intervention, *Txp* transplant, *DM* diabetes mellitus

During an average follow-up of 4.3 years, 3247 major vascular events occurred in patients with diabetes. All-cause mortality was reduced by 9% per 1 mmol/L reduction in LDL-C in patients with diabetes (RR 0.91 [99% CI 0.82–1.01], $p = 0.02$), which was similar to patients without diabetes. As expected, the mortality reduction was attributable to lower vascular mortality (RR 0.87 [99% CI 0.76–1.00], $p = 0.008$) with no effect on nonvascular mortality. Major vascular events were reduced by 21% per 1 mmol/L reduction in LDL-C (RR 0.79 [99% CI 0.72–0.86], $p < 0.0001$). Individually, each component end point was reduced: myocardial infarction or coronary death (RR 0.78 [99% CI 0.69–0.87], $p < 0.0001$), coronary revascularization (RR 0.75 [99% CI 0.64–0.88], $p < 0.0001$), and stroke (RR 0.79 [99% CI 0.67–0.93], $p = 0.0002$). Findings were not dependent on pre-treatment

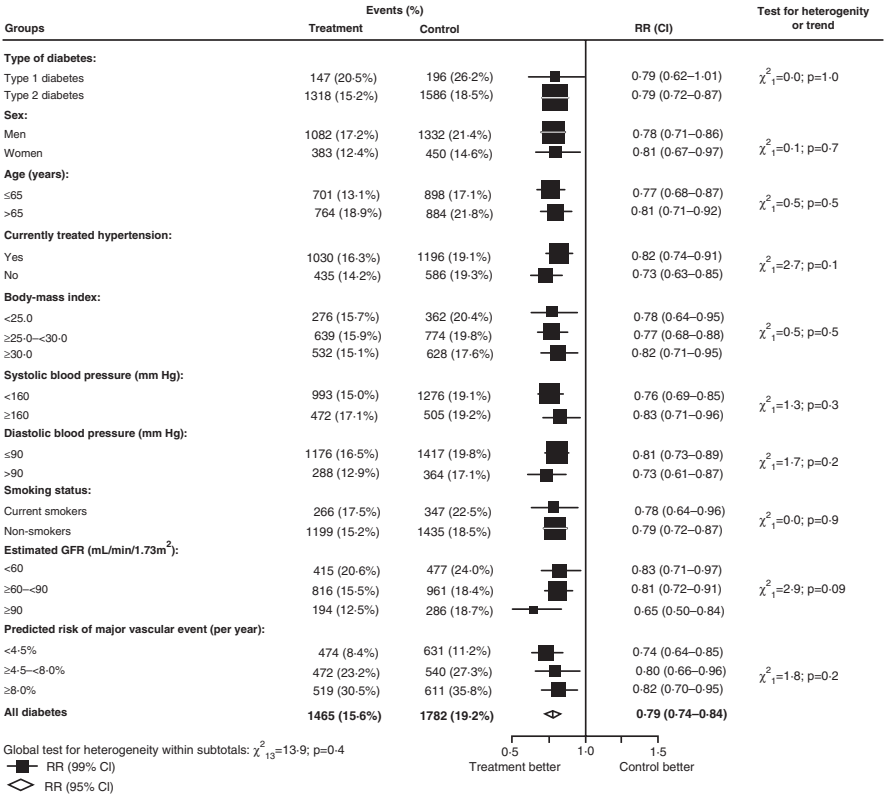

Fig. 22.1 Proportional effects on major vascular events per mmol/L reduction in LDL cholesterol by baseline subgroups in patients with diabetes. Rate ratios (RRs) are plotted comparing the outcome in participants who were allocated statin treatment to control, along with their CIs. The area of each square is proportional to the amount of statistical information in that particular category. Diamonds or squares to the left of the solid line indicate benefit with treatment, which is significant (i.e., $p < 0.05$ and $p < 0.01$, respectively) if the diamond or horizontal line does not overlap the solid line. The RRs are weighted to represent the reduction in the rate per 1 mmol/L LDL cholesterol reduction achieved by treatment at 1 year after randomization. *GFR* glomerular filtration rate. (Figure reproduced with permission from Elsevier [14])

lipoprotein parameters, and there was no threshold below which benefit was absent. The proportional therapeutic benefits of statins in patients with diabetes were also similar irrespective of the type of diabetes, sex, age, hypertension, body mass index, smoking, kidney disease, or overall risk category (Fig. 22.1).

Potential Risks of Statin Therapy

There is an association between the initiation of statin therapy and new-onset type 2 diabetes mellitus, which is modest, on the order of 1 new case per 1000 patient years [15]. Patients who develop incident diabetes on statin therapy are frequently

insulin resistant, so small incremental increases in glucose levels attributable to statin therapy may be sufficient to unmask a diagnosis of diabetes. Further, many of these patients tend to have progressive insulin resistance and develop overt diabetes mellitus even without statin therapy, as shown by a less than 1-year difference in median time to onset of diabetes mellitus in the rosuvastatin-treated group versus the placebo group in the JUPITER trial [16]. The potential harm of new-onset diabetes is outweighed by concurrent reduction in cardiovascular morbidity and mortality on therapy, especially in high-risk patients. Therefore, the epidemiologic link between statin initiation and type 2 diabetes mellitus should not alter the decision to initiate statin therapy. There is an extremely small risk of rhabdomyolysis with statin therapy; an analysis of 252,460 patients' claims data found a 0.44 per 10,000 patient years incidence of statin-induced rhabdomyolysis requiring hospitalization [17]. The American Heart Association scientific statement on the safety of statin therapy additionally notes a 0.001% risk of hepatotoxicity, and a possibly increased risk of hemorrhagic stroke, but notably a reduction in total risk of stroke overall [18]. However, recent evidence from the FOURIER and ODYSSEY OUTCOMES trials, which achieved very low LDL-C levels, provides reassurance about a lack of association of low LDL-C and hemorrhagic stroke [19]. There is also no evidence for a relationship between statin therapy and cancer, cataracts, cognitive dysfunction, neuropathy, erectile dysfunction, arthritis, or tendonitis [18, 20].

Putting the Evidence in Perspective

Based on the CTT meta-analysis [14], in adults who have diabetes, it was estimated that a low-potency statin would prevent approximately 45 patients per 1000 from having a major vascular event over 5 years. Given that high-potency statins are roughly two and one-half times as effective as low-potency ones, a high-potency statin prevents approximately 113 patients per 1000 from having a major vascular event over 5 years with a number needed to treat (NNT) of 9. This is approximately half the 5-year number needed to treat of 20 for a major vascular event found in the Justification for the Use of statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial [21], a primary prevention trial of a potent statin, rosuvastatin 20 mg daily, that excluded patients with diabetes. Economic analyses of randomized trials, including the HPS [22], have shown that statin therapy is cost effective, if not cost saving, for a wide range of patients with diabetes.

Implementing the Evidence in Practice

In the Steno-2 study, investigators from Denmark randomly assigned 160 patients with type 2 diabetes and microalbuminuria to a multifactorial intervention (lipid-lowering therapy, aspirin, renin-angiotensin inhibition, and tight glucose

control) versus conventional therapy [23]. The study completed follow-up in 2006 after a mean duration of treatment of 7.8 years and additional mean observation period of 5.5 years. During the intervention phase, 85% of the treatment group took statins (mean attained LDL-C 83 mg/dL from 133 mg/dL at baseline) compared with 22% of the conventional therapy group (mean attained LDL-C 126 mg/dL from 137 mg/dL at baseline). More than eight in ten patients in both groups went on to take statins in the observation phase with mean LDL-C concentrations converging near 70 mg/dL; however, survival curves continued to diverge.

Upon completion of follow-up, compared with 40 deaths in the conventional therapy group, only 24 patients who received multifactorial intervention died (hazard ratio 0.54 [95% CI 0.32–0.89], $p = 0.02$). Multifactorial intervention reduced cardiovascular mortality (hazard ratio 0.43 [95% CI 0.19–0.94], $p = 0.04$) and cardiovascular events (hazard ratio 0.41 [95% CI 0.25–0.67], $p < 0.001$). Even with imperfect implementation (proportion of patients achieving ideal treatment targets was modest), the NNTs over the full study period (7.8 years of intervention and an additional 5.5 years of follow-up) were impressively low: three patients to prevent one cardiovascular event, five patients to prevent death from any cause, and eight patients to prevent a cardiovascular death. It was concluded that statins and antihypertensive therapies were the two most influential therapies in reducing risk. In sum, Steno-2 demonstrates that early implementation of statin therapy as part of a multifaceted approach to risk reduction achieves dramatic reductions in absolute risk, and thus low numbers needed to treat, making primary prevention strategies incorporating statin therapy in patients with diabetes second to few if any other medical therapies in modern medicine.

It is important to note that despite this established efficacy and profound effect of early implementation of statin therapy, there are still vast care gaps in day-to-day practice that should be addressed. A study of 32,400 adults with diabetes mellitus in the Community Health Applied Research Network indicated that female patients, those of Asian or Pacific Islander heritage, and those that primarily speak Spanish were less likely to be prescribed statin therapy in accordance with clinical guidelines [24].

Residual Risk of Cardiovascular Events in Patients with Diabetes on Statin Therapy

Residual Risk Data

In the HPS, there was a 22% relative risk reduction in major coronary events, stroke, or revascularization compared with placebo [8]. However, there remained a residual risk where 78% of events in patients with diabetes treated with simvastatin therapy were not prevented. In ASCOT-LLA, there was a similar 23% relative risk reduction in events, leaving a residual risk of 77% [10]. Both HPS and ASCOT-LLA showed

a reduction in LDL-C of approximately 40 mg/dL from baseline. Similarly, in the summary meta-analysis from the CTT Collaborators, for every approximate 40 mg/dL decrease in LDL-C, there was a relative risk reduction of major vascular events of 21% [14].

Several investigators have found differences in progression or regression of coronary atherosclerosis in patients with diabetes. Analysis of three-dimensional vascular imaging data from patients in the PREDICT trial indicated that despite reaching similar levels of LDL-C while on rosuvastatin 40 mg per day, patients with diabetes experienced higher progression of mean plaque area (0.47 versus 0.21 square millimeters), increased atheroma volume (+0.7% versus −1.4%), and more locations with thin-cap fibroatheroma (20.3% versus 12.5%) compared to patients without diabetes [25].

The residual risk in these treated patients with diabetes can be attributed to a number of factors, some of which may be related to lipoproteins. Accordingly, with regard to reducing residual risk with statin therapy, there are two potential areas of focus: (1) the target lipoprotein parameter measured (i.e., LDL-C, non-HDL-C, apoB, LDL-P) and (2) the target level of lipoprotein reduction.

Lipoprotein Epidemiology and the Ideal Therapeutic Target

Prospective observational studies have confirmed that vascular event risk is more accurately predicted by measurements of atherogenic particle concentration than total cholesterol or LDL-C [26]. Non-HDL-C is the currently recommended method of estimating risk and treatment in patients with hypertriglyceridemia, as there is considerable variation in the distribution of cholesterol content across particle classes [27]. In such instances, non-HDL-C provides a measure of the cholesterol content of all apoB-containing particles and has high correlations with apoB; however, on an individual patient basis, there is a significant degree of discordance between non-HDL-C and apoB [5].

Nevertheless, it has been substantially demonstrated that apoB and LDL particle measurements consistently outperform cholesterol measurements epidemiologically [26]. For example, in the Multi-Ethnic Study of Atherosclerosis (MESA), 6814 patients without cardiovascular disease were enrolled and followed for cardiovascular events. LDL particle concentration was measured and compared to LDL-C levels (Fig. 22.2). Discordance between the two measurements was defined as LDL-C and particle values differing by 12 percentile points (an arbitrary value so that 50% of the population was discordant). In patients with concordance, both LDL-C and particle concentration were associated with incident events. However, in those patients with discordance, only LDL particle concentration was associated with incident events [28]. This suggests that risk in those patients with elevated levels of circulating LDL particles may be underestimated by solely measuring cholesterol levels. In a comprehensive meta-analysis including 12 independent reports involving more than 230,000 individuals with nearly 23,000 events, as markers of

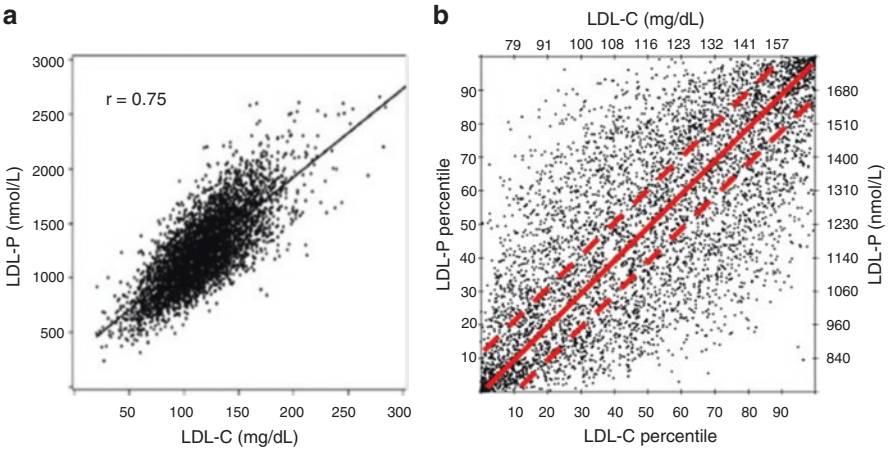

Fig. 22.2 Relations between LDL-C and LDL-P among 5598 MESA participants. (**a**) Relation of LDL-C and LDL-P concentrations. (**b**) Relation of LDL-C and LDL-P levels given in percentile units. The dashed lines bracket concordant LDL-C and LDL-P values defined as those within ±12 percentile units. (Reprinted with permission from Elsevier [28])

cardiovascular risk, apoB (RRR 1.43; 95% CI 1.35–1.51) outperformed non-HDL-C (RRR 1.34; 95% CI 1.24–1.44) which outperformed LDL-C (RRR 1.25; 95% CI 1.18–1.33) [29].

Despite the imperfections of reliance solely on LDL-C, a recent observational cohort study of 19,095 patients with type 2 diabetes mellitus without established atherosclerotic cardiovascular disease showed lower risk of cardiovascular disease events with lower levels of LDL-C achieved on statin therapy, with the lowest event rate for patients who achieved LDL-C levels less than 50 mg/dL [30]. To guide the intensity of lipid-lowering therapy, an emerging clinical tool is coronary artery calcium (CAC) scoring, which may allow for more tailoring of lipid-lowering treatment in patients with diabetes. Based on data from MESA and other cohort studies, a low or zero CAC score can be useful in downgrading the estimated risk of cardiovascular disease in patients with diabetes, for instance [31]. The ongoing RosCal study will evaluate the effect of rosuvastatin 20 mg on the density score of CAC and thus plaque density in patients with type 2 diabetes [32].

Guidelines

Table 22.2 compares dyslipidemia guidelines in patients with diabetes by recommendations for statin therapy, LDL-C targeting, other lipid targets, and approach to therapeutic intensification. In targeting lipoprotein-based risk to reduce cardiovascular events, the target of choice has progressed from total cholesterol followed by LDL-C to the evolving recommendation of non-HDL-C levels. It remains unsettled whether more aggressive reduction of atherogenic particles, as measured by particle

Table 22.2 Comparison of major dyslipidemia management guidelines in patients with diabetes

Recommendation	AHA/ACC (2018)	ESC (2019)	ADA (2021)	ACE (2017)
Empiric statin therapy	Yes, at least moderate intensity in all patients aged 40–75	No, statin therapy only if at least at "moderate risk"	Yes, at least moderate intensity in all patients aged 40–75	No, only if LDL-C is greater than 100 mg/dL
LDL-C target	Reduction 50 or more % from baseline, if 10-year ASCVD risk 20 or more %	<55 mg/dL if "very high" risk[a] <70 mg/dL if "high risk" <100 mg/dL if "moderate risk"	Reduction 50 or more % from baseline, if 10-year ASCVD risk 20 or more %	<55 mg/dL if "extreme" risk <70 mg/dL if "very high" risk <100 mg/dL if "high" risk
Other lipid targets	No	No specific targets in diabetes, but recommendations for apoB and non-HDL-C in the general population	Triglyceride level <135 mg/dL with maximally tolerated statin therapy, with addition of icosapent ethyl if still elevated	ApoB <70 mg/dL and non-HDL-C <80 mg/dL if "extreme risk" ApoB <80 mg/dL and non-HDL-C <100 if "very high" risk ApoB <90 mg/dL and non-HDL-C <130 mg/dL if "high risk"
Intensification of therapy	Consideration of ezetimibe to maximally tolerated statin if above LDL-C threshold and unable to reach LDL-C reduction target Consideration of PCSK9 inhibitor if above LDL-C threshold on statin and ezetimibe and clinical ASCVD	Consideration of ezetimibe or PCSK9 inhibitor to maximally tolerated statin to reach LDL-C numerical target	Consideration of ezetimibe or PCSK9 inhibitor to maximally tolerated statin to reach LDL-C reduction target	Consideration of ezetimibe or PCSK9 inhibitor to maximally tolerated statin to reach LDL-C numerical target Consider bile acid sequestrants to reduce LDL-C if unable to reach numerical target

[a] Risk categories defined in the text below

concentration or apoB, would more completely reduce residual risk [33] by formal prospective trial design. However, given the known biology of atherosclerosis, there is a compelling scientific basis to research this question with observational evidence consistently showing risk reduction with progressive reduction in atherogenic apoB-containing lipoprotein concentration [14].

In 2018, the American College of Cardiology (ACC) and the American Heart Association (AHA) Task Force on Clinical Practice Guidelines released the latest Cholesterol Clinical Practice Guidelines in conjunction with ten other organizations, including the ADA [34]. In these guidelines, use of the Martin-Hopkins equation to calculate LDL-C is recommended, as it is more accurate than the established Friedewald equation at lower LDL-C levels (<70 mg/dL) and at higher triglyceride levels (≥150 mg/dL). These guidelines are also notable for recommending empiric moderate-intensity statin therapy in all patients with diabetes aged 40–75 due to their overall high lifetime risk for ASCVD, and for recommending intensification of statin therapy and addition of ezetimibe to maximally tolerated statin to reduce LDL-C by 50% or more if the 10-year ASCVD risk is 20% or more.

The European Society of Cardiology (ESC) released similar guidelines in 2019, but with LDL-C targets for these patients [35]. For those in the "very high risk" category (established cardiovascular disease, other target organ damage, or three or more risk factors, or early-onset type 1 diabetes mellitus of more than 20 years' duration), the target LDL-C is <55 mg/dL. For patients in the "high risk" category (diabetes mellitus of 10 years or more duration plus one additional risk factor), the ESC recommends a target LDL-C of <70 mg/dL. Finally, for those at "moderate risk" (patients with type 1 diabetes mellitus younger than 35, or type 2 diabetes mellitus younger than 50 years, with a duration of diabetes less than 10 years), ESC recommends an LDL-C target of <100 mg/dL.

The American Diabetes Association (ADA) releases guidelines yearly; the most recent guidelines for cardiovascular disease management in patients with diabetes largely mirror the most recent recommendations from the ACC and AHA [36]. For all patients with diabetes aged 40–75 without clinically established ASCVD, a moderate-intensity statin is recommended. In those aged 20–39, however, statin therapy is only recommended if additional ASCVD risk factors exist. In patients deemed "higher risk," e.g., those with multiple ASCVD risk factors, who are 50–75 years of age, or who have a calculated 10-year ASCVD risk of 20% or more, high-intensity statin therapy is recommended. Further, in this group, ADA recommends the addition of ezetimibe or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors to maximally tolerated statin therapy to reduce LDL-C by 50% or more from baseline, noting that ezetimibe may be preferable due to lower cost. Regarding other lipoprotein fractions, the ADA recommends addition of icosapent ethyl to statin therapy in patients who have elevated triglycerides to 135–499 mg/dL with established ASCVD or ASCVD risk factors. The ADA generally recommends against the use of statin-fibrate or statin-niacin combinations in routine clinical practice, although the ADA notes that statin-fibrate combinations may be useful in patients who have both hypertriglyceridemia and diabetes.

The American Association of Clinical Endocrinologists and the American College of Endocrinology (ACE) recommend similar targets but with the addition of non-HDL-C and apoB as well [37]. Patients with diabetes and no other risk factors are deemed "high risk" and it is recommended to treat to LDL-C <100 mg/dL, non-HDL-C <130 mg/dL, and apoB <90 mg/dL. Patients with diabetes and one or more risk factors are deemed "very high risk" with targets including LDL-C <70 mg/dL, non-HDL-C <100 mg/dL, and apoB <80 mg/dL. Patients with diabetes and

established cardiovascular disease are termed “extreme risk” and assigned target LDL-C <55 mg/dL, non-HDL-C <80 mg/dL, and apoB <70 mg/dL, largely in accordance with the ESC guidelines.

Combination Therapy

Statin and non-statin agent combinations may be uniquely useful in patients with diabetes mellitus. Combination therapy with simvastatin and ezetimibe, a Niemann-Pick C1-like 1 protein inhibitor, resulted in greater reduction in LDL-C, more than statin dose doubling, and was comparable to high-intensity rosuvastatin 10 mg per day in patients with diabetes [38]. In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), ezetimibe reduced cardiovascular mortality, major adverse cardiovascular events, all-cause mortality, coronary revascularization, and hospitalization for unstable angina when added to simvastatin after acute coronary syndrome [39]. Further, the reduction in major adverse cardiovascular events with this drug combination appears to be greater in patients with diabetes than in those without diabetes [40]. This may be due to increased cholesterol absorption in patients with diabetes mellitus, suggesting that they may uniquely benefit from the addition of ezetimibe [41].

In recent years, major advances have been made with other non-statin therapies in addition to ezetimibe, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and omega-3 polyunsaturated fatty acids. In the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) and the Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trials, the PCSK9-inhibiting monoclonal antibodies evolocumab and alirocumab, respectively, reduced adverse cardiovascular events and markedly reduced LDL-C levels among patients already on statin therapy [42, 43]. Additionally, the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) demonstrated that in the subset of patients with persistent hypertriglyceridemia (triglycerides 135–499 mg/dL) despite the use of maximally tolerated statin therapy, the risk of ischemic events was reduced with the omega-3 polyunsaturated fatty acid ester, icosapent ethyl [44]. This medication may be particularly useful in lowering residual risk in a patient with diabetes who has multiple cardiovascular risk factors or has clinical atherosclerosis. These medications are covered in much more detail in Chaps. 28–30.

Conclusions

In association with atherogenic lipoprotein reduction, there is robust evidence that statin therapy significantly lowers cardiovascular event rates in patients with diabetes mellitus. Multiple randomized clinical trials, spanning the last two decades, have

consistently and unequivocally made this case. The beneficial impact of statins on cardiovascular events includes reductions in myocardial infarction, need for coronary revascularization, strokes, and cardiovascular mortality. The evidence supports a class effect as a number of statins have been tested. Regarding individual statins, atorvastatin, simvastatin, and pravastatin have been clinically tested in the largest number of patients with diabetes. The tens of thousands of patients with diabetes who have participated in randomized clinical statin trials have taught us that the proportional benefit of therapy, or relative risk reduction, is relatively constant across a wide array of diabetes subgroups and predictably related to the proportion of LDL-C lowering. With this wealth of data, we have witnessed a paradigm shift, dating back to the publication of the Heart Protection Study, in the way we manage cardiovascular risk in patients with diabetes. Statins are now justifiably commonplace in the management of patients with diabetes at various levels of risk. Further reduction of risk may be achieved by combining statins with non-statin therapy to drive LDL-C and atherogenic burden, such as measured by apoB, to very low levels.

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Chapter 23
Statin Intolerance: An Overview for Clinicians

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Introduction

The most common forms of cardiovascular disease (CVD) are ischemic heart disease (IHD), 49.2%, and ischemic stroke, 17.7%, which are classified as atherosclerotic cardiovascular disease (ASCVD). CVD is the leading cause of death globally, and ASCVD is responsible for 70% of all cardiovascular (CV) deaths [1, 2]. The latest statistics of the European Society of Cardiology (ESC) confirm that among both men and women, the main causes of premature death in 2021 were IHD (17% for both sexes) and stroke (12% for women and 8% for men) [3]. In 2019, 17.9 million people died of CVD, which represents 32% of all global deaths [2]. Such a large global burden of ASCVD is related to the high prevalence of well-recognized, mostly modifiable risk factors for these diseases. Increased level of low-density lipoprotein cholesterol (LDL-C) has been ranked as the third most common cardiovascular risk factor in the world [1]. An increase of LDL-C by every 1 mmol/L is associated with a significant increase in the risk of ASCVD by 16% (HR = 1.16; 95% CI: 1.12–1.21), while among people aged 20–49, this increase is higher, i.e., by 47% (HR = 1.47; 95% CI: 1.32–1.64) [4]. A study by Navar-Boggan et al. showed that the incidence of moderate dyslipidemia in young adults who were not treated with statins increased the risk of coronary artery disease (CAD) by 67% (HR = 1.67; 95% CI: 1.06–2.64) over 15 years of follow-up [5]. The atherogenic effect of LDL-C appears to be dependent on both the level of circulating LDL-C and the duration of the exposure (Fig. 23.1) [6].

Considering such a significant influence of the increased level of LDL-C on the risk of ASCVD, recent Polish guidelines (2021) on the diagnosis and therapy of lipid disorders indicated that LDL-C concentration is a key lipid parameter determining the CV risk and defining the goals of lipid-lowering therapy (class: I; level: A) [10]. Lowering low-density lipoprotein cholesterol (LDL-C) by 38.7 mg/dL (1.0 mmol/L) results in 21% decrease in CVD morbidity and mortality [11]. It is recommended that lipid-lowering therapy (LLT) follows the principle of "the lower, the better," but it is also critically important to achieve the therapeutic goal for LDL-C as soon as possible in accordance with the "the earlier, the better" principle and to maintain it for as long as possible ("the longer, the better") [10, 12, 13]. Currently, it is recommended to use intensive lipid-lowering therapy, and for the selected group of patients at high and extremely high CVD risk—up-front combination therapy [10, 14]. This approach brings greater CV benefits, especially in patients with higher baseline LDL-C levels [10] as confirmed by the results of the meta-analysis of 34 RCTs conducted by Navarese et al. These researchers showed that more intensive LDL-C lowering was associated with greater reductions in all-cause mortality and CVD mortality among patients with LDL-C levels ≥100 mg/dL (all-cause mortality: change in RRs per 40 mg/dL increase in baseline LDL-C, 0.91; 95% CI: 0.86–0.96; CVD mortality: change in RRs per 40 mg/dL increase in baseline LDL-C, 0.86; 95% CI: 0.80–0.94) [15]. Similar results were obtained in a

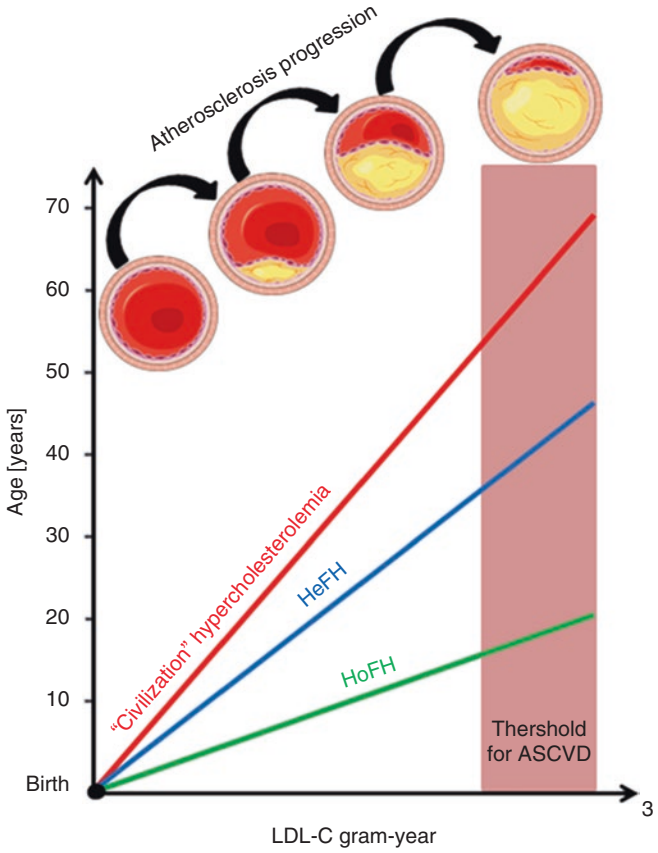


Fig. 23.1 Relationship between LDL-C accumulation over time and risk of ASCVD. *Abbreviations*: *LDL-C* low-density lipoprotein cholesterol, *ASCVD* atherosclerotic cardiovascular disease, *HeFH* heterozygous familial hypercholesterolemia, *HoFH* homozygous familial hypercholesterolemia. (Data taken from Refs. [6–9])

meta-analysis of 46 RCTs by Ma et al., showing that more intensive treatment was associated with a lower risk of all-cause mortality (RR = 0.91; 95% CI: 0.88–0.95), CV mortality (RR = 0.89; 95% CI: 0.86–0.92), MI (RR = 0.79; 95% CI: 0.77–0.81), coronary revascularization (RR = 0.80; 95% CI: 0.76–0.84), and cerebrovascular events (RR = 0.84; 95% CI: 0.80–0.88) compared with the less intensive treatment [16]. Current LDL-C targets are determined by CV risk and may require LDL cholesterol reduction to <1.4 mmol/L (<55 mg/dL) and ≥50% of baseline (primary and secondary prevention in patients of very high CV risk) (class: I, level: C, and class: I, level: A, respectively), and even lower to <1.0 mmol/L in those at extremely high CVD risk [10, 17].

Statins: A Brief Clinical Overview

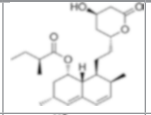
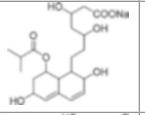
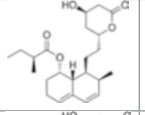
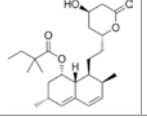
Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] (Table 23.1) are the gold standard, first-line agents in the treatment of hypercholesterolemia, and among all lipid-lowering agents, statins have the best documented efficacy in the primary and secondary prevention of CVD in patients with acute coronary syndromes (ACS), dyslipidemia, CAD, hypertension, diabetes mellitus (DM), stroke, and chronic kidney disease (CKD), irrespective of cholesterol levels [10]. As already mentioned, effective treatment should be based on optimal, intensive lipid-lowering therapy. It is recommended that high-intensity statins are prescribed in tolerated doses to achieve the goals set for specific CV risk level (class: I, level: A) [10]. Among the statins, only rosuvastatin at a dose of 20–40 mg and atorvastatin at a dose of 40–80 mg reduce the baseline LDL-C level by 50% [22–24]. As demonstrated by Zhang et al. in a network meta-analysis of 50 RCTs, rosuvastatin had the strongest effect on LDL-C reduction, followed by atorvastatin and pitavastatin [25].

The efficacy of statin use in the primary prevention of CVD has been summarized in a meta-analysis of randomized clinical trials (RCTs) by Yebyo et al., which included 94,283 subjects. Statins have been shown to reduce the risk of nonfatal MI by 38% (RR = 0.62; 95% CI: 0.53–0.72), CVD mortality by 20% (RR = 0.80; 95% CI: 0.71–0.91), all-cause mortality by 11% (RR = 0.89; 95% CI: 0.85–0.93), nonfatal stroke by 17% (RR = 0.83; 95% CI: 0.75–0.92), unstable angina by 25% (RR = 0.75; 95% CI: 0.63–0.91), and composite major CV events by 26% (RR = 0.74; 95% CI: 0.67–0.81) [26]. A meta-analysis of 9 RCTs conducted by Tramacere et al. in patients with stroke or transient ischemic attack (TIA) showed that statin use (with 2.5-year follow-up) reduced the risk of ischemic stroke by 19% (OR = 0.81; 95% CI: 0.70–0.93), ischemic stroke or TIA by 25% (OR = 0.75; 95% CI: 0.64–0.87), and CV events by 25% (OR = 0.75; 95% CI: 0.69–0.83) [27]. Moreover, a meta-analysis of 16 RCTs by Yu et al. showed that intensive statin therapy in patients with ACS reduced the risk of major adverse CV events by 23% (RR = 0.77; 95% CI: 0.68–0.86) [28]. Finally, a meta-analysis of 5 RCTs by de Vries et al. in patients with diabetes and CVD showed that the use of standard-dose statins reduced any major CV or cerebrovascular event by 15% (RR = 0.85; 95% CI: 0.79–0.91). Intensive statin therapy reduced this risk by a further 9% (RR = 0.91; 95% CI: 0.84–0.98) [29].

Importantly, a meta-analysis of 15 RCTs by Koskinas et al. showed that statins reduced the risk of major vascular events by 19% (RR = 0.81; 95% CI: 0.76–0.86) in secondary prevention patients [30]. Summarizing the effectiveness of statins in the primary and secondary prevention of CVD, we should also mention the results of the meta-analysis of 76 RCTs by Mills et al., which showed that treatment with these drugs reduced the risk of all-cause mortality by 10% (RR = 0.90; 95% CI: 0.86–0.94) and CVD mortality by 20% (RR = 0.80; 95% CI: 0.74–0.87) [31].

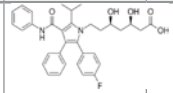
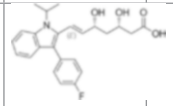
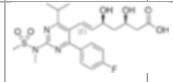
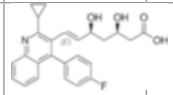
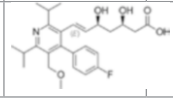
It is critically important to note that statin use is highly effective in both men and women with a similar CV risk. Fulcher et al. in their meta-analysis of 27 RCTs with

Table 23.1 Statins' chemical and pharmacological characteristics

Class of statins		Statin	Chemical structure	Dose range and half-life	Solubility and bioavailability	Metabolism	Clearance	Max. effect on LDL-C (%)
Type 1	Natural	Lovastatin		10–80 mg 2 h	Lipophilic 5%	CYP3A4 OAT1B1 P-gp	Hepatic	80 mg **−41%**
		Pravastatin		20–80 mg 2 h	Hydrophilic 15%	Non-CYP450 OAT1B1 OAT1B3	Hepatic and kidney	80 mg **−41%**
		Mevastatin/compactin (first statin)			Lipophilic	CYP3A4	Hepatic	
	Semisynthetic	Simvastatin		5–40 mg 2 h	Lipophilic 5%	CYP3A4 OAT1B1 P-gp	Hepatic	80 mg **−48%**

(continued)

Table 23.1 (continued)

Class of statins		Statin	Chemical structure	Dose range and half-life	Solubility and bioavailability	Metabolism	Clearance	Max. effect on LDL-C (%)
Type 2	Synthetic	Atorvastatin		10–80 mg 14 h	Lipophilic 15%	CYP3A4 OAT1B1 P-gp	Hepatic	80 mg **−54%**
		Fluvastatin		20–80 mg 3 h	Lipophilic 25%	CYP2C9 OAT1B3	Hepatic	80 mg **−34%**
		Rosuvastatin		5–40 mg 19 h	Hydrophilic 20%	CYP2C9 OAT1B1 OAT1B3	Hepatic and kidney	40 mg **−60%**
		Pitavastatin		1–4 mg 12 h	Lipophilic 50%	CYP2C9 OAT1B1 P-gp	Hepatic	4 mg **−48%**
		Cerivastatin			Lipophilic	Various CYP3A	Hepatic	Withdrawn from the market

Data taken from Refs. [18–21]

Abbreviations: *LDL-C* low-density lipoprotein cholesterol, *CYP* cytochrome P_{450}, *OAT1B1* organic anion transporting polypeptide B1, *OAT1B3* organic anion transporting polypeptide B3, *Pgp* P-glycoprotein

174,000 subjects showed that the proportional reductions per 1.0 mmol/L reduction in LDL-C in major CV events were similar overall for women (RR = 0.84; 99% CI: 0.78–0.91) and men (RR = 0.78; 99% CI: 0.75–0.81). These net benefits translated into reductions in all-cause mortality with statin therapy for both women (RR = 0.91; 99% CI: 0.84–0.99) and men (RR = 0.90; 99% CI: 0.86–0.95) [32].

Thus, statins are very effective drugs in the primary and secondary prevention of CV and are well established in the recommendations for lipid-lowering therapy. Taking into account the demonstrated effectiveness of these drugs, it is not surprising that these drugs are the most commonly used lipid-lowering drugs in the world. In 2018, 172.6 million people worldwide were using lipid-lowering drugs, 145.8 million of whom were taking statins (85.5%). Moreover, the frequency of statin use is increasing every year [33]. In addition to the well-documented lipid-lowering effect of statins (in addition to their many pleiotropic effects), their beneficial properties on the improvement of the prognosis in COVID-19 patients have recently been emphasized [34–37].

Statins: Safety of Use

Taking into account the important role of statins in CVD prevention, an important issue from the clinical point of view is the safety of their use. According to the position paper from an International Lipid Expert Panel (ILEP) (Fig. 23.2), the main potential side effects of statins are statin-associated muscle symptoms (SAMSs), temporary elevation of aminotransferase alanine (ALT), and new-onset diabetes (NOD) [38].

Fig. 23.2 Professor Maciej Banach is the founder and president of the International Lipid Expert Panel (ILEP): www.ilep.eu

The safety of statin therapy in primary prevention was assessed in a meta-analysis of 62 RCTs by Cai et al., which included 120,456 subjects who were followed for an average of 3.9 years. It was shown that statin use was significantly associated with the risk of muscle symptoms (OR = 1.06; 95% CI: 1.01–1.13), liver dysfunction (OR = 1.33; 95% CI: 1.12–1.58), and kidney dysfunction (OR = 1.14; 95% CI: 1.01–1.28). There was no significant association between statin use and risk of developing diabetes and clinically confirmed muscle disorders. Importantly, no dose-response relationship between statins and side effects was found. The authors of the meta-analysis concluded that the risk of adverse events attributable to statins was low and definitely did not outweigh their efficacy in preventing CVD [39]. The abovementioned meta-analysis by Yebyo et al. showed that the use of statins in primary prevention was associated with a borderline significant increase in the risk of myopathy (RR = 1.08; 95% CI: 1.01–1.15), kidney dysfunction (RR = 1.12; 95% CI: 1.00–1.26), and liver dysfunction (RR = 1.16; 95% CI: 1.02–1.31). A network meta-analysis showed that atorvastatin had the best safety profile [26], in contrast to the findings of the PRIMO study, in which hydrophilic statins—pravastatin and rosuvastatin—were found to have the best safety profile [40]. Considering the results of the REAL-CAD study, it may be that pitavastatin has the best safety profile, as the prevalence of SAMS and NOD for this statin was found to be comparable to placebo [41]. Finally, the largest meta-analysis on the prevalence of statin intolerance (SI), with almost 4.2 million patients, clearly showed that there is no difference in the prevalence of statin intolerance between hydrophilic and lipophilic statins [42].

SAMS

The study by Navar et al., covering 7938 patients from 140 primary care, cardiology, and endocrinology practices in the United States, showed that the most frequently reported adverse event in patients using statins was muscle aches/cramps (29%) [43]. On the other hand, as shown by a meta-analysis by Davis and Weller involving 153,000 patients, the use of statins regardless of the dose did not significantly affect the risk of any muscle problems (RR = 1.02; 95% CI: 1.00–1.04) [44]. A meta-analysis of 22 studies by Riaz et al. with a mean follow-up time of 4.1 years (statins = 66024, placebo = 63656) indicated that there was no significant difference in the risk of myopathy between statins and placebo (OR = 1.20; 95% CI: 0.88–1.62) [45]. The safety of statins was also assessed in a meta-analysis of 135 RCTs by Naci et al. involving 246,955 subjects. It was shown that the effect of statins on the risk of myalgia was not significant (OR = 1.07; 95% CI: 0.89–1.29). It was also found that statins did not significantly affect the risk of elevated levels of creatine kinase (CK) (OR = 1.13; 95% CI: 0.85–1.51) [46]. In a study by Herrett et al., involving 200 patients (randomized N-of-1) recruited from 50 general practices in England and Wales, it was shown that muscle symptoms were not significantly different between 2-month periods of treatment with 20 mg of atorvastatin or placebo (MD statin minus placebo: −0.11, 95% CI: −0.36 to 0.14) [47]. Thus, the prevalence of

SAMS among statin users does not appear to be high, as clearly confirmed in the meta-analysis by Bytyci et al. mentioned above [42]. As indicated in the Scientific Statement from the American Heart Association (AHA), the risk of statin-induced serious muscle injury, including rhabdomyolysis, is <0.1%, and the risk of rhabdomyolysis is 1.6 cases per 100,000 patient-years [48].

It seems that some of the SAMSs reported in studies may result from the coexistence of predisposing factors, including comorbidities (see later) [42, 49] or genetic polymorphisms (e.g., solute carrier organic anion transporter, *SLCO1B1*) [50]. Drug interactions with statins (e.g., macrolides, HIV/AIDS drugs, antifungal drugs, warfarin, amiodarone, anticancer drugs) may play an important role in the development of SAMS. The risk of statin toxicity is increased by drug-drug interactions that increase the concentration of statins in the plasma, with up to 50% of statin-mediated adverse events thought to be because of drug-drug interactions [49].

Kidney Dysfunction

It is worth noting that the increased risk of kidney failure reported in some meta-analyses in patients using statins may not be directly related to the action of these drugs. There is no data confirming the causal relationship between statin therapy and acute kidney injury [51]. Rhabdomyolysis is an important risk factor for acute kidney injury. In a study by Yang et al. of 329 patients with rhabdomyolysis, the incidence of acute kidney disease in this group of patients was 61.4% [52]. The incidence of statin-induced rhabdomyolysis was assessed by Safitri et al. in an analysis of 1,129,477 patients. Statin-induced rhabdomyolysis has been shown to occur in 0.009% of patients [53]. As indicated in the Scientific Statement from the AHA, statins do not cause or worsen proteinuria in the long term, do not cause acute kidney injury in individuals without rhabdomyolysis, and do not worsen kidney function [48], and indeed may improve renal functional parameters [54].

The forementioned meta-analysis by Davis and Weller showed that, regardless of the intensity of statin therapy, the risk of developing rhabdomyolysis was not statistically significant (RR = 1.41; 95% CI: 0.80–2.51) [44]. It should be emphasized that the use of statins may have a positive effect on kidney function. A meta-analysis of 33 RCTs by Zhao et al. of 37,391 patients with chronic kidney disease (CKD) showed that statins improved kidney function by significantly reduced urinary albumin (WMD: −2.04; 95% CI: −3.53 to −0.56) and protein (WMD: −0.58; 95% CI: −0.95 to −0.21) excretions and increased creatinine clearance (WMD: 0.86; 95% CI: 0.32–1.41) [55]. This beneficial effect of statins is due, inter alia, to the antioxidant and anti-inflammatory properties of these drugs [56]. Moreover, in a meta-analysis of 9 RCTs by Lv et al., including 3426 patients with diabetic nephropathy, it was shown that after statin treatment, estimated glomerular filtration rate (eGFR) in the experimental group was higher than in the control group (MD = 5.80; 95% CI: 2.21–9.40), and serum creatinine was lower than in the control group (MD = −0.46; 95% CI: −0.69 to −0.24) [57]. These findings may be associated

with significantly improved outcomes, especially in patients who do not require dialysis. Barylski et al. showed that statin therapy in subjects with non-dialysis-dependent CKD resulted in a marked reduction in death from all causes (RR: 0.66; 95% CI: 0.55–0.79; $P < 0.0001$), cardiac causes (0.69; 95% CI: 0.55–0.68), cardiovascular events (0.55; 95% CI: 0.4–0.75), and stroke (RR: 0.66; 95% CI: 0.5–0.88) [58].

Thus, the impact of statin use on the kidney disfunction seems doubtful and is probably due to other comorbid factors. Moreover, the results of clinical studies show that statins may significantly improve kidney function.

Liver Dysfunction

The increased risk of liver dysfunction with statins reported in some studies is also controversial and overestimated. Here, it is critically important to always pay attention to the definition of liver dysfunction and to remember that statin-related elevation of ALT is temporary in almost all cases, and that after 4–6 weeks, all patients may be treated again with statins.

Naci et al. showed that statin users were at higher risk of elevated ALT and AST levels (OR = 1.51; 95% CI: 1.24–1.84) [46]. In a meta-analysis of 16 studies conducted by Liang et al., which included 74,078 individuals, a marginally statistically significant correlation was found between statin use and risk of hepatic injury (OR = 1.18; 95% CI: 1.01–1.39). It was found that only intensive statin therapy was associated with an increased risk of liver injury (OR = 3.62; 95% CI: 1.52–8.58). A safety sub-analysis of specific types of statins showed that only fluvastatin, which is now *de facto* not used in clinical practice, significantly increased the risk of liver injury (OR = 3.50; 95% CI: 1.07–11.53). Importantly, it was found that long-term statin therapy was not associated with the risk of liver injury (OR = 1.15; 95% CI: 0.98–1.36) [59]. Another meta-analysis of 5 studies by Masson et al., including 2548 patients with abnormal liver tests at baseline, found that more intensive statin-based LLTs were associated with a similar occurrence of serious alteration of liver tests (OR = 0.90; 95% CI: 0.21–3.99) compared to less intensive treatment or placebo [60]. As indicated in the Scientific Statement from the AHA, risk of serious hepatotoxicity during statin therapy is ≈0.001%, which means that the number needed to harm (NNH) is 1:1,000,000 (with NNT = 30 for the reduction of CVD events) [48].

It should be emphasized that the use of statins in patients with hepatic dysfunction may be beneficial. In a meta-analysis conducted by Vahedian-Azimi et al., including 195,602 patients with chronic viral hepatitis, it was shown that statin use significantly reduced the risk of death by 39% in a 3-year follow-up. Moreover, the risk of hepatocellular carcinoma (HCC), fibrosis, and cirrhosis in those on statins decreased by 53% (OR = 0.47; 95% CI: 0.28–0.81), 45% (OR = 0.55; 95% CI: 0.34–0.87), and 41% (OR = 0.59; 95% CI: 0.55–0.62), respectively. Although alanine transaminase (ALT) and aspartate transaminase (AST) were reduced slightly

following statin therapy, this reduction was not statistically significant [61]. Similar results were obtained in patients with chronic liver disease (CLD). A meta-analysis by Kim et al., including 121,058 patients with CLD, showed that statin use did not significantly reduce the risk of liver fibrosis progression and cirrhosis. Moreover, in patients with cirrhosis, statin use was associated with 46% lower risk of hepatic decompensation (RR = 0.54; 95% CI: 0.46–0.62) and 46% lower mortality (RR = 0.54; 95% CI: 0.47–0.61) [62]. A meta-analysis of 14 studies by Fatima et al., involving 1,247,503 subjects, showed that statins may significantly reduce the risk of developing nonalcoholic fatty liver disease (NAFLD) (OR = 0.69; 95% CI: 0.57–0.84). Furthermore, statins were found to significantly reduce ALT levels (WMD: −27.28; 95% CI: −43.06 to −11.51), AST levels (WMD: −10.99; 95% CI: −18.17 to −3.81), and GGT levels (WMD: −23.40; 95% CI: −31.82 to −14.98) in patients presenting with NAFLD at baseline. Statin therapy was also found to significantly reduce steatosis grade ($P = 0.01$), NAFLD activity score ($P < 0.00001$), necro-inflammatory stage ($P < 0.00001$), and fibrosis ($P = 0.04$) [63]. Similar results were obtained by Pastori et al. in a meta-analysis of 22 studies covering 2345 NAFLD patients. In all interventional studies, except one, patients had raised ALT, AST, and GGT at baseline. It was found that ALT, AST, and gamma-glutamyl transferase (GGT) were reduced after statin treatment with a percentage mean difference of −35.41% (95% CI: −44.78 to −26.04), −31.78% (95% CI: −41.45 to −22.11), and −25.57% (95% CI: −35.18 to −15.97), respectively [64]. A recently published study by Wang et al., including 601,733 cancer patients and 2,406,932 patients in control, showed that those patients who used statins had a significantly lower risk of liver cancer (OR = 0.43; 95% CI: 0.40–0.47) [65].

Thus, clinically significant liver damage from statins is a very rare side effect of these drugs, for which causality has not been confirmed besides transient elevation of ALT, and fluvastatin (which is no longer recommended). Statins are safe in patients with liver dysfunction and may improve liver function and prognosis in these patients. Therefore, there is a clear recommendation for statin therapy in all patients with chronic liver diseases, and the only contraindication is acute liver disease.

NOD

As Scientific Statement from the AHA statin therapy modestly increases the risk of developing NOD, HR is ≈1.1 for moderate-dose and 1.2 for intensive statin therapy for 5 years. The risk is largely confined to patients with multiple preexisting risk factors for diabetes mellitus. The absolute risk of statin-induced NOD in major trials is ≈0.2% per year. The size of any effect in routine clinical practice will depend on the baseline risk for developing NOD in the patient population [48].

A meta-analysis of 5 statin trials with 32,752 participants conducted by Preiss et al. showed that odds ratios were 1.12 (95% CI: 1.04–1.22) for NOD among participants receiving intensive therapy compared with moderate-dose therapy. As

compared with moderate-dose statin therapy, the NNH per year for intensive-dose statin therapy was 498 for NOD while the number needed to treat (NNT) per year for intensive-dose statin therapy was 155 for CV events (over 3× higher benefit) [66]. A similar relationship was demonstrated in the meta-analysis of 29 RCTs by Thakker et al. It was found that statin use was statistically borderline significantly associated with the risk of NOD (OR = 1.12; 95% CI: 1.05–1.21) [67]. Naci et al. showed that statin users were at low risk—only 9% of the increase of NOD (OR = 1.09; 95% CI: 1.02–1.16) [46]. Finally, in the meta-analysis of 17 RCTs by Navarese et al., no significant effect of statin use (vs. placebo and comparison of different statins at different doses) on the risk of NOD was found [68]. In turn, Kamran et al. in a meta-analysis of patients with CVD and kidney disease showed that statin use is significantly but still relatively weakly associated with the risk of NOD (OR = 1.61; 95% CI: 1.55–1.68). The authors indicate that the observed results may be overestimated since statin users are people who often have concomitant risk factors for diabetes [69]. It is also worth noting the results of the meta-analysis by Danaei et al., including 285,864 subjects, which showed that the risk of statin-induced NOD was significantly influenced by other risk factors. Hazard ratio NOD in crude analysis was 1.45 (95% CI: 1.39–1.50), while only 1.14 (95% CI: 1.10–1.19) after multiadjustment [70].

Thus, the results of many clinical studies indicate that the use of statins may be associated with the risk of NOD, but the effect is small and probably related to the morbidity of people using these drugs. The profit and loss balance (NNT vs. NNH) indicate that the low risk of NOD should not be a reason for not using statins.

Efficacy and Safety of Statin Use Among Older People

A meta-analysis of 8 studies by Savarese et al. including 24,674 elderly subjects without established CVD showed that statins significantly reduced the risk of MI by 39.4% (RR = 0.606; 95% CI: 0.434–0.847) and the risk of stroke by 23.8% (RR = 0.762; 95% CI: 0.626–0.926) compared with placebo [71]. A meta-analysis of 8 studies by Teng et al. also demonstrated the efficacy and safety of statins among elderly people in primary prevention. It was shown that statins significantly reduced the risk of composite major adverse CV events (RR = 0.82; 95% CI: 0.74–0.92), nonfatal MI (RR = 0.75; 95% CI: 0.59–0.94), and total MI (RR = 0.74; 95% CI: 0.61–0.90) [72]. In a meta-analysis of 35 RCTs by Kostis et al., it was shown that statins reduced the risk of death from any cause (P = 0.03) among subjects >75 years of age in primary prevention [73]. Moreover, the meta-analysis of 23 studies by Ponce et al. involving 60,194 elderly patients assessed the effectiveness of statins in both primary and secondary prevention. It was shown that statins in primary prevention reduced the risk of CAD (RR = 0.79; 95% CI: 0.68–0.91) and MI (RR = 0.45; 95% CI: 0.31–0.66). In secondary prevention, it was found that statins reduced all-cause mortality (RR = 0.80; 95% CI: 0.73–0.89), CV mortality (RR = 0.68; 95% CI: 0.58–0.79), CAD (RR = 0.68; 95% CI: 0.61–0.77), MI (RR = 0.68; 95% CI:

0.59–0.79), and revascularization (RR = 0.68; 95% CI: 0.61–0.77) [74]. A meta-analysis of 28 RCTs by Armitage et al. showed that statin therapy or a more intensive statin regimen produced an 18% (RR = 0.82; 95% CI: 0.77–0.81; 0.70–0.95) proportional reduction in major CV events per 1.0 mmol/L reduction in LDL-C in patients >75 years of age. This relationship was significant only in patients with preexisting CVD [75]. In a meta-analysis of 10 observational studies by Awad et al. involving 815,667 elderly people in primary prevention, statin use was shown to reduce the risk of stroke (HR = 0.85; 95% CI: 0.76–0.94), all-cause death (HR = 0.86; 95% CI: 0.79–0.93), and death from causes of CV (HR = 0.80; 95% CI: 0.78–0.81), and the significant effect was maintained also in those >75 and even 85 years of age [76]. In summary, we have no doubt on the benefits of statin therapy in older adults, including those >75 years of age in primary and secondary prevention, however with stronger EBM for those with established CVD.

The meta-analyses cited above found no significant association between statin use and risk of new cancer onset, myalgia, elevation of liver transaminases, NOD, and serious adverse events [71, 72, 74, 76]. A meta-analysis of 11 RCTs by Zhou et al. of 18,192 older adults found no significant association between statin use and risk of SAMS, or other serious adverse events [77]. As demonstrated by Ott et al. in a meta-analysis of 25 RCTs including 46,836 subjects, statins did not significantly affect the risk of cognitive impairment [78]. Indeed, in a meta-analysis of 25 studies, Chu et al. obtained different results, showing that statins were significantly associated with a reduced risk of all-cause dementia (RR = 0.849; 95% CI: 0.787–0.916) [79].

Thus, the results of clinical studies show that statin use in the elderly is of significant benefit to CV prognosis and is very well tolerated. However, it needs to be strongly emphasized that taking into account that the metabolism of both cholesterol and drugs changes with age, owing to changes in pharmacokinetics and pharmacodynamics, statin doses should be increased gradually in elderly patients, as age itself is a significant risk factor of statin intolerance.

Efficacy and Safety of Statin Use Among Children

Statins are also effective in treating children and adolescents with familial hypercholesterolemia (FH). As indicated by the recommendations from the National Lipid Association (NLA) Expert Panel on Treatments for Pediatric Familial Hypercholesterolemia, statins are preferred for initial pharmacologic treatment in children after initiation of diet and physical activity management. Moreover, they indicate that clinical studies with medium-term follow-up suggest safety and efficacy of statins in children [80]. In a study by Luirink et al. involving 184 children with FH and 77 unaffected siblings who were followed for 20 years, the effectiveness of statin use was assessed. The mean progression of carotid intima-media thickness (CIMT) over the entire follow-up period was 0.0056 mm per year in patients with FH and 0.0057 mm per year in siblings. The incidence of CV events

and of death from CV causes at 39 years of age was lower among the patients with FH than among their affected parents (1% vs. 26% and 0% vs. 7%, respectively) [81]. A literature review by Peterson et al. found lower rates of ASCVD-related events and death in individuals with FH who were treated with statins from childhood, compared to those who initiated statins in adulthood [82]. A study by Kavey et al. involving 289 children treated with statins for severe LDL-C elevation demonstrated that after 2.7 years of follow-up, there was a significant reduction in LDL-C ($P < 0.001$) and non-HDL-C ($P < 0.001$). Therapy was not associated with a significant increase in the risk of elevated ALT ($P = 0.45$/year), AST ($P = 0.73$/year), CK ($P = 0.09$), and glucose levels ($P = 0.87$/year). Potentially, statin-related symptoms were recorded for 7% of patients (muscle pain, fatigue, rash, abdominal pain, and "yellow eyes") [83]. A meta-analysis of 10 RCTs by Anagnostis et al. of 1191 children and adolescents with FH summarized the efficacy and safety of statins. Compared with placebo, statins led to a mean relative reduction in total cholesterol, low-density LDL-C, triglyceride, and apolipoprotein B (apo-B) concentrations by −25.5% (95% CI: −30.4% to −20.5%), −33.8% (95% CI: −40.1% to −27.4%), −8.4% (95% CI: −14.8% to −2.03%), and −28.8% (95% CI: −33.9% to −23.6%), respectively. HDL-C was increased by 3.1% (95% CI: 1.1–5.2%). Statins were well tolerated, with no significant differences in ALT/AST and CK levels or other adverse effects compared with placebo. Statins exerted no effect on growth or sexual development [84]. In our analyses, we clearly showed that children with FH presented subclinical atherosclerosis manifested as decreased arterial wall elasticity. We also confirmed that the efficacy of LLT is very low, however with a very good safety profile [85, 86].

Thus, the use of statins is recommended in sick children with FH and is highly effective in the prevention of CVD and is safe and well tolerated. All the abovementioned aspects have been extensively discussed in the recent Position Paper of the Mighty Medic and ILEP on the risk assessment and clinical management of children and adolescents with heterozygous FH [87].

Safety of Statin Use Among Pregnant Women

As indicated by PoLA/CFPiP/PCS/PSLD/PSD/PSH guidelines on diagnosis and therapy of lipid disorders in Poland 2021: (1) statins are not recommended due to the risk of teratogenicity, despite the lack of evidence unequivocally confirming such a relationship; (2) there are more and more reports on the lack of risk of using statins and their benefits, especially for pregnant women with an underlying disease that threatens the life of the mother and the fetus (diagnosed cardiovascular disease and homozygous FH) [10]. The need to reconsider the views on the safety of statin use during pregnancy is confirmed by the results of recent meta-analyses.

A meta-analysis of 9 studies by Vahedian-Azimi et al. found no significant association between statin therapy and stillbirth (OR = 1.30; 95% CI: 0.56–3.02). While

statin exposure was significantly associated with increased rates of spontaneous abortion (OR = 1.36; 95% CI: 1.10–1.68), it was nonsignificantly associated with increased rates of induced abortion (OR = 2.08; 95% CI: 0.81–5.36) and elective abortion (OR = 1.37; 95% CI: 0.68–2.76). A nonsignificant numerically reduced rate of preterm delivery was observed in statin users (OR = 0.47; 95% CI: 0.06–3.70) [88]. In a systematic review of 136 pregnant women and 35 placental samples from hypertensive and normotensive women, Vahedian-Azimi et al. showed that statins might be beneficial for preventing or treating preeclampsia [89]. Moreover, another meta-analysis by Vahedian-Azimi et al. of 6 studies (1,267,240 participants) showed that statin use in pregnancy does not increase the risk of birth defects (OR = 1.48; 95% CI: 0.90–2.42), including cardiac anomalies (OR = 2.53; 95% CI: 0.81–7.93) and other congenital anomalies (OR = 1.19; 95% CI: 0.70–2.03) [90].

In conclusion, the use of statins during pregnancy is not currently recommended, but the results of recent studies may change this view in the near future, especially in line with complete lack of new lipid-lowering drugs (including the most innovative ones) for this more and more challengeable group of patients with many concomitant diseases, who may have high CVD risk.

Statin Intolerance: Definition and Real Global Prevalence

Taking into account the above critical discussion on the safety of statins, it seems that true (=confirmed, primary) intolerance to these drugs is not (contrary to popular belief) a common phenomenon. Statin intolerance should be defined as the inability to receive statin therapy adequate (with respect to the product or the dose) to manage the existing cardiovascular risk [91]. In other words, statin intolerance is not only the lack of statin treatment due to clinical or biochemical symptoms, but also the phenomenon of underdosage or the use of a statin too weak in relation to the cardiovascular risk [91]. There are several formal definitions of statin intolerance (Table 23.2).

The largest meta-analysis in the world by Bytyçi et al., published in the European Heart Journal in 2022, summarizes the prevalence of global statin intolerance and factors that increase the risk of developing this disorder. The meta-analysis covered 176 clinical studies (112 RCTs and 64 cohort studies) with 4,143,517 patients. It has been shown that the **overall prevalence of statin intolerance worldwide is 9.1% (8.1–10%)**. It means, in other words, that statin intolerance is overdiagnosed, and that 91% of patients on statin can be treated effectively without any safety concerns. Moreover, the prevalence was even smaller when defined using the National Lipid Association (NLA), the ILEP, and the European Atherosclerosis Society (EAS) criteria [7.0% (6.0–8.0%), 6.7% (5.0–8.0%), 5.9% (4.0–7.0%), respectively]. The prevalence of statin intolerance in RCTs was significantly lower compared with cohort studies [4.9% (4.0–6.0%) vs. 17% (14–19%)]. In primary prevention, statin intolerance was slightly less frequent than in secondary prevention [8.2% (6–10%)

Table 23.2 Approved definitions of statin intolerance

Society, year [Ref #]	Definition of statin intolerance
National Lipid Association (NLA), 2014 [92]	"Inability to tolerate at least two statins: one statin at the lowest starting daily dose and another statin at any daily dose, due to either objectionable symptoms (real or perceived) or abnormal laboratory determinations, which are temporally related to statin treatment and reversible upon statin discontinuation"
European Atherosclerosis Society (EAS), 2015 [93]	"The assessment of statin-associated muscle symptoms (SAMS) includes the nature of muscle symptoms, increased creatine kinase levels and their temporal association with initiation of therapy with statin, and statin therapy suspension and rechallenge"
International Lipid Expert Panel (ILEP) Unified Definition, 2015 [38]	1. The inability to tolerate at least two different statins—one statin at the lowest starting average daily dose and the other statin at any dose 2. Intolerance associated with confirmed, intolerable statin-related adverse effect(s) or significant biomarker abnormalities 3. Symptom or biomarker change resolution or significant improvement upon dose decrease or discontinuation 4. Symptoms or biomarker changes not attributable to established predispositions such as drug-drug interactions and recognized conditions increasing the risk of statin intolerance
Canadian Consensus Working Group, 2016 [94]	"A clinical syndrome, not caused by drug interactions or risk factors for untreated intolerance and characterized by significant symptoms and/or biomarker abnormalities that prevent the long-term use and adherence to statins documented by challenge/dechallenge/rechallenge, where appropriate, using at least two statins, including atorvastatin and rosuvastatin, and that leads to failure of maintenance of therapeutic goals, as defined by national guidelines"
Luso-Latin American Consortium, 2017 [95]	"(I) Pharmacologic (Ia) inability to tolerate at least two statins at any dose, OR (Ib) inability to tolerate doses higher than 5 mg of rosuvastatin; 10 mg atorvastatin; 20 mg of simvastatin; 20 mg of pravastatin; 20 mg of lovastatin; 40 mg of fluvastatin; or 2 mg of pitavastatin, AND (Ic) symptoms or CK changes NOT attributable to established drug-drug interactions and recognized conditions increasing the risk of statin intolerance; (II) symptomatic (IIa) intolerable muscle symptoms (muscle pain, weakness, or cramps, even with normal or mildly changed CK) OR (IIb) severe myopathy (SAMS 4); (III) etiologic (IIIa) plausible time relationship (0–12 weeks) with the introduction of statin, dose increase or introduction of a drug competing for the same metabolic pathway, AND/OR (IIIb) resolution or improvement of symptoms after discontinuation of statin (usually in 2–4 weeks), AND (IIIc) with worsening in less than 4 weeks after the new exposure (rechallenge)"

Data taken from Refs. [92–95]

vs. 9.1% (6–11%)]. It is also worth mentioning that statin lipid solubility (Table 23.1) did not affect the prevalence of statin intolerance [4.0% (2.0–5.0%) vs. 5.0% (4.0–6.0%)]. This meta-analysis identified and for the first time confirmed (it was hitherto mainly an expert opinion) a number of factors and conditions that influenced the risk of statin intolerance (Fig. 23.3) [42].

So, based on this analysis of >4 million patients, the prevalence of statin intolerance is low when diagnosed according to international definitions, and the authors

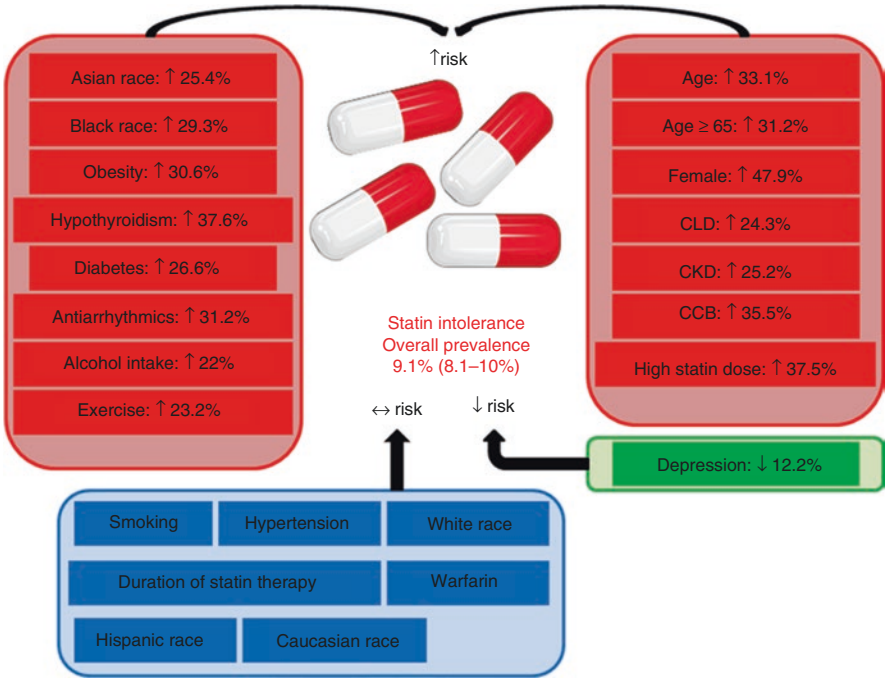


Fig. 23.3 Factors that influence the risk of statin intolerance. *Abbreviations*: *CLD* chronic liver disease, *CKD* chronic kidney disease, *CCB* calcium channel blockers. (Data taken from Ref. [42])

strongly recommend diagnosing SI based on these definitions, as this may represent an effective way to exclude nocebo/drucebo effect. These results support the concept that the prevalence of complete statin intolerance is overestimated and highlight the need for a careful step-by-step assessment of patients with potential symptoms related to statin intolerance.

Nonadherence/Discontinuation of Statin Therapy: Prevalence, Causes, and Consequences

Although true statin intolerance is not a common finding, patients either will find themselves unwilling to use these drugs or may stop treatment with these drugs. Statin discontinuation and nonadherence are the main reasons for the low effectiveness of lipid-lowering treatment. It is worth noting that only one in three patients in Europe achieves therapeutic goal; only 18% of patients in Europe, and only 13% in Central and Eastern European countries, achieve the therapeutic goal among very-high-risk patients (<55 mg/dL/<1.4 mmol/L); in patients with extreme risk, less than 10% achieve their therapeutic goal (<40 mg/dL/<1 mmol/L) [96, 97].

Prevalence

The prevalence of statin discontinuation is changeable. A literature review by Hope et al. found that the proportion of patients classed as "adherent" to statin ranged from 17.8% to 79.2% [98]. In a study by Bradley et al., including 5693 patients who had indications for the use of statins, it was found that 464 (30.7%) had discontinued therapy. Fear of side effects and perceived side effects were the most common reasons cited for declining or discontinuing a statin [99]. Huber et al., in a RCT of 486 patients after ACS, obtained different results. It was shown that after 3.9 years of follow-up, 10.5% of them were nonadherent to statin treatment (this is clearly related to the type of study—RCT—and the extent of patients' monitoring and management) [100]. Similar results were obtained in a study by Giral et al. involving 120,173 elderly people, which demonstrated that 14.3% of participants discontinued statin use during the 2.4-year follow-up [101]. However, the authors did not evaluate what percentage of patients were administered statins irregularly or at ineffective doses. Moreover, a study by Sigglekow et al., involving 289,666 new statin users, compared the level of adherence in patients with primary and secondary prevention. It was found that primary prevention patients discontinued statin use more frequently (29.8% vs. 19.7%) [102]. In the study by Vinogradov et al., covering 431,023 patients with primary prevention (137-week follow-up) and 139,314 patients with secondary prevention (181-week follow-up), it was shown that 47% and 41%, respectively, discontinued statin use [103]. Rezende Macedo do Nascimento et al. in a study involving 73,716 adult patients followed for approx. 7 years showed that the percentage of nonadherence patients was lower in the secondary prevention group (48.0% vs. 65.4%) with the lowest percentage of nonadherence among patients undergoing intensive statin therapy for both primary (55.9%) and secondary (36.3%) prevention [104]. A study by Booth et al., including 158,795 patients with MI who were followed for 182 days, showed that 15.4% of patients discontinued statin therapy after this period. Moreover, it was found that moderate- and high- vs. low-intensity statins were associated with a lower risk for statin discontinuation (moderate intensity: relative risk RR = 0.93; 95% CI: 0.89–0.96; high intensity: RR = 0.95; 95% CI: 0.91–0.99). It is worth mentioning that statin persistence after reinitiation (rechallenge) was also low (only 45.8% had high persistence) [105]. However, the relationship between the intensity of statin therapy and the level of adherence is inconsistent. A study by Rodriguez et al., including 347,104 adults with ASCVD, showed that patients taking moderate-intensity statin therapy were more adherent than patients taking high-intensity statin therapy (OR = 1.18; 95% CI: 1.16–1.20) [106]. In a study by Colantonio et al., involving 29,932 patients aged 66–75 years, it was shown that 6 months and 2 years after MI, 17.3% and 19.1% had low adherence, and 12.4% and 18.8% discontinued their statin, respectively [107]. A meta-analysis of 22 cohort studies by Mann et al. found that age had a reverse U-shaped association with adherence; the oldest (≥70 years) and youngest (<50 years) subjects had lower adherence than the middle-aged (50–69 years) subjects. A history of CVD predicted better adherence to statins (odds of nonadherence 0.68;

95% CI 0.66–0.78) [108]. A meta-analysis of 82 studies by Ofori-Asenso et al., including three million older (≥65 years) statin users from 40 countries around the world, assessed adherence and persistence in therapy with these drugs. It was shown that after a 1-year follow-up, 59.7% (primary prevention 47.9%; secondary prevention 62.3%) of users were adherent. Among new statin users, 48.2% were nonadherent and 23.9% discontinued within the first year [109]. A meta-analysis of 67 studies conducted by Lemstra et al. showed that the level of adherence to statin medications depended on the type of study (what is obviously not a surprise). Among observational studies, 49.0% of patients were adherent to statin medications at 1 year of follow-up, and among RCTs 90.3%. Importantly, this meta-analysis found that the factors increasing the level of nonadherence included primary prevention, new statin users, copayment, lower income status, fewer than two lipid tests performed, and not having hypertension [110]. A review of the literature by Ingersgaard et al. attempted to summarize the factors contributing to nonadherence among patients using statins. These factors include female sex, older and younger age, non-white race, low socioeconomic position, high copayments, being a new statin user, comorbidities, side effects, regimen complexity, type and intensity of statin dose, smoking, alcohol consumption, imperceptible benefits, and medical distrust [111].

Causes

It is worth noting that the cause of the lack of adherence is not always caused by the side effects of statins, as indicated by the results of clinical studies, but on the other hand SI seems to be one of the most common reasons of statin nonadherence. The previously cited meta-analysis by Teng et al. did not show a significant relationship between the side effects of statins and the risk of treatment discontinuation in the group of older patients (RR = 1.10; 95% CI: 0.85–1.42) [72]. Similar findings were reported in the previously cited meta-analysis by Zhou et al. (RR = 1.05; 95% CI: 0.83–1.33) [77]. The risk of statin therapy discontinuation due to side effects was also not significant in the pediatric group, as reported by Kavey et al. [83]. In a meta-analysis by Anagnostis et al., it was found that the percentage of individuals discontinuing statin therapy in the pediatric group was very low and amounted to 0–1.9% [84]. The abovementioned meta-analysis by Riaz et al. showed no significant difference in the risk of discontinuation of statin use between placebo and drugs (13.9% vs. 13.3%; OR = 0.99; 95% CI: 0.93–1.06). The sub-analysis including 14 RCTs also showed no significant difference (OR = 0.99; 95% CI: 0.9–1.1). Moreover, the analysis by specific statin types also showed no difference in the risk of treatment discontinuation compared to placebo [45].

Based on the available data, the most important reason for statin nonadherence is a lack of suitable patient education. A study by Wouters et al., involving 229 patients, showed that 40–70% doubted the necessity of or lacked knowledge about the efficacy of statins, 27–35% of the patients were worried about joint and muscle side effects, and 23% had encountered practical problems regarding information about

statins, taking of tablets, or problems with the package, or the blister [112]. Good communication with patients, appropriate education on the disease, and explanation of the necessity of statin therapy and its efficacy and safety are also the best solution to exclude the nocebo/drucebo effect [113]. Experiencing more practical problems was also associated with increased unintentional nonadherence (OR = 1.54; 95% CI: 1.13–2.10), whereas worrying about side effects was associated with increased intentional nonadherence (OR = 1.90; 95% CI: 1.17–3.08) [112]. The important role of the lack of sufficient information by the physician on the safety of statin use in the development of nonadherence was also raised by Tarn et al. The researchers stated that 27.2% of 173 patients were afraid of side effects and therefore did not comply with medical recommendations [114].

It is therefore very important to educate patients on the benefits of statin use based on the principles of evidence-based medicine (EBM). This point was extensively discussed in the recent ILEP recommendations on nocebo/drucebo effect management—the first recommendations of their kind in the world [115]. The public is very susceptible to all kinds of information and misinformation on television, in newspapers, or on social media. For example, a study by Matthews et al. showed that media coverage in the United Kingdom meant that patients already taking statins were more likely to stop taking them for both primary and secondary prevention after the period of high media coverage (OR = 1.11, 95% CI: 1.05–1.18, and OR = 1.12, 95% CI: 1.04–1.21, respectively). The elderly, and those who had used statins for a long time, had the highest risk of withdrawing from statin therapy [116]. A literature review by Nelson et al. indicated that the media has a key role in informing discussion on the public agenda but also on how issues are framed. Most studies evaluating news coverage suggest that the content on statins is predominantly negative and focused on potential harm (which receives 8–10 times more coverage than benefits of therapy). Studies utilizing quasi-experimental and interrupted time series design have shown that periods of negative news stories on statins in multiple countries are associated with (1) less statin commencement in eligible patients, (2) high rates of discontinuation, and (3) poor long-term adherence [117]. As noted in their study by Golder et al., the topic of statins is widespread in various types of social media, where users of these drugs exchange views and advice [118]. As indicated by Jones et al., statin-related websites vary widely in the quality of consumer-facing information they present. Moreover, individuals engaging in a search of statin-related information are not likely to treat pertinent information equally, differentially weighting the information that informs their medical decisions [119]. A very important role in creating a negative attitude towards statins is played by fake news spread, among others, by “antistatin movements.” A study by Scherer et al. showed that a person who is susceptible to online misinformation about one health topic may be susceptible to many types of health misinformation. Individuals who were more susceptible to health misinformation had less education and health literacy, less healthcare trust, and more positive attitudes towards alternative medicine [120]. It should also be emphasized that the cause of fake news may

be misinterpretations of the results of clinical studies or direct extrapolation of the results of experimental studies to humans (it is important to emphasize that only 1% of drugs tested on animals/cell cultures are appropriate for clinical use in humans) [121].

Thus, the lack of sufficient knowledge and the spread of fake news about the safety of statins play an important role in nonadherence of these drugs. Table 23.3 summarizes the factors associated with statin nonadherence.

Table 23.3 Factors associated with statin nonadherence

Patient-related factors 	Voluntary • Lack of understanding of current disease condition • Difficulty accepting disease severity • Previous negative experience to therapy • Skeptical on recommended treatment efficacy • Poor trust in the healthcare provider • Cultural and ethnic beliefs • Susceptibility to false information about statins on the internet and on TV Involuntary • Low level of health literacy or education • Increased susceptibility to medication adverse effects • Other comorbidities or concomitant conditions such as "psychological problems or cognitive impairments" • Unstable family background • Difficulty affording therapy
Physician-related factors 	• Complex medication regimen • Poor awareness about patient adherence • Insufficient explanation to patients about their medical condition and medications (benefits, side effects, time needed for medication to work, etc.) • Multiple physicians providing varying and possibly conflicting details to the patients • Specialty of prescriber • Poor understanding between patient and physician
Healthcare system-related factors 	• The economics of healthcare systems restricts the time spent between the physician and the patient. This results in insufficient time to: – Provide proper patient education (about their medical condition or medication) – Assess patient medication-taking behavior – Address patients' concerns – Offer encouragements and tips to improve adherence • Cost of medication • Insufficient clinical monitoring

Data taken from Ref. [122]

Complications

The consequences of noncompliance and discontinuation of statin use are critically important in everyday clinical practice. Rodriguez et al. showed that in comparison with the patients most adherent to statin therapy, those less adherent to medical recommendations were characterized by an 8–30% increase in the risk of death [106]. Giral et al. found that statin discontinuation led to a significant increase in the risk of any CV event (HR = 1.33; 95% CI: 1.18–1.50), coronary event (HR = 1.46; 95% CI: 1.21–1.75), and cerebrovascular event (HR = 1.26; 95% CI: 1.05–1.51) [101]. The consequences of statin discontinuation on the risk of major CV event (MACE: MI, ischemic stroke or TIA, coronary revascularization, and death due to MI or ischemic stroke) were also assessed by Thompson et al. in a study involving 67,418 older long-term statin users, including 27,463 in the primary prevention and 39,955 in the secondary prevention. It was shown that patients who discontinued statin therapy were characterized by a 32% and 28% higher risk of MACE during the 6-year follow-up, respectively [123]. In turn, a study by Rea et al. of 29,047 older patients exposed to polypharmacy showed that patients who discontinued statin use had a higher risk of hospital admissions for heart failure (HR = 1.24; 95% CI: 1.07–1.43), any CV outcome (HR = 1.14; 95% CI: 1.03–1.26), deaths from any cause (HR = 1.15; 95% CI: 1.02–1.30), and emergency admissions for any cause (HR = 1.12; 95% CI: 1.05–1.19) [124]. In a study by Rannanheimo et al., covering 97,575 new statin users aged 45–75 years, followed for 3 years, it was shown that those with better adherence had a significantly better prognosis (25% lower risk of any CV event or death) than those with low adherence. Patients with good adherence had also a lower incidence of ACS (HR = 0.56; 95% CI: 0.49–0.65) and acute cerebrovascular events (HR = 0.67; 95% CI: 0.60–0.76) [125]. Serban et al. investigated 105,329 Medicare beneficiaries who began a moderate- or high-intensity statin dosage after hospitalization for MI between 2007 and 2013. Statin intolerance was defined as down-titrating statins and initiating ezetimibe therapy, switching from statins to ezetimibe monotherapy, having ICD diagnostic codes for rhabdomyolysis or an antihyperlipidemic adverse event, followed by statin down-titration or discontinuation, or switching between ≥3 types of statins within 1 year after initiation. High adherence to statin therapy over the year following hospital discharge was defined as the proportion of days covered ≥80% [126]. Overall, 1741 patients (1.65%) had statin intolerance, and 55,567 patients (52.8%) had high statin adherence. The multivariate-adjusted hazard ratios (HR) comparing beneficiaries with statin intolerance versus those with high statin adherence were 1.50 (95% CI 1.30–1.73) for recurrent MI, 1.51 (1.34–1.70) for CHD events, and 0.96 (0.87–1.06) for all-cause mortality [126]. Finally, a meta-analysis by Martin-Ruiz et al. found that patients with the best adherence to statin had a significant reduction in risk: IHD by 18%, CVD by 47%, cerebrovascular disease by 26%, and death by 49% compared to patients with worst adherence to these drugs [127].

Thus, statin discontinuation or insufficient adherence to medical recommendations significantly worsens the prognosis of patients. In conclusion, it should be stated that the degree of compliance with medical recommendations regarding statin

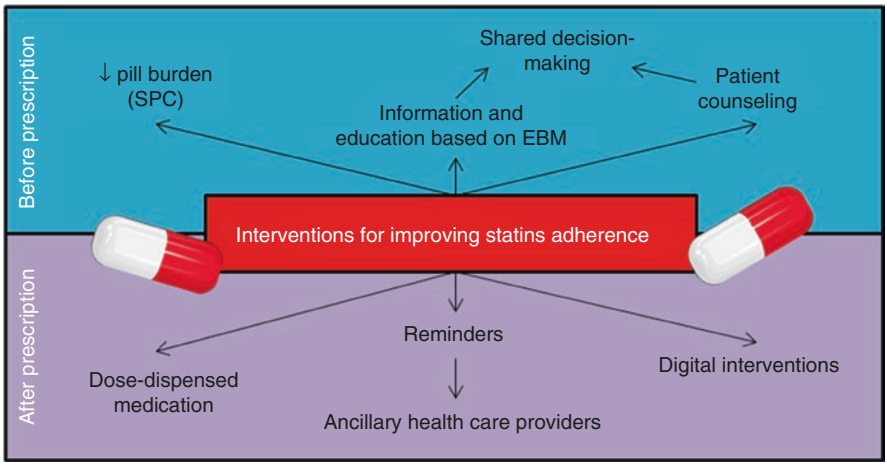


Fig. 23.4 Interventions for improving statin adherence. *Abbreviations*: *SPC* single pill combination, *EBM* evidence-based medicine. (Data taken from Ref. [122])

therapy is insufficient. A significant percentage of patients discontinue statin therapy. In most cases, the discontinuation of statin therapy seems not to result from the occurrence of side effects, but from insufficient knowledge and prejudice against these drugs. Insufficient adherence to medical recommendations and discontinuation of statin therapy significantly increase the risk of CV and worsen the prognosis of patients, and this is now considered as an important risk factor of CVD events. Figure 23.4 shows ways to improve adherence to statin use.

It is also worth mentioning that a very effective method of improving compliance with recommendations is the use of preparations based on a single pill combination (polypills, fixed combination, SPC) [128]. Patients with CVD often take several tablets (e.g., lipid-lowering drug, antihypertensive drug) or require several lipid-lowering drugs, and the combination of active substances in one SPC may significantly improve adherence. In a study by Rea et al., involving 256,012 patients, the effect of statin and ezetimibe in single tablets and as SPC on adherence was assessed. It was shown that the use of SPCs was associated with an 87% (95% CI: 75–99%) greater likelihood of high adherence and a 79% lower risk of poor adherence to treatment [129]. In the RCT by Lafeber et al., which included 78 patients with CVD, the effectiveness of the use of aspirin 75 mg, simvastatin 40 mg, lisinopril 10 mg, and hydrochlorothiazide 12.5 mg in the form of SPC or individual drugs was assessed. The authors showed that therapy with a SPC was associated with an increased adherence and that the SPC was highly preferred by patients [130]. It is also worth mentioning the meta-analysis of 44 studies by Parati et al., which showed that SPC therapy leads to improved adherence and persistence compared with free-equivalent combination therapy and may lead to better blood pressure control in patients with hypertension [131].

Thus, to effectively increase adherence and persistence, SPC-based therapy should always be considered (class: IIa, level: C), which is also reflected in the clinical recommendations [10].

Statin Intolerance: Diagnosis and Therapeutic Management

Management of patients with statin intolerance should consider the ILEP 2015 and 2022 recommendations [38, 115]. The management of statin intolerance has also been discussed in detail in the Polish guidelines 2021 on diagnosis and management of lipid disorders [10]. Additionally, in the management of statin intolerance, the ILEP position in the field of statin therapy in athletes and patients performing regular intense exercise can be used [132].

As shown earlier, genuine statin intolerance is not a common occurrence. Complete statin intolerance occurs in only a small minority of treated patients (estimated prevalence of only **3%**) [115]. Many perceived adverse effects are misattributed (e.g., physical musculoskeletal injury and inflammatory myopathies), and subjective symptoms occur as a result of the fact that patients expect them to do so when taking medicines (the nocebo/drucebo effect)—which may account for 50–70% of all patients with muscle weakness/pain [115]. The drucebo effect (a combination of DRUg and plaCEBO or noCEBO) relates to beneficial or adverse effects of a drug, which result from expectation and are not pharmacologically caused by the drug. The concept of the drucebo effect was first designed and introduced by Professor Maciej Banach and the ILEP [113]. Penson et al., based on a literature review, showed that the contribution of the drucebo effect to statin-associated muscle pain ranged between 38% and 78% [133].

When discussing the phenomenon of statin intolerance, attention should be paid to several key elements. When intolerance occurs, symptoms appear in 90% of cases within the first 6 months after initiation of statin therapy or dose increase, and in 75% within the first 12 weeks of this therapy [134]. Symptoms of intolerance are unlikely to occur 1 year after treatment initiation or dose increase, unless a new factor increasing this risk appears (disease exacerbation or initiation of a new medication which interacts with statins) [134]. The most common reasons of statin intolerance are SAMS [135]. In statin-intolerant patients, the appropriate management (so-called step-by-step approach, i.e., thorough history taking and gradual exclusion of reasons for intolerance, prompt initiation of appropriate management) may contribute to the fact that more than 95% of those patients may still receive statins [136]. Currently, in the management of patients with statin intolerance, the dominant rule of thumb for statins is to try to keep even the smallest statin dose that is tolerated and/or used even every 2–3 days (data indicate this as a possibility for atorvastatin and rosuvastatin [137]), and in the case of complete intolerance to statins, ezetimibe should be started immediately [12] and for high-risk patients other available non-statin therapies (bempedoic acid, PCSK9 inhibitors, inclisiran, as well as nutraceuticals or their combinations with proven lipid-lowering effect) should be considered [138]. Among the nutraceuticals that can be used in patients with statin intolerance, it is worth remembering that curcumin has been recognized to have lipid-lowering properties [10, 139, 140].

A detailed management algorithm for patients with suspected statin intolerance is presented in Fig. 23.5 [10]. The diagnostic process should take into account a number of factors that increase the risk of statin intolerance (Fig. 23.3) [42].

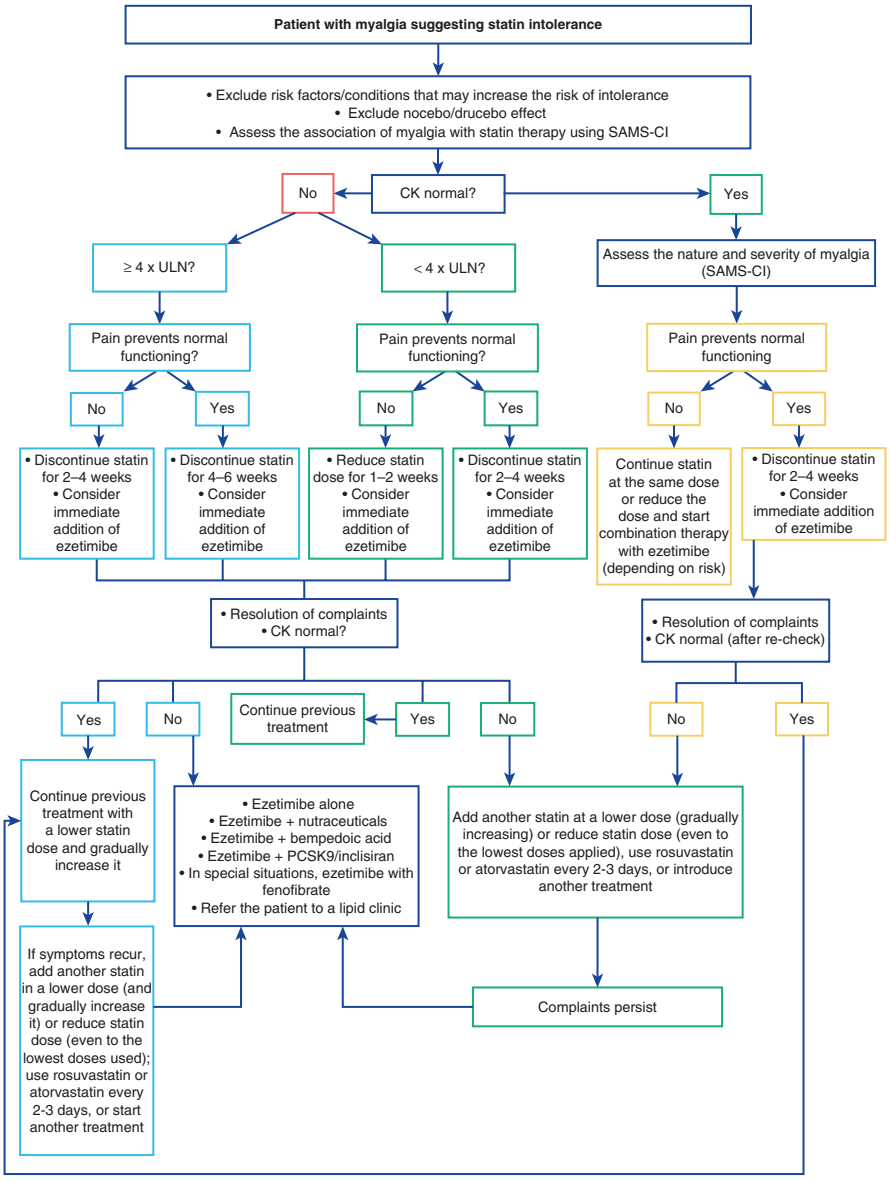


Fig. 23.5 Polish Lipid Association (PoLA) 2021 detailed recommendations for the management of patients with statin intolerance. *Abbreviations*: *SAMS-CI* Statin-Associated Muscle Symptom Clinical Index, *CK* creatine kinase, *ULN* upper limits of normal. (Reproduced with permission from Ref. [10])

It is also worth mentioning that pitavastatin, due to its bioavailability of 50% and metabolism practically without the participation of CYP450 (Table 23.1), is associated with the lowest risk of intolerance. In a study by Jeong et al., including 502 patients with high risk of developing diabetes, observed for 3 years, it was shown

that the incidence of NOD was similar between the pitavastatin 1 and 4 mg groups (4.2% vs. 2.8%, $P = 0.36$) [141]. In a study by Liu et al., including 8337 nondiabetic patients taking moderate-intensity statins (2 mg/day pitavastatin, 10 mg/day atorvastatin, and 10 mg/day rosuvastatin), it was shown that during 4 years of follow-up, pitavastatin group had a higher probability of being NODM free than the atorvastatin and rosuvastatin groups [142]. Pitavastatin also has the lowest potential risk of myalgia (estimated at about 2% for 4 mg), which is similar to placebo based on the available studies [10].

SAMS: Management Tips

One of the most difficult challenges is not only the proper management, but most of all the correct diagnosis, which will make it more probable that our patient is statin intolerant. In this context, the authors recommend the use of the SAMS scale-Clinical Index (Table 23.4), which makes it possible to give credence to the pain you are experiencing muscle has been associated with the use of statins [143]. This also, in a relatively easy way, helps to exclude the drucebo effect.

Table 23.4 Modified statin-associated muscle symptom-clinical index (SAMS-CI)

SAMS-CI	Score
1. Location and pattern of muscle symptoms *(if more than one category applies, record the highest number)*	
Symmetric, hip flexors, or thighs	3
Symmetric, calves	2
Symmetrical, proximal upper extremity[a]	2
Asymmetric, intermittent, or not specific to any area	1
2. Timing of muscle symptom onset in relation to starting statin regimen	
<4 weeks	3
4–12 weeks	2
>12 weeks	1
3. *Dechallenge*—timing of muscle symptom improvement after withdrawal of statin	
<2 weeks	2
2–4 weeks	1
No improvement after 4 weeks	0
4. *Rechallenge*—timing of recurrence of similar muscle symptoms in relation to starting second regimen	
<4 weeks	3
4–12 weeks	1
>12 weeks or similar symptoms did not reoccur	0
Interpretation *(likelihood that the patient's muscle symptoms are due to statin use)*	**Probable 9–11 Possible 7–8 Unlikely 2–6**

Adapted from Refs. [10, 143]

[a] The coracobrachialis muscle, the biceps brachii muscle, the brachialis muscle

The ILEP recommendations for the management of SAMS are summarized in Tables 23.5, 23.6, 23.7, 23.8, 23.9, and 23.10.

In the differential diagnosis of elevated CK levels, a number of other causes should be considered (Table 23.9) [115].

Table 23.5 ILEP recommendations on the management with patients **with intolerable SAMS and CK <4 ULN**

Recommendations	Class	Level
If intolerable muscle pain occurs, discontinue statin therapy for 2-4 weeks until symptoms have resolved.	IIb	C
Immediately start ezetimibe in high-risk and very high-risk patients.	IIb	C
Rechallenge with statin therapy is recommended.	I	C
SLAP algorithm is recommended to maximize long-term adherence to lipid-lowering therapy.	I	C

Reproduced with permission from Ref. [115]
Abbreviations: *SAMS* statin-associated muscle symptom, *CK* creatine kinase, *ULN* upper limits of normal

Table 23.6 SLAP approach to managing partial statin intolerance

	Step	Brief description	Rationale
S	Switch statin	Rechallenge patient with a different statin Consider using a drug with alternative partitioning chemistry (hydrophilic vs. lipophilic) or metabolic pathway to the drug which caused intolerance	Some adverse effects may be drug rather than class specific Patient may be unwilling to be rechallenged with a drug they associate with adverse effects
L	Lower dose	Reduce daily dose of statin	Adverse effects are dose dependent Adequate LDL-C reduction may be possible with a lower dose
A	Alternate-day dosing	Consider alternate-day dosing	Adverse effects are dose dependent Adequate LDL-C reduction may be possible with alternate-day dosing
P	Polypharmacy	Add another lipid-lowering drug with proven efficacy on hard outcomes	If adequate LDL-C reduction cannot be achieved with monotherapy, polypharmacy is appropriate

Reproduced with permission from Ref. [115]
Abbreviations: *LDL-C* low-density lipoprotein cholesterol

Table 23.7 ILEP recommendations on the management **with SAMS with CK >4 ULN**

Recommendations	Class	Level
Where serious muscle damage is suspected, or CK >10 ULN, statin therapy should be stopped immediately and (multi)specialist advice sought.	I	B
After symptoms release, treatment should follow the guidance for individuals with complete statin intolerance (*Figure 5*)	IIa	C

Reproduced with permission from Ref. [115]
Abbreviations: *CK* creatine kinase, *ULN* upper limits of normal, *SAMS* statin-associated muscle symptom

Table 23.8 ILEP recommendations on the management **with patients without SAMS and CK >4 ULN**

Recommendations	Class	Level
In patients with CK ≥4x ULN without SAMS, statin therapy should be stopped for at least 4 weeks, after which biomarkers should be re-investigated.	IIa	C
Statin rechallenge at a lower dose or combination therapy with ezetimibe may be considered after CK normalization.	IIb	C
SLAP algorithm is recommended to maximize long-term adherence to lipid-lowering therapy.	I	C

Reproduced with permission from Ref. [115]
Abbreviations: *CK* creatine kinase, *ULN* upper limits of normal, *SAMS* statin-associated muscle symptom

NOD: Management Tips

As detailed above, NOD is not a common side effect of statins. The ILEP recommendations for NOD are summarized in Table 23.11 [115].

When planning lipid-lowering therapy with statins in patients with a higher risk of NOD, it is worth remembering about pitavastatin, which has a lower diabetogenic effect compared to other statins [10].

ALT Elevated Level: Management Tips

As discussed in detail above, statin hepatotoxicity is not a common side effect of statins. ILEP recommendations for elevated ALT levels in patients treated with statins are presented in Table 23.12.

The lipid-lowering properties of nutraceuticals that may be helpful in the management of statin-intolerant patients are shown in Table 23.13 [115].

Table 23.9 The most common causes of CK elevation

Chronic diseases	Medications	Toxins	Metabolic disturbances	Muscle trauma/disorders	Others
Endocrine disorders Hyperthyroidism Hypothyroidism Hypoparathyroidism Acromegaly Cushing's syndrome Connective tissue disorders Rheumatological diseases Cardiac disease (heart failure, valvular, tachycardia, myocarditis, acute coronary syndrome) Acute kidney disease Viral illnesses Celiac disease	Statins Fibrates Antiretrovirals Beta-blockers Clozapine Angiotensin receptor-blocking agents Hydroxychloroquine Isotretinoin Colchicine Steroids	Ethanol Cocaine Heroin Amphetamine	Hyponatremia Hypokalemia Hypophosphatemia	Muscle dystrophies Metabolic and mitochondrial disorders of muscle Inflammatory myopathies Others Familial elevated CK Sarcoid myopathy Motor neuron diseases Charcot-Marie-Tooth disease Other congenital diseases Intramuscular injections Needle electromyography Seizures	Ethnicity (black Americans may have elevated baseline CK levels) Intensive exercise Surgery Malignancy Macro-CK Severe chills Predisposition to malignant hyperthermia Idiopathic hyperCKaemia

Reproduced with permission from Ref. [115]

Table 23.10 Summary of the ILEP recommendations on the management with SAMS

Recommendations	Class	Level
If a statin-based regimen is not tolerated at any dosage (even after rechallenge), ezetimibe should be considered.	I	C
In patients with the family history of statin intolerance, and those being on the SI risk, starting with the combination therapy of lower dose of statin and ezetimibe (with the doses suitable for the given CVD risk) might be considered.	IIb	C
In patients with complete statin intolerance, ezetimibe may be considered immediately after statin discontinuation.	IIa	C
In secondary prevention, patients with acute coronary syndrome (ACS) and with complete statin intolerance, combination therapy with ezetimibe and PCSK9 inhibitors may be considered immediately after statin discontinuation.	IIb	C
If a statin-based regimen is not tolerated at any dosage (even after rechallenge), a PCSK9 inhibitor added to ezetimibe should be considered.	IIa	C
If a statin-based regimen is not tolerated at any dosage (even after rechallenge), bempedoic acid or fixed combination of bempedoic acid with ezetimibe may be considered.	IIb	C
If a statin-based regimen is not tolerated at any dosage (even after rechallenge), inclisiran added to ezetimibe may also be considered.	IIb	C

Reproduced with permission from Ref. [115]
Abbreviations: *SI* statin intolerance, *CVD* cardiovascular disease, *PCSK9* proprotein convertase subtilisin kexin type 9 inhibitors

Table 23.11 ILEP recommendations on the management with new-onset diabetes (NOD)

Recommendations	Class	Level
If NOD occurs, it is recommended to continue statin therapy at the indicated dose.	I	B
In patients at risk of developing NOD, moderate-intensity statin therapy and/or combination therapy, depending on the risk, may be considered.	IIb	C
All individuals on a statin who have major risk factors for NOD, particularly impaired fasting glucose, should be informed about the risk and monitored for hyperglycemia.	IIa	A

Reproduced with permission from Ref. [115]

Table 23.12 ILEP recommendations on the management with elevated level of ALT

Recommendations	Class	Level
If ALT rises to <3× ULN, statin therapy should be continued, and re-checking liver enzymes may be considered after 4 weeks, especially with ALT >2x ULN.	IIa	C
If ALT rises to >3× ULN statin therapy at a lower dose (step-by-step dechallenge) may be considered. Ezetimibe may be started immediately, taking into account the patient's baseline risk and lipid profile.	IIb	C
Re-challenge of statin therapy with original dose may be considered after 2-4 weeks.	IIb	C
SLAP algorithm is recommended to maximize long-term adherence to lipid-lowering therapy	I	C

Reproduced with permission from Ref. [115]
Abbreviations: *ALT* alanine aminotransferase, *ULN* upper limits of normal

Table 23.13 Summary of the ILEP recommendations on the application of nutraceuticals in statin-intolerant patients

Nutraceuticals	Active Daily Doses	Expected Effects on LDL–C	Safety Issues	Recommendations	
				Class	Level
Red Yeast Rice	Up to 1,200 mg (*up to 3 mg of monacolin K*)*	–15% to –25%	Due to content of monacolin K some adverse effects typical for statins might appear	I	A
Phytosterols	800–2,400 mg	–7% to –10%	Should be avoided in patients with phytosterolemia and those who are heterozygous for variants of *ABCG5* and *ABCG8* and other genes	IIa	B
Bergamot	500–1,500 mg	–15% to–25%	No safety concerns	IIb	B
Soy Products	25–100 g	–6% to –10%	Possible interfering with thyroid function and fertility; ↓absorption of calcium, magnesium, copper, iron, and zinc	IIb	B
Polyunsaturated Omega–3 Fatty Acids**	2–4 g	–3% to –7%	Fish oil supplementation might be proarrhythmic (the risk of atrial fibrillation) especially in patients at the risk of arrhythmias	IIa	B
Berberine	500–1,500 mg	–15% to –25%	No safety concerns	IIa	C
Artichoke	1,800 mg/day	–15 to –23 %	Good tolerability in short-medium term	IIa	B

Reproduced with permission from Ref. [115]
Abbreviations: *LDL-C* low-density lipoprotein cholesterol
[a] Maximum recommended doses as dietary supplement recommended by the draft (2021) recommendations by the European Food Safety Authority (EFSA)
[b] Attention should be paid to increased risk of atrial fibrillation

Conclusions

Lipid disorders are the most important risk factor for ASCVD (the leading cause of premature death in the world), because they are both common and poorly managed. Effective LLT is the basis of the primary and secondary prevention of CVD. Statins are the gold standard in lipid-lowering therapy. These drugs are highly effective and, most importantly, prolong life. Statins are usually very well tolerated; however, in common with all medicines, statins may cause adverse events in some patients. The most common side effects of statins, for which the causality has been confirmed, are SAMS, NOD, and elevated ALT. Genuine statin intolerance is uncommon—globally, it affects 9.1% of treated patients. A number of risk factors can increase the risk of developing statin intolerance. Widespread negative attitudes towards statins and the drucebo effect negatively affect adherence. A significant percentage of patients discontinue statin use or exhibit a nonadherence attitude. It has been clearly shown that nonadherence and discontinuation of statin therapy significantly increase the risk of CV. Therefore, proper diagnosis and management of statin-intolerant patients are extremely important. In statin-intolerant patients, the appropriate management (so-called step-by-step approach, i.e., thorough history taking and gradual exclusion of reasons for intolerance, prompt initiation of appropriate management) may contribute to the fact that more than 95–97% of those patients may still receive statins. In the management of patients with statin intolerance, the recommendations of the ILEP should be applied.

The authors of this chapter wish to highlight that due to the constant progress of knowledge in the field of lipid-lowering treatment and statin intolerance issue [144], there is a continual and permanent need for updated information in this area.

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Chapter 24
Fibrate Therapy: Impact on Dyslipidemia and Cardiovascular Events in Patients with Diabetes Mellitus Type 2

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Introduction

Coronary heart disease (CHD), ischemic stroke, and lower extremity arterial disease (LEAD) are primarily due to atherosclerosis and, given their common pathophysiology, together are called atherosclerotic cardiovascular disease (ASCVD). ASCVD is the leading cause of morbidity and mortality in the USA, with over three-quarters of a million new cases of CHD and nearly comparable incidence of stroke plus LEAD each year [1]. Lowering low-density lipoprotein-cholesterol (LDL-C) levels with statin monotherapy is well proven to reduce ASCVD events by about 20–50% [2]. Importantly, the degree of risk reduction is proportional to the degree of LDL-C decrease—roughly a 21% ASCVD event decrease per 39 mg/dL (1 mM/L) LDL-C decrease [2]—with a similar relative reduction in patients with diabetes mellitus type 2 (DM-2) [3]. Due to the higher ASCVD risk in DM-2 (other risk factors being equal), the similar relative reduction with DM-2 translates to a higher absolute risk reduction with a given LDL-C lowering in those patients. Although statin treatment is clinically very useful and essentially always cost effective, it is important to note that the majority of ASCVD events still occur despite statin monotherapy [4–7]. How to address this large residual risk, especially in patients with high baseline ASCVD risk due to strong risk factors such as DM-2 and insulin-resistant states such as the metabolic syndrome?

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Much of the excess ASCVD risk in patients with DM-2 and insulin resistance appears to come from their characteristic "atherogenic dyslipidemia," traditionally denoted by high plasma triglyceride (TG) levels, low levels of high-density lipoprotein cholesterol (HDL-C), and increased number of small, dense LDL particles (sdLDL), and these three are usually also accompanied by increased levels of apolipoprotein B (apo B) [8, 9]. A primary mechanism by which statins reduce ASCVD risk appears to be reducing elevated LDL-C levels by preferentially promoting clearance of larger LDL particles, while they have only modest effects on high TG or low HDL-C, and minimal reduction of sdLDL. Thus, the atherogenic dyslipidemia is characterized by lipoprotein abnormalities for which statins have limited efficacy. In contrast, the peroxisome proliferator activator receptor (PPAR)-alpha agonists, commonly called fibrates, are very effective for the abnormalities characteristic of the atherogenic dyslipidemia, and so they are often added to statins in patients with DM-2. Much more common than DM-2 is prediabetes, which is more or less interchangeable with insulin resistance, which may also be called the metabolic syndrome. The interrelationship between these entities and the atherogenic dyslipidemia is such that two of the five criteria for the metabolic syndrome, low HDL-C and HTG, are also the two most widely noted aspects of the atherogenic dyslipidemia.

This chapter focuses on the effects of fibrates as monotherapy and in addition to statins on the atherogenic dyslipidemia and related ASCVD risk factors, and most importantly on atherosclerosis and on the incidence of ASCVD events in patients with DM-2 and insulin resistance.

Pathophysiology of "Atherogenic" and Other Dyslipidemias and of ASCVD in DM-2

Hypertriglyceridemia (HTG) is the key element of the atherogenic dyslipidemia and is strongly associated with accelerated atherosclerosis and ASCVD risk [10, 11]. While cholesterol is a primary component of atherosclerotic plaque and driver of its progression, TG, the other major neutral lipid component of lipoproteins, is not found in excess in the plaque. A fundamental question then is what are the biologically plausible mechanisms by which high circulating TG levels could cause atherosclerosis and ASCVD?

Over the past two or three decades, much of the research regarding HTG and ASCVD risk has focused on TG-rich remnant lipoproteins (remnants) and on their cholesterol content (remnant lipoprotein cholesterol, or RLPC) [12]. Remnants are now considered to be primary drivers of the relationship between HTG and ASCVD, and indeed there is good evidence for direct atherogenicity of TG-rich lipoproteins, remnants in particular, in the artery wall (see [12–14] for three of many excellent reviews on this subject). TG-rich lipoproteins consist of two families: chylomicrons, carrying TG of exogenous/dietary origin from the small intestine, and VLDL,

carrying endogenous TG from the liver [14, 15]. When newly secreted, these particles appear to have little or no atherogenicity, but once in the circulation, they rapidly undergo extensive lipolysis of their phospholipid surface and especially their TG core, primarily by the action of endothelial bound lipoprotein lipase (LPL) [12, 14, 15]. This lipolytic modification of TG-rich lipoproteins makes them lipoprotein "remnants," which are far more atherogenic than their nascent counterparts for several reasons. First, being smaller, they more readily pass through the endothelium into the artery wall, and second, once inside the wall, they are more readily taken up by artery wall macrophages than are other atherogenic lipoproteins, specifically LDL [12, 13, 15]. In part, this is because LDL particles are not readily taken into macrophages until they are oxidized or otherwise modified, while remnants need no modification for avid uptake [11, 13, 15]. Further, macrophage uptake of LDL (even perhaps modified LDL) appears to be downregulated by increased macrophage cholesterol content, whereas uptake of remnant lipoproteins is not so regulated and thus it progresses as long as remnant particles are present [13]. Further, remnant lipoproteins contain, and thus deliver, more cholesterol per particle to artery wall cells than does LDL [11, 13]. Therefore, even though LDL particles vastly outnumber remnants in the circulation, remnants appear to play an outsized role in cholesterol loading of the macrophages, which in turn is a primary atherogenic factor in the artery wall [13]. Given this compelling rationale for atherogenesis of TG-rich remnants, why does this chapter not dwell extensively on remnant lipoprotein cholesterol levels (RLPC) and fibrate effects on them? First, as is unfortunately ignored by many commentators, most studies touting the strong prediction of ASCVD events by RLPC levels did not measure those levels, but instead calculated them as very-low-density lipoprotein cholesterol (VLDL-C) by the Friedewald equation, which estimates VLDL-C by simply dividing total plasma or serum TG by 5 [16]. Thus, "RLPC" in most scientific publications is just TG divided by 5, such that results owe solely to total TG levels [17–24]. Second, significant problems persist with the methodology of measuring RLPC levels. There are many methods for calculating and measuring RLPC, but they can be rather discrepant in their results [25], and sometimes the methods for direct RLPC assessment are no better than Friedewald-calculated VLDL-C (total TG divided by 5) in predicting ASCVD risk [26]. Most importantly, there is still no broad consensus regarding the best method to measure RLPC, and until there is such, progress with RLPC-specific risk assessment and treatment will remain tortuous [14, 15, 27]. Despite the abovementioned logistical difficulties with RLPC, in one additional regard, it is clinically useful to remember that remnants are shown to be strongly atherogenic. Beyond the above remnant-related mechanisms and observations, there is an underappreciated dyslipidemia called type III, or familial dysbetalipoproteinemia [15, 28, 29]. Type III dyslipidemia is characterized by a large excess of circulating remnant lipoproteins due to impaired hepatic removal [15, 28, 29]. Although it is rare, clinicians should remember to consider it in the differential diagnosis for all patients with moderate elevations in both TG and total cholesterol, since it is associated with a striking increase in ASCVD risk and, as discussed below, it is readily treated with fibrates [15, 28, 29].

Given the above-discussed limitations of RLPC levels for monitoring ASCVD risk related to the atherogenic dyslipidemia, it is important to acknowledge total plasma TG (traditionally after an overnight fast) as a powerful and practical predictor of ASCVD risk in the setting of first-line statin-based LDL-C lowering [11, 13, 30–33]. Earlier studies showed a substantial increase in ASCVD risk with a fasting TG above 200 mg/dL [28, 34]. Importantly, a recent prospective study of two large US-based cohorts has additionally shown a steep increase in ASCVD risk beginning well below the traditional cutoff for elevated TG levels [35]. Strikingly, risk rose starting at just 60 mg/dL and continued rapidly upward to about 200 mg/dL, using an average fasting TG measured more than once over time (Fig. 24.1a). This was especially true when accompanied by an HDL-C <40 mg/dL (Fig. 24.1b, c) [35]. Thus, clinical attention to elevated fasting TG levels should likely begin at a far lower point than previously considered, as further emphasized by the fact that low HDL-C [35] and also elevated sdLDL [8, 9] typically begin well within or even below a "borderline" TG range of 100–200 mg/dL. An earlier meta-analysis of observational studies found a 32% and 76% increased risk of ASCVD in men and women, respectively, for each 88 mg/dL increase in fasting TG, independent of HDL-C levels [36]. The association of HTG with ASCVD is also strong with non-fasting TG levels [17, 19, 20, 22–24], including total mortality [19], and especially in women [18, 37]. Further, and perhaps most importantly, HTG (measured fasting or non-fasting) is associated with ASCVD risk in Mendelian randomization studies, evidence that HTG may cause atherosclerosis [22, 38]. How might HTG per se be atherogenic? High plasma TG levels are naturally associated with elevated TG content of the artery wall and, although excessive TG accumulation is not found in atherosclerotic plaque, even just modestly increased artery wall TG-rich lipoproteins are likely atherogenic by the following mechanism. LPL activity for TG lipolysis is present not only on the vascular endothelium but also on the surface of artery wall macrophages [13, 39]. LPL-mediated lipolysis of circulating lipoproteins generates free fatty acids (FFAs), but these are usually bound and neutralized avidly by albumin; however, very little albumin is present between cells in the artery wall. Therefore, the FFAs generated from lipolysis in the artery wall are minimally bound and neutralized, so they are strongly pro-inflammatory and thus also pro-atherogenic [13, 39].

Another key TG-related atherogenic factor is apolipoprotein (apo) C-III, which is the primary regulator of lipoprotein lipase (LPL) activity, and thus of lipolysis of TG in circulating lipoproteins. Elevated apo C-III levels, therefore, are commonly and causally associated with HTG [12, 40, 41]. Beyond this important association, however, apo C-III is also well established as a powerful pro-atherogenic factor in the artery wall [40, 42], although the details of these mechanisms are not yet well understood. Fibrates, especially fenofibrate, are well documented to reduce circulating apo C-III levels, and the resulting disinhibition of LPL is one of the most important mechanisms by which they lower TG [43]. Thus, decreasing apo C-III may be an important mechanism by which fibrates directly can reduce atherosclerosis and ASCVD event risk. In addition to the TG-lowering effect of LPL activity, the other major regulator of plasma TG is hepatic production of very-low-density

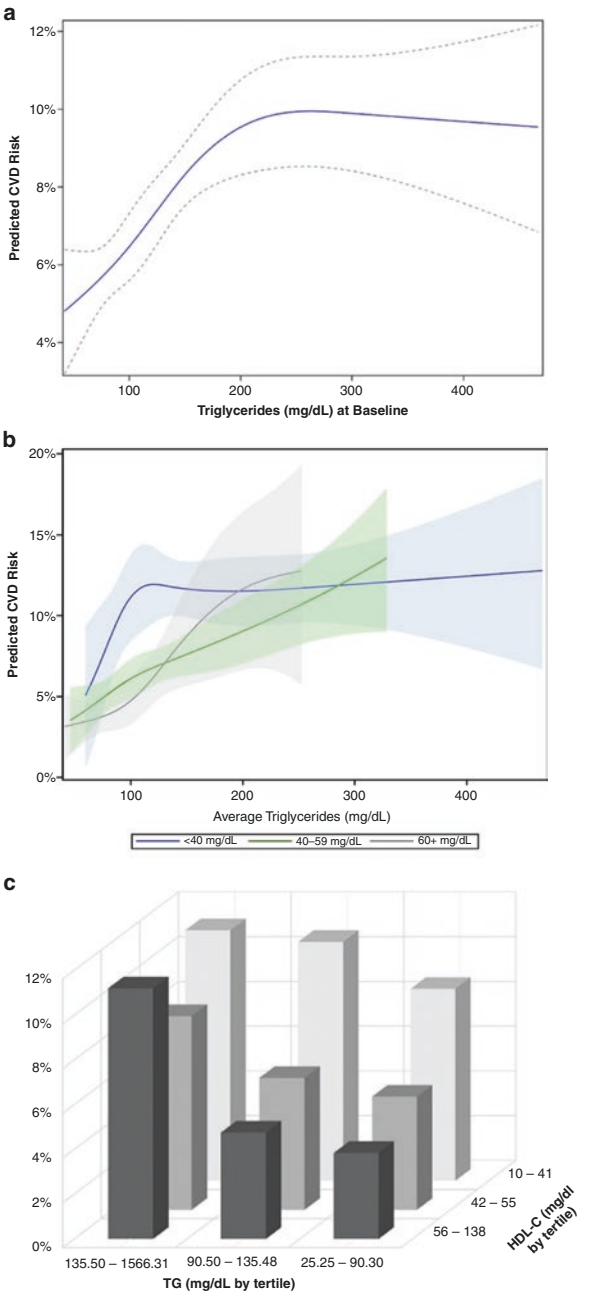

Fig. 24.1 (**a**–**c**) Predicted 10-year ASCVD risk by TG and HDL-C Levels. (**a**) ASCVD risk by average of two fasting TG levels by the cubic splines model. (**b**) ASCVD risk by TG levels (average of two fasting samples) separately by three HDL-C categories: <40, 40–59, and ≥60 mg/dL by the cubic splines model. (**c**) Three-dimensional bar graph of ASCVD risk by tertiles of TG and HDL-C. (Figures taken from Aberra T. et al. [35])

lipoproteins (VLDL). This is largely driven by hepatic content of fatty acids and TG but can also be inhibited by insulin effects on the liver [8]. Although fibrates are not known to reduce hepatic fat content, they do enhance insulin action [8] and may thus reduce hepatic VLDL production, as further discussed below.

The second major element of the atherogenic dyslipidemia is low HDL-C levels, and epidemiological studies consistently show that HDL-C is inversely associated with atherosclerosis and ASCVD event risk [44–48]. Low HDL-C is part of the definition of metabolic syndrome, and it is particularly prevalent in patients with DM-2 [49]. HDL deficiency is a strong predictor of increased ASCVD risk in DM-2, even when aggressive statin therapy leads to low LDL-C levels. HDL-C levels are strongly and inversely correlated with TG via various mechanisms [50]. Due to this collinearity, it is hard to separate the effects of low HDL-C from those of HTG, but low HDL-C predicts ASCVD risk even after correction for elevated TG levels [51, 52], even TG above just 135 or 150 mg/dL despite good LDL-C control with statin therapy [53–55]. Both epidemiologic [35] and mechanistic studies [56] show a synergistic interaction between low HDL-C and HTG in promoting arterial cholesterol content and inflammation, and thus also atherosclerosis and ASCVD risk. There are many anti-atherosclerotic mechanisms specific to HDL particles, such as promoting stabilization [48] or regression [57], and this interesting and much-studied topic has been the subject of several reviews [58–63]. Although plasma HDL-C levels predict ASCVD risk well, not surprisingly, measurements of antiatherogenic HDL function, such as its capacity to promote cholesterol efflux or to inhibit LDL oxidation, appear to be even stronger predictors. A key anti-atherogenic function of HDL is promotion of cholesterol efflux from cells, which can be measured in stored plasma [64], and which activity was shown to correlate with ASCVD risk much more closely than HDL-C levels in a large prospective cohort study [65]. The general difficulty and lack of standardization of such assays, however, have sharply limited any application in a clinical setting [66, 67]. Advanced assays of HDL composition, on the other hand, are of potential clinical interest since compositional assays are generally simpler and more reproducible than functional assays, and because HDL particle composition is a key determinant of HDL function. The relationship between HDL composition and function is not simple, however, because composition relates not only to future lipoprotein function but also to prior particle activity. Nevertheless, a well-documented HDL composition assay is the level of apo A-I, the principal apoprotein on HDL (often analyzed concurrently with apo B), and it is superior to HDL-C levels for ASCVD risk prediction in many [68, 69] although not all studies [70, 71]. Importantly, impaired metabolism and low levels of apo A-I may be an important cause of insulin resistance and DM-2 [72]. Despite its potential advantages over HDL-C levels, however, apo A-I is not measured routinely in the clinic because clinic-friendly assays were developed only after the standard lipid panel was already well established, so it requires the inconvenience and expense of an extra test. Another advanced HDL composition assay is HDL particle concentration (HDL-P) estimated by nuclear magnetic resonance (NMR), which is available commercially and has been shown to predict ASCVD risk better than HDL-C and even HDL-mediated cholesterol efflux in a large clinical trial [73]. As for apo A-I, however, HDL-P also requires an extra test beyond the basic lipid panel. Further research is needed to better validate the clinical utility of these and other special assays of HDL composition both for ASCVD risk prediction and for evaluating treatment response, especially in populations with insulin resistance and/or DM-2.

The third element of atherogenic dyslipidemia is excess levels of smaller, denser LDL particles, which is common in insulin resistance and DM-2 [51]. Importantly, sdLDL is also usually associated with elevated levels of apolipoprotein B (apo B) in these states [74], as discussed in the next paragraph. HTG is strongly associated with sdLDL, apparently in part due to their mutual relationship with hepatic overproduction of apo B, and also in part due to exchange of TG for cholesteryl ester (CE) in the core of LDL via cholesteryl ester transfer protein (CETP) [13, 14]. CETP avidly exchanges CE out from, and TG into, LDL in patients with HTG. This exchange is always rapidly followed by lipolysis of TG in LDL (by both LPL and hepatic TG lipase) [13, 14]. This TG lipolysis prevents substantial TG enrichment of LDL but causes a net decrease in LDL core lipid, resulting in smaller, denser LDL (sdLDL) [13, 14, 75]. (Parenthetically, HTG causes similar action of CETP and lipolysis on HDL, which also results in small, dense HDL particles by the same mechanisms [76]. Unique to HDL, this core-lipid depletion also leads to loss of apo A-I from the HDL particle, accelerating renal clearance of apo A-I, thus lowering apo A-I levels directly) [13, 50, 76]. Excess sdLDL is associated with increased ASCVD risk [77–80], apparently by several mechanisms: (1) increased penetration from plasma through the endothelium into the subendothelial space, (2) greater adhesion to the subendothelial matrix (where atherogenesis mainly occurs), (3) greater susceptibility to oxidation, and (4) less binding to and clearance by the LDL receptor [15, 27, 81].

A fourth element of the atherogenic dyslipidemia, strongly related to sdLDL, is elevated levels of apo B. Although it is less often mentioned in this context than the other three elements, it is usually present with these. Apo B is the main protein on all atherogenic particles [82, 83] and thus is a very strong predictor of elevated ASCVD risk in all patients, including those with insulin resistance and DM-2 [84]. Interestingly, much of the increased ASCVD risk with elevated apo B levels may be attributable to elevated TG [23]. Importantly, elevated apo B levels were suggested to be an important causal factor for DM-2 in a recent Mendelian randomization study [85]. Meanwhile, in the reverse direction, insulin resistance appears to cause increased apo B levels, according to two lines of evidence. First, elevated circulating insulin levels (the *sine qua non* of insulin resistance before DM-2 onset) are strongly associated with both increased secretion and decreased catabolism of apo B [86], and second, patients with DM-2 (but not DM-1) onset before 20 years of age have both increased sdLDL and apo B, implying insulin resistance to be a cause of both [74]. Thus, elevated apo B appears to be mutually causal with insulin resistance and DM-2, given these strong interrelationships in their metabolism [72]. As discussed above for HDL-C vs. apo A-I levels, there is controversy regarding the utility of non-HDL-C levels (or the mathematically similar total cholesterol) vs. apo B for optimal ASCVD risk assessment and management. Although many studies show apo B levels to be better [69, 87], other studies do not show any added benefit beyond non-HDL-C (or total cholesterol) [70, 71, 88]. Despite the potential advantages of apo B levels over LDL-C or non-HDL-C, apo B is not measured routinely in the clinic because automated assays were developed after the basic lipid panel was already well established, such that apo B determination requires the inconvenience and expense of an extra test [89].

Fibrate Effects on Lipoprotein Levels, Especially in Insulin Resistance and DM-2

Several studies have reported the lipid effects of gemfibrozil and fenofibrate (the two fibrates currently available in the USA), data from two reviews being shown in Table 24.1 [90] and Fig. 24.2 [91]. The greatest and most consistent lipid effect of fibrates is TG lowering, its degree varying directly with the baseline TG level. The decrease in TG levels with fenofibrate is related to a significant reduction in the large, buoyant very-low-density lipoprotein 1 (VLDL1) (−46.5%; $p < 0.001$), which has far more TG molecules per particle than do sdVLDL2 (−33.3%; $p < 0.001$) [81]. VLDL1-TG levels are primary determinants of plasma TG, and their excess is related to an excess of sdLDL particles (see below). Fibrates may often increase LDL-C levels, especially when baseline TG levels are high, although fenofibrate can lower LDL-C modestly when baseline TG is low (Fig. 24.3) [90]. The increase in LDL-C seen with fibrate use in the setting of a high baseline TG level likely relates, however, to an increase in average LDL particle size ($p < 0.001$) [92–94], rather than an increase in LDL particle concentration. This is strongly suggested by the finding that fenofibrate generally decreases plasma apo B levels (for example by 13% in 11 men with metabolic syndrome, $p < 0.001$ [95], and by 18%, $p < 0.001$, in 29 subjects with familial combined hyperlipoproteinemia) [96]. Further, (1) there is only one apo B molecule per VLDL, intermediate-density lipoprotein (IDL) or LDL particle, and (2) the vast majority of circulating apo B-containing particles are LDL. Importantly, the number and TG content/size of the large VLDL1 particles (a primary determinant of total plasma TG) are also directly related to sdLDL levels. This could be due to direct conversion (somehow) of large VLDL1 to sdLDL. More likely, however, it is because VLDL1 particles drive CE depletion/TG enrichment and resulting lipid depletion/core shrinkage of LDL, as explained above [14, 15, 27, 81]. Fenofibrate reduces sdLDL by these same mechanisms. Fenofibrate also lowers RLPC whenever it is calculated from TG by the Friedewald calculation and, of course, to the same degree that it lowers TG (generally in the 20–50% range). There are, unfortunately, only a few studies reporting the effects of fibrates on directly measured RLPC. In the Diabetes Atherosclerosis Intervention Study (DAIS), 204 subjects with DM-2 and coronary atherosclerosis visible on angiogram were treated for an average of 40 weeks with fenofibrate or placebo during which RLPC (by homogenous auto-assay) decreased 24.6% with fenofibrate (placebo corrected, $p < 0.001$) [97]. In a small single-arm trial, 20 men selected for fasting TG > 150 and no obesity (BMI < 30 kg/m^2) had a 75.4% decrease in RLPC (using a different homogenous auto-assay) [98]. In another study, 26 patients with moderate HTG (314 mg/dL) had a 33% decrease in fasting RLPC by immunoprecipitation (cholesterol in the supernatant after precipitation of plasma with anti-apo B-100 and anti-apo A-I mAbs, thus specific for chylomicrons). A subset of six of these subjects also showed a 69% decrease in area-under-the-curve for RLPC after a fat test meal [99]. Very similar fasting and postprandial RLPC effects were also seen with gemfibrozil in other, comparable, subjects in this study.

Table 24.1 Baseline TG, LDL-C, and HDL-C levels, and placebo-corrected percent change in these three parameters from 11 fenofibrate trials and 7 gemfibrozil published randomized placebo-controlled trials

					TG		LDL-C		HDL-C	
First author	Journal	Year published	*N*	Trial duration (weeks)	Baseline TG (mg/dL)	Placebo-corrected % change TG	Baseline LDL-C (mg/dL)	Placebo corrected % change LDL-C	Baseline HDL-C (mg/dL)	Placebo corrected % change HDL-C
Fenofibrate										
Keech [107]	Lancet	2005	9795	260	153.5	−23%	118.5	−6%	42.5	1%
Vakkilainen [92]	Circulation	2003	405	172	225.6	−24%	130.5	−9%	39.6	3%
Farnier [182]	Eur. Heart J.	2005	253	12	276.5	−26%	165	−3%	42.5	16%
Farnier [182][a]	Eur. Heart J.	2005	372	12	274.3	−33%	160.2	−5%	42.5	15%
Farnier [183]	Amer. Heart J.	2007	244	12	231.1	−38%	162.6	−11%	45.3	19%
Farnier [183][b]	Amer. Heart J.	2007	367	12	226.4	−20%	162.1	−1%	44.7	1%
Knopp [187]	Amer. J. Med	1987	227	24	193.1	−44%	NR	NA	47.5	21%
Davidson [188]	Clinical Cardiol	2006	146	8	479.7	−37%	119.3	16%	35.7	16%
Krempf [189]	Diab. and Metab	2000	138	13	127.9	−40%	225.7	−33%	56.8	4%
Seidehamel [190]	Cardiology	1989	147	8	614.6	−56%	109.9	34%	30.8	22%
Nissen [191]	JAMA	2007	102	12	246	−37%	NR	NA	37.4	15%
Gemfibrozil										
Frick [141]	NEJM	1987	4081	262	176	−36%	188.7	−9%	47.3	9%

(continued)

Table 24.1 (continued)

First author	Journal	Year published	*N*	Trial duration (weeks)	TG		LDL-C		HDL-C	
					Baseline TG (mg/dL)	Placebo-corrected % change TG	Baseline LDL-C (mg/dL)	Placebo corrected % change LDL-C	Baseline HDL-C (mg/dL)	Placebo corrected % change HDL-C
Rubins [128]	NEJM	1999	2531	52	160.5	−32%	111.5	0%	32	6%
Frick [185]	Annals of Med	1993	628	260	183.2	−41%	188.1	−11%	46.3	10%
Vinik [192]	Diab. Care	1993	442	20	272.3	−32%	NR	NA	NR	NA
Schaeffer [193]	Atheroscl	1996	305	13	177.2	−42%	206.5	−10%	34.8	10%
Avogaro [194]	Acta Diabetol	1999	217	20	317	−52%	NR	NA	NR	NA
Wiklund [195]	Amer. J. Med	1993	137	12	159.8	−44%	198.8	−14%	46.3	19%

Data taken from Abourbih S. et al. [90]

First author, journal, year of publication, subject number, and trial duration are noted, along with reference number in this chapter

NR not reported, *NA* not available

[a] Fenofibrate was added to background treatment with ezetimibe 10 mg/day

[b] Fenofibrate was added to background treatment with ezetimibe 10 mg/day + simvastatin 20 mg/day

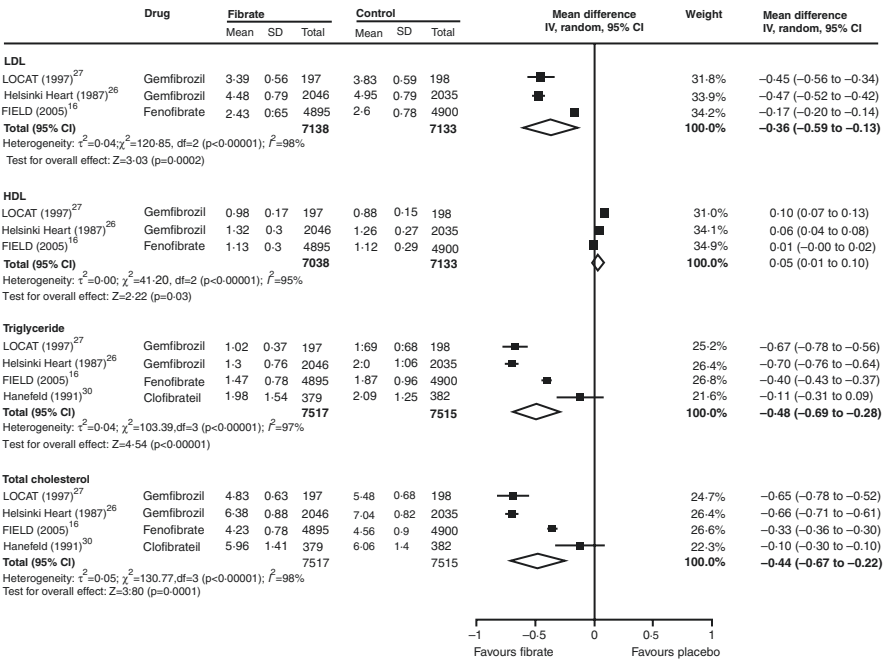

Fig. 24.2 Fractional change in major lipid parameters with gemfibrozil and fenofibrate treatment (and some data from one small trial of clofibrate), adapted from a meta-analysis of several large randomized controlled fibrate trials. (Reproduced with permission from Jun M. et al. [91] The reference numbers in the figure are from original publication)

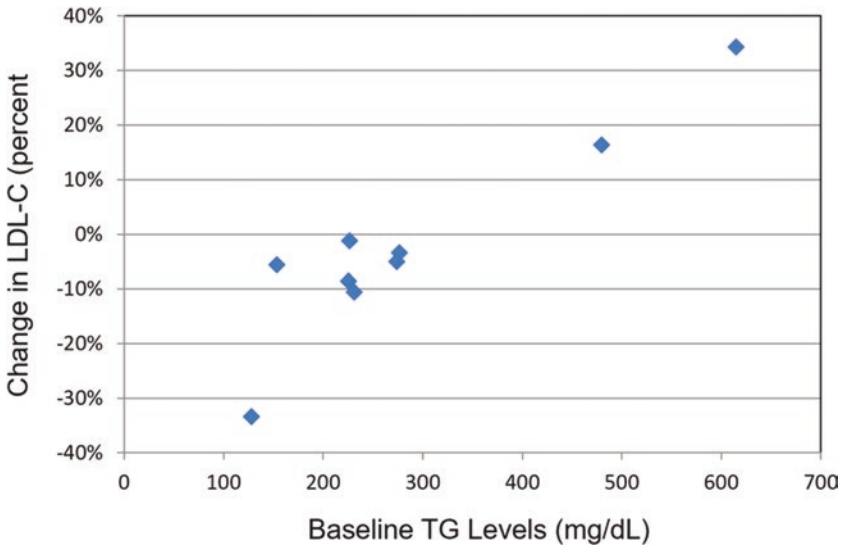

Fig. 24.3 Baseline TG levels (mg/dL) vs. the placebo-corrected change in LDL-C with fenofibrate (as a percent of the baseline LDL-C). (Data taken from Abourbih S. et al. [90] as shown in Table 24.1. There is one point per trial, or one point per trial arm, in the case of the two trials by Farnier M. et al. [182, 183])

Fibrate treatment nearly always raises HDL-C levels, likely in part by the mechanisms discussed in the prior paragraph and farther above [76, 100], For example, fenofibrate (160 mg/day) or simvastatin (40 mg/day) was given for 8 weeks to 52 patients with moderate to very high CHD risk, selected for HDL-C levels <40 mg/dL [94]. Fenofibrate had dramatic effects on TG and HDL-C, with a 43% decrease and 22% increase, respectively, and baseline HDL-C was a strong inverse predictor of the HDL-C increase ($R = -0.56$, $p = 0.003$). As with most HDL-raising agents, the degree of HDL-C increase is strongly related to the degree of increases in HDL particle size and apo A-I levels. Typically, both HDL size and apo A-I levels are inversely related to apo A-I fractional catabolic rate, which in turn is directly related to TG levels and insulin resistance, even in subjects without DM-2 [76]. Paradoxically, however, predictors of the degree of HDL-C increase and types of changes in HDL composition with fibrates appear to differ sharply from those of other HDL-raising agents. For example, despite the large increase in HDL-C with fenofibrate in the abovementioned trial [94], average HDL particle size did not increase. Further, levels of apo A-II increased, but A-I did not, and the concentration of HDL particles with apo A-I but lacking apo A-II (Lp A-I) also decreased. Both these changes appear to be specific to fibrates among HDL-C-raising agents, being absent with simvastatin in this same trial [94]. Many other studies have confirmed that fibrates may actually decrease average HDL size [97], while increasing apo A-II but not apo A-I levels (these again being seen only with fibrates among HDL-raising agents) [93, 94, 101–103]. To further emphasize the uniqueness of fibrate effects on HDL, niacin, although lowering plasma TG roughly as well as fenofibrate and raising HDL-C more effectively, has very different effects on HDL size and composition. Niacin treatment leads to larger HDL particles and a striking increase in levels of apo A-I and of Lp A-I particles (HDL with apo A-I but lacking apo A-II) [104, 105].

Finally, elevated levels of lipoprotein(a) [Lp(a)] are worth mentioning in the context of dyslipidemias in insulin resistance and DM-2. Although not an element of the atherogenic dyslipidemia, Lp(a) is strongly pro-atherogenic in DM-2 and it may be lowered by fibrate therapy. A meta-analysis of 16 trials comparing fibrates to statins head-to-head, including three trials in patients with DM-2, found that fibrates were consistently superior to statins in their effects on Lp(a) levels [106]. Although fibrates were found to lower Lp(a) only modestly, they contrast with statins, which usually raise Lp(a).

Long-Term Fibrate Effects on Lipids and Lipoproteins

In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, in patients with DM-2, fenofibrate increased HDL-C levels modestly for the first 2–3 years after treatment initiation, but they then returned to near baseline by the end of the study, at about 5 years [107]. This loss of lipid effect may have partially been due to the greater drop-in use of statins in the placebo arm of the trial, as

discussed below, but also might have been related to long-term physiological adaptation to fenofibrate, since statin effects on HDL-C levels are quite modest. The Action to Control Cardiovascular Risk in Diabetes (ACCORD)-Lipid trial, with constant statin therapy throughout, also tended to show a similar trend. Instead, however, the narrowing of the gap between treatment arms appeared to be more the result of progressive HDL-C increase and TG reduction in the placebo arm than loss of fenofibrate effect [108]. Of note, the 11% increase in HDL-C with gemfibrozil vs. placebo in the Helsinki Heart Study (HHS) was sustained across the several years of that trial [109]. Similarly, there was no apparent attenuation of the 30% TG lowering or the 6% HDL-C increases over 40 months of fenofibrate treatment in the DAIS trial [110].

Lipid and Lipid-Related Effects of Fibrates vs. Statins and in Combination with Them

The lipid effects of fibrates tend to be complementary to those of statins as noted above [94] and presented in two additional reviews [111, 112]. For example, fenofibrate 160 mg/day had dramatic effects on TG and HDL-C, with a 43% decrease and 22% increase, respectively, in contrast to modest to negligible changes with simvastatin 40 mg/day (−15% and +6%, respectively) [94]. Conversely, simvastatin significantly reduced LDL-C and total cholesterol levels (−28% and −19%, respectively), whereas fenofibrate did not significantly affect these parameters [94]. A large contrast was seen in lipid effects between fenofibrate and atorvastatin 40 in a small trial of 12 subjects with elevated TG and LDL-C, which actually reported a 15% increase in fasting apo B levels with fenofibrate vs. a 43% decrease with atorvastatin [113]. Since the vast majority of dyslipidemic patients are treated with statins when trying to reduce ASCVD risk, the additivity of fibrate effects on lipids to those of a statin is of great clinical importance. FIELD is a complicated case of fibrate-statin combination in that it tried to study fenofibrate in the absence of background statin therapy by excluding subjects on or likely to need statins in the near future [107]. Unfortunately, as should have been expected, substantial numbers of subjects did receive "drop-in" statin treatment, disproportionately more in the placebo arm. To attempt to clarify lipid effects of fenofibrate alone, Hiukka et al. examined lipid changes among FIELD subjects at the Helsinki site who were randomized to fenofibrate and whose results were not confounded by drop-in statin treatment during the 5 years of the study (mean age 62 ± 5.7 years and mean DM-2 duration 6 years) [93]. Lipid effects with fenofibrate (placebo corrected) were as expected for total cholesterol (−18.7%; $p < 0.001$) and TG (−25.8%; $p < 0.001$), while surprisingly LDL-C decreased substantially (−20.5%; $p < 0.001$, perhaps related to the low baseline TG of 171 mg/dL), while HDL-C was unchanged. The lack of HDL-C increase may relate in part to the relatively normal baseline HDL-C in both study arms (42.9 mg/dL, above the NCEP ATP-III cutoff for low HDL-C, <40 mg/dL)

[114] given that HDL-C increases with any agent are generally inversely related to baseline HDL-C. It must be remembered, however, that these two non-randomized subject groups are not necessarily comparable, and further, they are not likely representative of FIELD subjects in general. Thus, interpretation of lipid effects of fenofibrate alone in FIELD is unavoidably confounded. Finally, a key consideration regarding lipid changes with fibrate therapy is the frequent, paradoxical lack of connection between those effects and any ASCVD benefits, as noted in the second-to-last section of this chapter. Only one large long-term fibrate trial used statin background therapy in all patients, ACCORD-Lipid, which employed simvastatin per current guidelines, adjusted slightly during the trial as clinically needed [108]. In that setting, fenofibrate had rather modest placebo-corrected lipid effects: a TG decrease of 15.6% (although from a low baseline of only 164 mg/dL), an HDL-C increase of only 2.4%, and a slight LDL-C increase of 2.0% [108].

Fibrate Effects on Other Atherosclerosis-Related Mechanisms

A major anti-atherogenic function of HDL appears to be the promotion of cholesterol efflux from extrahepatic cells to HDL, which is mediated by its interaction with specific cell membrane transport proteins, primarily the ATP-binding cassette transporter (ABCA1) [115]. This cholesterol is then passed from HDL to the scavenger receptor B1 (SR-B1) in the liver, as a last step of reverse cholesterol transport [66]. Using plasma from patients treated with fenofibrate or simvastatin (as a source of HDL), cholesterol efflux from peripheral cells to lipoproteins was determined in macrophages for ABCA1-mediated efflux, and then from lipoproteins to hepatic cells for SR-B1-mediated flux. ABCA1-mediated cholesterol efflux to HDL was significantly increased using plasma from fenofibrate- but not simvastatin-treated patients [94]. Conversely, SR-B1-mediated cholesterol flux was significantly increased with plasma from simvastatin- but not fenofibrate-treated patients [94]. Thus, a combination fenofibrate and statin therapy may be better than either drug alone to enhance the full process of reverse cholesterol transport from the periphery to the liver. Two additional factors related to HDL concentration, composition, particle size distribution, and reverse cholesterol transport are CETP and lecithin cholesterol acyl transferase (LCAT). Fenofibrate and simvastatin were both reported in one study to significantly increase CETP by 17% and 9%, respectively [94]. In contrast, another study reported decreased CETP activity with fenofibrate, which was related both to increased LDL particle size and to decreased coronary intimal hyperplasia after angioplasty and stent placement [116]. An explanation for the contrast between the results of these studies is not readily available. Meanwhile, LCAT activity is also reported to trend slightly, but nonsignificantly, upward with both fenofibrate and simvastatin therapy, by 7% and 6%, respectively [94].

Increased inflammation is common in DM-2 and appears to contribute greatly to its excess ASCVD risk. Several lines of evidence suggest that the inverse relationship between HDL-C levels and atherosclerosis may be mediated in part by an anti-inflammatory effect of HDL particles [117]. Thus, the low HDL-C levels usually

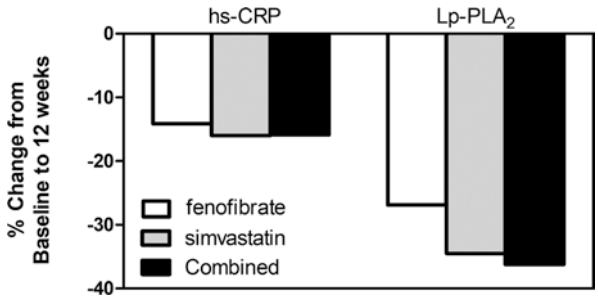

Fig. 24.4 Comparison of treatment with fenofibrate, simvastatin, both, or neither on the inflammatory biomarkers high-sensitivity C-reactive protein (hs-CRP) and lipoprotein phospholipase A_2 (Lp-PLA_2). Data are shown as percent change from baseline after 12 weeks of treatment. (Data taken from Muhlestein JB, et al. [112])

seen in DM-2 may be pro-inflammatory, while increased HDL-C with fibrate therapy may be anti-inflammatory. DM-2 is associated with increased levels of an inflammatory biomarker, C-reactive protein (CRP, measured by high-sensitivity assay or hsCRP), and an inflammatory factor, lipoprotein-associated phospholipase A_2 (Lp-PLA_2) [118, 119]. Importantly, fibrate therapy can reduce both hsCRP and Lp-PLA_2 [118, 120], as well as VCAM-1 and other inflammatory factors (see a review by Elkeles R [121]). These anti-inflammatory effects were further addressed by Muhlestein et al., in 300 patients with DM-2 and mixed dyslipidemia [112]. Treatment with fenofibrate, simvastatin, or combined therapy reduced hsCRP by 14.1% ($p = 0.17$), 16% ($p = 0.04$), or 15.9% ($p = 0.01$), respectively, and significantly decreased Lp-PLA_2 by 26.9%, 34.5%, and 36.2%, respectively (all $p < 0.001$), although there was no apparent additive effect with combination therapy (Fig. 24.4) [112]. Finally, another likely anti-atherosclerotic effect of fibrates in DM-2 is reduced insulin resistance, seen particularly in patients with HTG, low HDL-C, and other elements of the metabolic syndrome [122] (see also a review by Elkeles R [121]). This has led to suggestions that fibrates be tested for a possible ability to prevent new-onset DM-2 [121].

Fibrate Effects on Atherosclerosis

Three fibrate trials have studied fibrate effects on carotid intima-media thickness (CIMT) by carotid ultrasound as endpoint. In the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention (SENDCAP) trial, bezafibrate showed no effect on CIMT [123]. A similar lack of efficacy on carotid atherosclerosis was found with fenofibrate in the Helsinki cohort of the FIELD trial [124]. The third study, however, found that fenofibrate blocked the progression of CIMT seen in an untreated control group over a 24-month study period [125]. Interestingly, the two trials without evident CIMT benefit were exclusively in patients with DM-2, while the single study showing a beneficial effect excluded DM-2. In contrast to the

generally neutral findings with carotid atherosclerosis, particularly in patients with DM-2, fibrates consistently have been found to reduce atherosclerosis in the coronary tree. Trials assessing coronary atherosclerosis as minimum lumen diameter (MLD) by quantitative angiography reported that bezafibrate [126], gemfibrozil [127], and fenofibrate (in DAIS) [110] all improved this parameter and the benefit with fenofibrate was strongly related to increased LDL particle size [92]. Finally, it is important to note that fibrates are considered very effective in reducing both the dyslipidemia and atherosclerosis in patients with the rare type III dyslipidemia [28, 29].

The mechanisms by which fibrates might fail to reduce carotid atherosclerosis while reducing coronary atherosclerosis are unknown. Interestingly, however, these apparent regional differences in effects on atherosclerosis per se correspond with the differing regional effects on ASCVD events. That is, as discussed below, fibrates appear to reduce coronary heart disease events, but have little if any favorable effect on ischemic stroke. Further, the one relatively small fenofibrate trial showing reduced progression of carotid atherosclerosis (by CIMT) is also the only clinical fibrate trial to report reduction in clinical stroke events [125]. Unfortunately, however, this trial excluded patients with DM-2 [125], meaning that there appear to be no published data that fibrates reduce stroke in DM-2.

Fibrate Effects on ASCVD Event Risk in the General Patient

Two meta-analyses of fibrate trials across broad patient groups have shown evidence for reduced ASCVD events. Abourbih et al. [90] looked at 20 trials with 25,655 subjects, using bezafibrate ($n = 4984$), fenofibrate ($n = 12{,}398$), or gemfibrozil ($n = 8273$). Focusing on the five trials with myocardial infarction (MI) data, they found a significant 22% decrease in nonfatal MI (Fig. 24.5a) [90]. In troubling contrast, however, the six trials with mortality data showed a nonsignificant trend towards a 5% increase in all-cause mortality (Fig. 24.5b) [90]. A second meta-analysis focused on studies selected for presenting ASCVD event data in at least 100 patient-years' follow-up and included the ACCORD-Lipid study [108], absent from the Abourbih report. They included 18 trials with 45,058 subjects who had 2870 major ASCVD events and 3880 deaths [91]. Among the four major trials using currently available fibrates (one each with gemfibrozil—Veterans Affairs High Density Lipoprotein Intervention Trial or VA-HIT [128], and bezafibrate—lower extremity arterial disease event reduction or LEADER [129] and two using fenofibrate—FIELD [107] and ACCORD-Lipid [108]), there was a highly significant 12% decrease in ASCVD events ($p = 0.002$), after exclusion of the one small, older, and clinically irrelevant clofibrate trial (Fig. 24.6) [91]. A pooled analysis of all fibrate studies with available data for each endpoint showed a highly significant 19% decrease in nonfatal coronary events ($p < 0.0001$), but only nonsignificant trends towards reductions in sudden death and cardiovascular death (11% and 7% decreases, and $p = 0.2$ and 0.1, respectively). Further, in the Jun et al. meta-analysis, there was no evidence for any benefit on total stroke (RR 1.03) and, reminiscent of the Abourbih publication, there was a modest, borderline statistically significant trend towards a 10% increase in nonvascular death (RR 1.10, $p = 0.06$) [91].

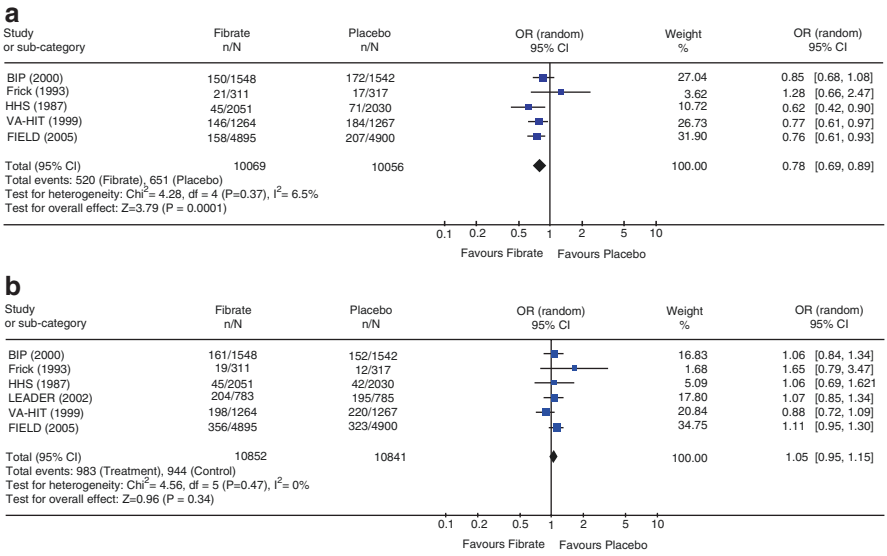

Fig. 24.5 (**a**, **b**) Forest plot of the effect of fibrate treatment on (**a**) nonfatal myocardial infarction and (**b**) all-cause mortality. BIP (2000) [184] and LEADER (2002) [129] were bezafibrate trials. Frick (1993) [185], HHS (1987) [141], and VA-HIT (1999) [128] were gemfibrozil trials. FIELD (2005) [107] was a fenofibrate trial. *BIP* Bezafibrate Infarction Prevention, *FIELD* Fenofibrate Intervention and Event Lowering in Diabetes, *HHS* Helsinki Heart Study, *LEADER* lower extremity arterial disease event reduction, *VA-HIT* Veterans Affairs HDL Intervention Trial. (Reproduced with permission from Abourbih et al. [90])

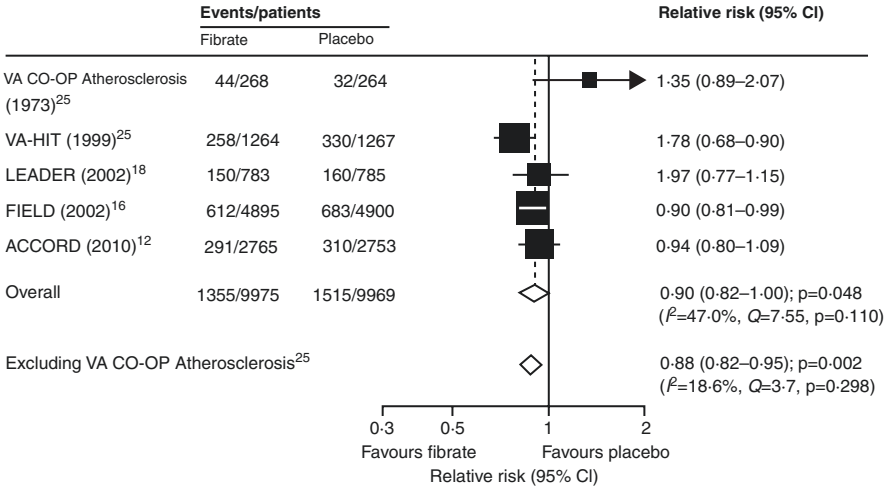

Fig. 24.6 Effects of fibrate treatment on major ASCVD events. The studies are noted by study name and year of publication. The VA CO-OP Atherosclerosis (1973) [186] used clofibrate, VA-HIT (1999) [128] used gemfibrozil, LEADER (2002) [129] used bezafibrate, and FIELD (2005) [107] and ACCORD (2010) [108] tested fenofibrate. (Reproduced with permission from Jun M. et al. [91]. The reference numbers in the figure are from original publication)

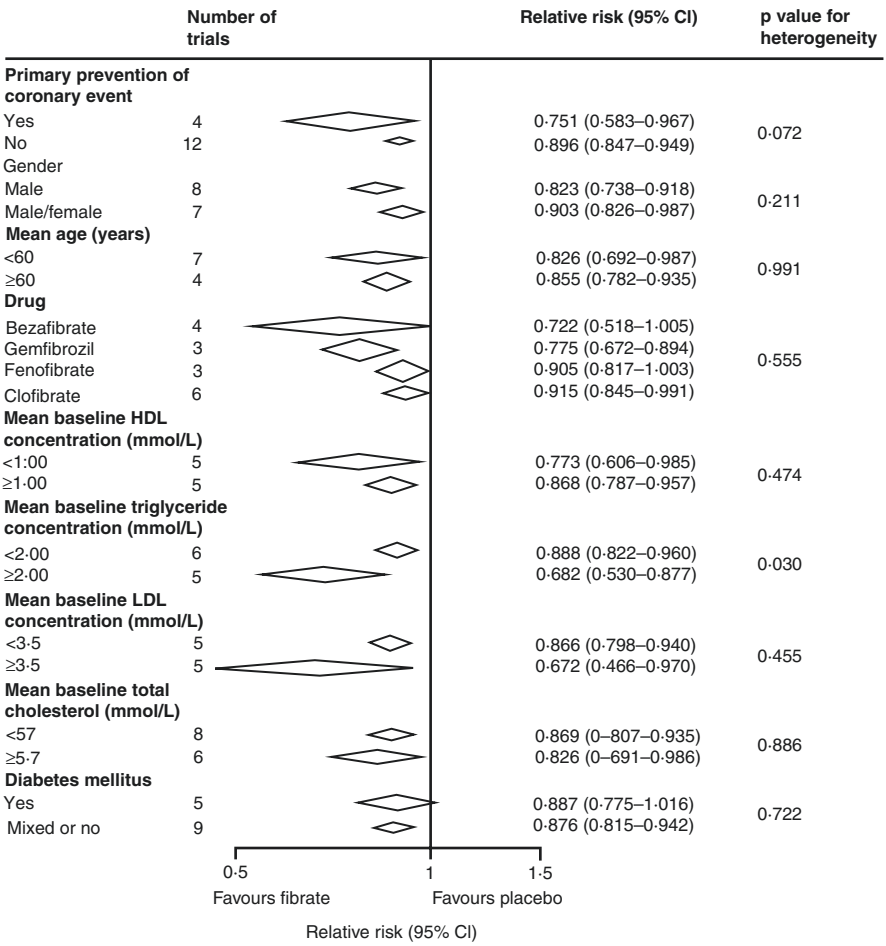

Fig. 24.7 Effects of fibrate treatment on coronary heart disease events alone, displayed as a forest plot of various subgroups of fibrate study subjects as noted on the figure. Data are taken from 18 trials in 45,058 participants, with 4552 coronary events. (Reproduced with permission from Jun M. et al. [91])

Curiously, with regard to stroke, one small, fairly short-term clinical trial reported a statistically significant reduction in stroke with fenofibrate therapy [125], although this trial does not appear in the Jun meta-analysis [91], despite appearing to have met the inclusion criteria.

In the Jun et al. meta-analysis, patient subgroup analyses were also performed using a composite of all coronary events, the broad endpoint most clearly reduced by fibrates. There was a suggestion of greater benefit in the treatment of patients without prior ASCVD vs. those with a prior history (primary prevention vs. secondary prevention, RR 0.75 vs. 0.90, respectively, p value for heterogeneity 0.07, Fig. 24.7) [91]. CHD benefits were greater (p value for heterogeneity = 0.03) in subjects with baseline TG levels ≥2.00 mmol/L (186 mg/dL—see Fig. 24.7), which

difference is discussed further below. Intercomparison among studies by the specific fibrate used suggested greater benefit with gemfibrozil (RR 0.78, 95% CI 0.67–0.89) than with fenofibrate (RR 0.91, 95% CI 0.82–1.00), although the overall heterogeneity among trials of the four fibrates had a *p* value of only 0.6 (Fig. 24.7) [91]. The potentially greater benefit with gemfibrozil is of very limited clinical utility in the statin era, and only one of the fenofibrate trials used standard-of-care background statin treatment. Despite general compatibility of fenofibrate in combination with statins, it should be noted that the U.S. Food and Drug Administration (FDA) withdrew its previously approved indication for combined use of fenofibric acid (the active form of fenofibrate and of niacin with a statin for TG <500 mg/dL) (nearly all use for ASCVD prevention) [130]. This withdrawal was based on the additive rates of myopathy of fenofibrate with statins, plus the lack of solid evidence for additive ASCVD reduction with this combination [130]. Finally, although three meta-analyses have shown ASCVD risk reduction with fibrates in patients with low HDL-C alone [131, 132], with HTG alone [132] or in patients with both [132, 133], these data are positive only when including non-fenofibrate results, and further, being subgroup analyses, they are only hypothesis generating and not directly clinically applicable.

Fibrate Effects on ASCVD in Patients with Insulin Resistance or Prediabetes

The VA-HIT study recruited subjects mainly on the basis of a low HDL-C level and was not primarily designed to test gemfibrozil effects in the insulin-resistant state. Nevertheless, due to the strong relationship between low HDL-C and disorders of glucose and insulin metabolism, 43% of VA-HIT patients had a clear manifestation of insulin resistance: either impaired fasting glucose (13%) or DM-2, whether newly diagnosed at the time of study entry (6%) or previously diagnosed (25%) [134]. A key subgroup analysis was performed among all subjects without DM-2, with or without impaired fasting glucose, to exclude the use of diabetes medications which would likely alter fasting plasma insulin levels. Among these patients, the quartile of fasting insulin level (a good surrogate for degree of insulin resistance) was a strong positive predictor of ASCVD risk ($p = 0.02$) [134]. Importantly, ASCVD event reduction with gemfibrozil increased progressively across quartiles of baseline fasting insulin levels, from a 15% increase in the lowest quartile to reductions of 20%, 22%, and 35% in the second through fourth quartiles, and this benefit remained after adjustment for other risk factors [134]. Although limited to gemfibrozil, this finding suggests the possibility of a similar benefit with fenofibrate. The latter, if true, would be of considerable clinical importance due to the high and rising prevalence of insulin resistance throughout the world, and due to its strength as an ASCVD risk factor. Paradoxically, however, both the decrease in TG and the increase in HDL-C were blunted in the presence of increasing insulin resistance [135].

Fibrate Effects on ASCVD in Patients with Diabetes Mellitus-2

With regard to the ability of fibrates to reduce ASCVD in patients with DM-2, 3 of 17 studies analyzed by Jun et al. did not report subjects' diabetes status [91]. In 9 of the remaining studies, between 0 and 66% of subjects had diabetes, while in the remaining 5, all had DM-2. Importantly, ASCVD event reduction in studies exclusively in patients with DM-2 was very similar to that in studies with mixed or non-DM-2 patient populations (RR 0.89 vs. 0.88, p value for heterogeneity 0.7) [91]. In light of the uncertainties of cross-study comparisons, it is instructive to note within-study results from the VA-HIT, which directly compared results in its relatively large DM-2 subgroup (769 or 31% of total subjects). As expected, those with established or newly diagnosed DM-2 had 87% and 72% more total ASCVD events, respectively, than those with normal fasting glucose [134], and importantly, the percent reduction of the primary combined ASCVD endpoint with gemfibrozil appeared to be much greater in those with DM-2 as in those without it (32% vs. 18%), although the suggested difference was not statistically significant. Further, the absolute risk reduction in patients with DM-2 was very high at 10%, suggesting that only 10 patients with DM-2 would need to be treated with gemfibrozil for 5 years to prevent one event [134]. Of interest and possible clinical significance, two individual components of the composite ASCVD endpoint which had not been found to be reduced by fibrates in the large meta-analyses, CHD death and stroke, were both shown to be reduced by gemfibrozil in subjects with DM-2 in VA-HIT (HR 0.59, $p = 0.02$, and HR 0.60, $p = 0.046$) [134]. Again, however, gemfibrozil data are of very limited clinical relevance in the statin era (due to adverse interactions), and the potential for reduction of stroke risk with fenofibrate treatment, whether in patients with or without DM-2, or whether population specific, remains unclear. As an interesting contrast to the robust ASCVD benefit seen in patients with gemfibrozil in DM-2 patients in VA-HIT, the nearly 10,000 FIELD subjects, all with DM-2, had only a nonsignificant trend towards reduction of the primary study endpoint of pooled major cardiovascular events [107]. There was a statistically significant reduction in the rate of nonfatal MI and coronary revascularizations, but these were secondary endpoints [107]. This apparent blunting of benefit with fenofibrate may have been due to greater off-study statin drop-in therapy in the placebo arm [107] and lack of focus on patients with HTG and low HDL-C [136] (despite earlier data from HHS [137] that such patients had far greater ASCVD reduction with fibrate than other patients) [138]. ACCORD-Lipid was conducted entirely in patients with DM-2 and with appropriate statin therapy as a uniform background, and thus constituted the best opportunity of any completed trial to show fibrate-mediated ASCVD reduction in the current statin era [108]. Unfortunately, neither the primary composite endpoint (major fatal or nonfatal cardiovascular events) nor any secondary endpoint came close to showing statistically significant benefit with fenofibrate. There was a statistically significant, albeit potentially troubling, difference in the primary composite result between men and women in that men had a statistically significant

16% decrease while women had a nonsignificant 38% increase, the sex difference itself being statistically significant ($p < 0.01$ for interaction) [108]. Fortunately, such a difference has not been seen in other fibrate trials, including a lack of between-sex difference among ACCORD-Lipid subjects with low HDL-C and HTG at baseline. Also, there is no known biological mechanism for this sex difference or for harm from fibrates in women. By extension, however, this unusual ACCORD-Lipid finding cannot be taken as an endorsement for fenofibrate use in men.

Prediction of Fibrate Effects on ASCVD by Baseline Lipids and On-Treatment Lipid Effects

Subgroup analyses within several individual fibrate trials have shown baseline TG and HDL-C levels to predict ASCVD benefit. For example, post hoc analysis of the HHS showed considerable ASCVD benefit of gemfibrozil in patients with HTG levels and low HDL-C at baseline [137]. A similar analysis of FIELD reported that study subjects with either TG > 200 mg/dL, HDL-C < 40 mg/dL, or both achieved statistically and clinically significant reduction in the primary ASCVD endpoint (HR 0.77, 0.86, and 0.73, $p = 0.01$, 0.03, and 0.005, respectively) [138]. ACCORD-Lipid showed a borderline significant trend to ASCVD benefit among subjects with the highest tertile of baseline fasting TG and lowest tertile baseline HDL-C ($p = 0.057$ for interaction) [108]. Combining data from several fibrate trials, the same pattern is clearly seen. In the large fibrate trial meta-analysis by Jun et al. [91], lower (vs. higher) baseline HDL-C and higher (vs. lower) baseline LDL-C appeared to predict greater ASCVD benefit with fibrate treatment (RR 0.77 vs. 0.87 and 0.67 vs. 0.87, respectively), but the p value for heterogeneity was far from significant (0.5 for both). In contrast, baseline HTG significantly predicted greater ASCVD reduction (RR 0.89 vs. 0.68, p value for heterogeneity 0.03) [91]. Three other meta-analyses of fibrate effects on ASCVD events focused primarily on the prediction of ASCVD benefit in five fibrate trials which reported baseline TG and HDL-C levels. One brief report showed a striking pooled ASCVD benefit in subjects with both HTG and low HDL-C (OR 0.65, 95% CI 0.54–0.78) but no benefit in those lacking both (OR 0.94, 95% CI 0.84–1.05) [133]. A second meta-analysis showed a similar effect in subjects with HTG [131]. A third, more detailed meta-analysis confirmed this finding, using a slightly different statistical method, with either baseline HTG (RR 0.75, 95% CI 0.65–0.86) or low HDL-C (RR 0.84, 95% CI 0.77–0.91) alone, or both (RR 0.71, 95% CI 0.62–0.82) predicting reduced ASCVD, while having neither predicted a lack of benefit (RR 0.94, 95% CI 0.82–1.08, Fig. 24.8) [132]. In additional analyses by the same authors, ASCVD benefit tended to be present across HTG and/or low HDL-C in the presence or absence of other conditions, such as primary vs. secondary prevention and background statin use or not (Table 24.2) [132]. In three other settings, however, reduced ASCVD benefit was suggested: (1) the presence of DM-2 (RR 0.74–0.87 with DM-2 vs. RR 0.61–0.80 without), a

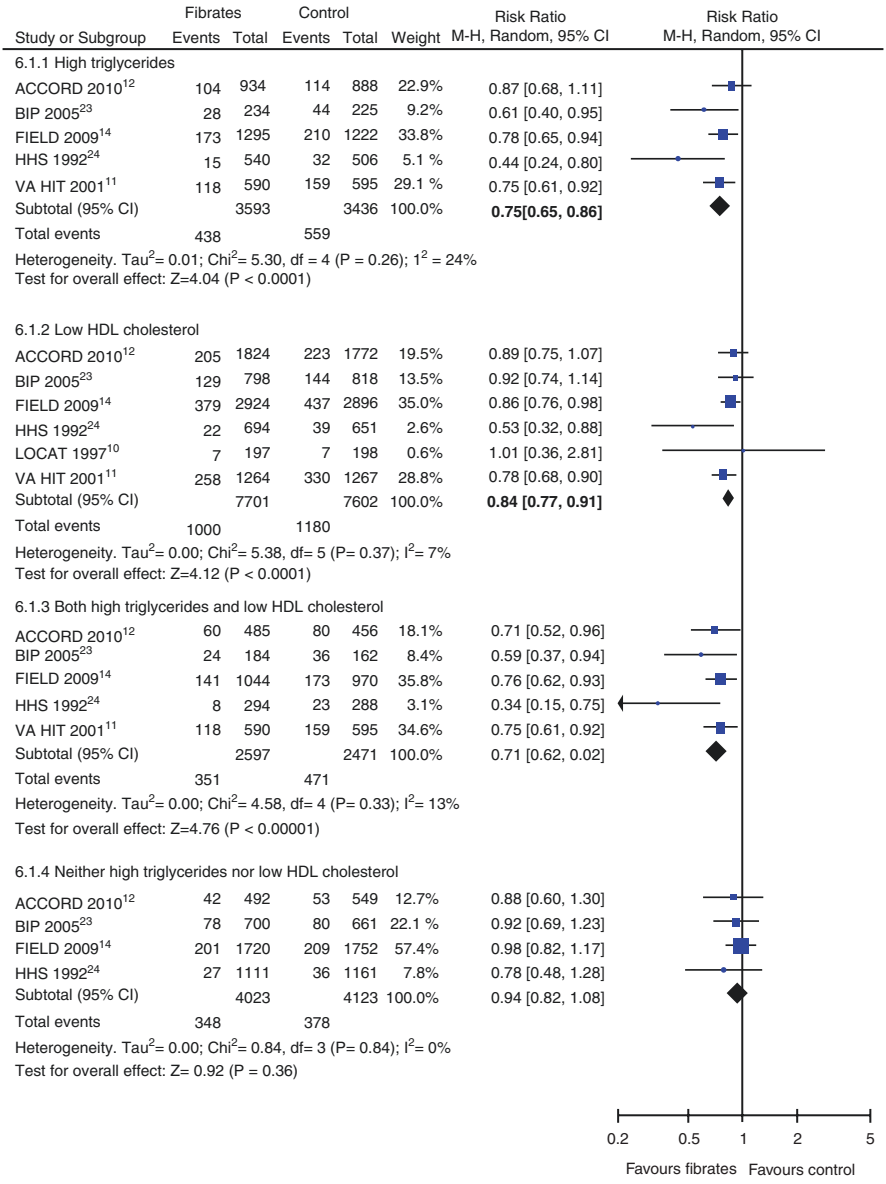

Fig. 24.8 Effects of fibrate treatment on cardiovascular events, by baseline TG and HDL-C levels, showing each study separately and also the pooled, weighted results of all 6 trials together. The risk ratio was calculated using the Mantel-Haenszel random-effects model (M-H, random). *CI* confidence interval, *df* degrees of freedom. (The studies are noted by study name and year of publication, and the reference numbers are reproduced with permission from Lee et al. [132], from which this figure is taken)

Table 24.2 The effects of fibrates on relative risk of ASCVD events in patients separated according to high TG (>200 mg/dL) and/or low HDL-C levels (<40 mg/dL)

Effect of fibrate on vascular risk in persons with atherogenic dyslipidemia in subgroup analyses			
	Triglyceride > 200 mg/dL or nearest equivalent, RR (95% CI)	HDL cholesterol < 40 mg/dl. or nearest equivalent, RR (95% CI)	Triglyceride > 200 mg/dl. and HDL cholesterol < 40 mg/dl. or nearest equivalent, RR (95% CI)
Population			
Diabetes mellitus as an entry criteria	0.81 (0.70–0.94)	0.87 (0.78–0.97)	0.74 (0.62–0.88)
Diabetes mellitus not as an entry criteria	0.65 (0.50–0.85)	0.80 (0.68–0.95)	0.61 (0.42–0.88)
Prevention			
Primary (< 50% people with CVD at entry)	0.75 (0.59–0.96)	0.84 (0.71–0.98)	0.68 (0.52–0.89)
Secondary	0.72 (0.60–0.87)	0.82 (0.73–0.93)	0.72 (0.59–0.87)
Treatment regimen			
Gemfibrozile	0.62 (0.37–1.02)	0.74 (0.59–0.93)	0.55 (0.26–1.18)
Bezafibrate	0.61 (0.40–0.95)	0.92 (0.74–1.14)	0.59 (0.37–0.94)
Fenofibrate	0.81 (0.70–0.94)	0.87 (0.78–0.97)	0.74 (0.62–0.88)
Mono-therapy vs. combination therapy			
Fibrate alone	0.72 (0.61–0.84)	0.83 (0.75–0.92)	0.70 (0.57–0.84)
Fibrate + statin	0.87 (0.68–1.11)	0.89 (0.75–1.07)	0.71 (0.52–0.96)
End point used for analysis			
CVD	0.79 (0.70–0.89)	0.84 (0.77–0.91)	0.74 (0.65–0.85)
CHD	0.55 (0.38–0.78)	0.78 (0.52–1.16)	0.49 (0.30–0.81)

CVD cardiovascular diseases, *CHD* coronary heart diseases, *RR* relative risk, *CI* confidence interval.

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Data are shown by the presence or absence of diabetes; primary or secondary prevention; treatment with gemfibrozil, bezafibrate, or fenofibrate; presence or absence of background statin therapy; and endpoint of CVD, which includes both coronary heart disease (CHD) and cerebrovascular events, or CHD alone

paradoxical finding in that DM-2 associates with both HTG and low HDL-C; (2) use of fenofibrate (RR 0.74–0.87 for fenofibrate vs. RR 0.55–0.74 for gemfibrozil), a concerning finding in light of the general lack of usefulness in the statin era; and (3) the general ASCVD endpoint, including stroke (RR 0.74–0.84 for CVD vs. RR 0.49–0.78 for CHD, see Table 24.2) [132]. In light of the first two of the abovementioned three points, it is difficult to recommend fenofibrate for ASCVD prevention in patients with DM-2. More importantly, despite remarkable consistency of possible ASCVD benefit from many analyses of patients with HTG and low HDL-C (summarized above and in Table 24.2 and Fig. 24.8), these are all subgroup analyses of generally neutral trials. Thus, the strong suggestion that patients with these two elements of the atherosclerotic dyslipidemia (and of the metabolic syndrome) may have ASCVD risk reduction with fibrate treatment is only hypothesis generating and cannot be considered of sufficient strength for clinical practice recommendations.

ASCVD outcome data from statin trials consistently have shown that LDL-C changes strongly predict the degree of ASCVD benefit both in general subjects [139] and in those with DM-2 [3]. In partial contrast to these results, however, lipid changes from certain fibrate trials were poor predictors of their ASCVD benefits. In the VA-HIT study, patients taking gemfibrozil had a 22% reduction in major cardiovascular events ($p = 0.006$) and a 24% decrease in nonfatal MI and stroke, and death from coronary disease ($p < 0.001$) compared to patients receiving placebo. The patients receiving gemfibrozil had a modest 6% increase in HDL-C levels compared to those receiving placebo ($p < 0.001$) but a 31% decrease in TG levels ($p < 0.001$) [135]. Although the HDL-C increase was much smaller than the TG decrease, the former predicted ASCVD risk reduction while the latter did not [135]. Related to this finding, fasting insulin levels (a surrogate for insulin resistance), which strongly

predicted ASCVD benefit, were paradoxically a strong inverse predictor of both TG and HDL-C change. That is, VA-HIT patients with higher baseline insulin had less lipid change but greater ASCVD reduction on gemfibrozil [135]. Meanwhile, more robust data from a meta-analysis of multiple trials by Jun et al. suggested that lipid changes with fibrates do indeed predict changes in ASCVD risk. On-treatment TG levels in ten trials significantly predicted ASCVD benefit ($p = 0.026$) with a 5% reduction per 88 mg/dL lower TG levels [91]. There was also a suggestion in data from seven trials of a 2% ASCVD reduction per 3.9 mg/dL lower on-treatment LDL-C, and of a 3% ASCVD reduction per 0.8 mg/dL higher HDL-C, although neither of these reached statistical significance ($p = 0.09$ and 0.13, respectively) [91]. Finally, analysis of lipoprotein particle concentrations by NMR in plasma samples from a nested case-control subgroup of the VA-HIT trial has suggested that both baseline and on-treatment levels of LDL-P and HDL-P predicted ASCVD events better than on-treatment apo B or HDL-C [102]. As pointed out, however, in an accompanying editorial [140], these observations are at best hypothesis generating, as they raised more questions than they answered, and their interpretation is uncertain in light of relatively neutral findings in larger datasets using more conventional lipoprotein measurements, reviewed above.

Fibrate Effects on ASCVD in Combination with Statins

Given the fact that statins are by far the best proven among all classes of dyslipidemia medications for reducing ASCVD event rates, and indeed are widely recommended and used in patients with DM-2 [114], it is critical to ask whether fibrates can further reduce ASCVD risk when added to statin therapy. This question was best addressed in the ACCORD-Lipid trial, in which all subjects received statin and half each were randomized to receive either fenofibrate or matching placebo [108]. Interestingly, as discussed above, in both ACCORD-Lipid [108] and FIELD [107], there was only a modest nonsignificant ASCVD reduction in the overall population, while in both studies that benefit appeared to be much greater (and statistically significant or near significant) in those with HTG and low HDL-C at baseline [132]. Unfortunately, however, intercomparison among studies according to the specific fibrate used has suggested greater benefit with gemfibrozil (RR 0.78, 95% CI 0.67–0.89) than with fenofibrate (RR 0.91, 95% CI 0.82–1.00), even though the overall heterogeneity among the four fibrates compared had a p value of 0.6 (Fig. 24.7). Further, this suggested difference may simply be an artifact of differential statin use. In the two largest gemfibrozil trials (HHS [141] and VA-HIT [128]), statins were scarcely or not at all yet available and so were not used by any subjects. In contrast, in the two largest fenofibrate trials (FIELD and ACCORD-Lipid), statins were used often in the placebo group (FIELD) [107] or were used in all subjects (ACCORD-Lipid) [108], thus likely making it harder to see incremental fibrate benefits. Does this mean that fibrates might not add to the ASCVD reduction obtained by statin monotherapy? Post hoc subgroup analysis by baseline lipid levels (see above) suggests that fibrates may be beneficial when added to statin treatment in

patients with baseline HTG and low HDL-C. These analyses, although remarkably consistent between these two large trials (and consistent with other fibrate trial data in the absence of statin use, see [132]), are only hypothesis generating. A valid test of this hypothesis is sorely needed and is now ongoing as a large international randomized double-blind trial with a novel agent, pemafibrate (not yet available outside Japan) vs. placebo in statin-treated subjects with DM-2 and low HDL-C plus HTG. This potentially exciting trial is nearly completed, as detailed below.

A key consideration in use of fibrates in combination with statins is the risk of myopathy, which is present with either agent alone and which tends to be further elevated with the use of their combination [142]. Although FDA-approved labeling includes a similar precaution for statin use with both gemfibrozil and fenofibrate, the effect on statin levels, and thus on the potential for increased myopathy risk, appears to be only about one-fifteenth as great with fenofibrate as with gemfibrozil [143]. Among the seven currently available statins, only fluvastatin (one of the least used statins) lacks this adverse interaction with gemfibrozil and so it is not encouraged for use as a statin adjunct. Largely for this reason in the current statin era, there is very little gemfibrozil use at present (except in monotherapy when a statin is not indicated or tolerated). Further, no large clinical trials using gemfibrozil in combination with statins have been, or are likely to be, conducted. In contrast, both FIELD [107] and ACCORD-Lipid [108] showed essentially no adverse safety signal for increased myopathy among thousands of patients taking fenofibrate with a statin. Even prior to the publication of the more robust of these two studies (ACCORD-Lipid [108]), fenofibrate was widely considered as safe in combination use with statins [142], although the FDA has subsequently withdrawn its indication for combined use of fenofibrate with statins [130].

Fibrate Effects on Diabetic Microvascular Disease

Although microvascular disease is not an element of ASCVD per se, it is included here because microvascular complications of diabetes are common and devastating and appear to be reduced by fenofibrate. Arguably, the most devastating of these is diabetic retinopathy, the most common cause of blindness in patients with DM-2. The effects of fenofibrate on diabetic retinopathy have been well addressed by a recent meta-analysis [144]. The outcome was laser treatment, with data from the large, long-term FIELD [145] and ACCORD-Lipid trials [146] as well as from a small third trial (the data from which were very limited by its premature termination due to the withdrawal of one of its study drugs, cerivastatin, from the market) [144]. There was a robust overall 23% decrease in this endpoint (OR 0.77, 95% CI 0.67–0.88, $p < 0.0001$) despite a lack of benefit during the first year [144].

Another diabetes complication related to microvascular disease is impaired renal function, evaluated both as reduced glomerular filtration (estimated glomerular filtration rate, eGFR, calculated from serum creatinine and demographic factors) and as increased albuminuria (expressed as the ratio of albumin to creatinine content, generally using a spot urine sample). Increased urinary albumin excretion generally

precedes decreased eGFR in the natural progression of diabetic nephropathy. Fenofibrate effects on albuminuria in three clinical trials [107, 108, 147] were evaluated in a meta-analysis [91], which reported a 14% decrease in the progression of albuminuria with this agent. Fenofibrate effects on eGFR are more complicated and unfortunately appear not to have been subjected to a published meta-analysis. DAIS found a 16% rise in serum creatinine, with a corresponding drop in eGFR with the initiation of fenofibrate treatment [147]. In FIELD, the largest of the three trials with such data, there were 14% fewer fenofibrate-treated subjects who had progression and 18% more with regression of albuminuria vs. those on placebo ($p < 0.001$) [148]. Although plasma creatinine remained higher on fenofibrate than on placebo throughout the study, the chronic rate of rise was significantly slower (1.62 vs. 1.89 μmol/L annually, $p = 0.01$), with far less estimated age-related GFR loss (1.19 vs. 2.03 mL/min per 1.73 m^2 annually, $p < 0.001$) [148]. Further, after an 8-week washout of fenofibrate treatment at the end of the trial, eGFR had fallen 72% less from baseline on fenofibrate (1.9 mL/min per 1.73 m^2, $p = 0.065$) than on placebo (6.9 mL/min per 1.73 m^2, $p < 0.001$), sparing 5.0 mL/min per 1.73 m^2 (95% CI 2.3–7.7, $p < 0.001$ for the difference) [148]. Of particular interest, this greater preservation of estimated GFR with fenofibrate was seen primarily in subjects with either (1) baseline HTG levels alone, (2) baseline HTG and low HDL-C together, or (3) TG reductions of ≥43 mg/dL on study drug. Curiously, however, progression to end-stage renal disease was not significantly reduced, occurring in 21 vs. 26 subjects with fenofibrate vs. placebo, respectively ($p = 0.48$) [148]. Finally, creatinine levels and eGFR were analyzed in the ACCORD-Lipid trial, separating all subjects according to their initial response to fenofibrate treatment during the 6-week run-in for all subjects. Nearly half (47%) had a ≥20% increase in serum creatinine, while 25% of subjects had less than a 2% [149]. After a 51-day washout of study drug at the end of the trial, subjects with a large initial rise in serum creatinine and drop in eGFR who were treated with fenofibrate had results similar to those on placebo, while those fenofibrate-treated subjects lacking an initial creatinine rise had levels 9% lower ($p = 0.00002$) and eGFR 7% higher ($p < 0.0001$) than comparable placebo controls [149]. Thus, fenofibrate acutely causes modest apparent renal glomerular impairment, but in long-term use provides improvement, at least in many subjects. This is analogous to the bidirectional effects of angiotensin-blocking antihypertensive medications. Thus, the overall net effect of fenofibrate on renal function in DM-2 appears to be at least modestly favorable. Of likely clinical importance and possible mechanistic meaning, these benefits are predicted by the same baseline lipid levels and on-treatment lipid changes as are the ASCVD effects [148, 149] (see above).

Finally, lower extremity amputation is a devastating complication of diabetes which appears related to both microvascular and macrovascular disease. In FIELD, lower extremity amputations occurred less often with fenofibrate than with placebo (45 vs. 70 events; hazard ratio HR 0·64, 95% CI 0.44–0.94; $p = 0.02$) [150]. This finding was driven entirely by fewer "minor" (below the ankle) amputations (18 vs. 34 events; 0.53, 0.30–0.94; $p = 0{\cdot}027$) with no difference between groups in "major" (ankle or above) amputations (24 vs. 26 events; 0.93, 0.53–1.62; $p = 0{\cdot}79$) [150]. Interestingly, these effects of fenofibrate were seen primarily among patients without known large-vessel lower extremity atherosclerotic disease, and the benefits were unrelated to on-study lipid levels [150].

Guideline Recommendations for Fibrate Use

Fibrates have excellent overall safety as monotherapy, there being no increase in serious drug-related adverse events compared to placebo (RR 1.21, $p > 0.2$), in a meta-analysis of 17,413 participants in multiple trials [91]. There is also reasonable evidence that fibrates may reduce ASCVD events, although, on average, the effects appear to be relatively modest and may be limited to patients not taking statins, as discussed above. The combination of a fibrate with a statin has been associated with increased risk of myopathy, especially with higher doses of statin in patients with renal insufficiency and when using gemfibrozil [143]. However, despite FDA withdrawal of the indication for combined fenofibrate plus statin use for TG < 500 mg/dL [130], published interventional and observational data with fenofibrate generally show minimal myopathy risk with statins, perhaps in part due to independent pharmacokinetic pathways of these agents [142]. Importantly, myopathy risk with a concurrent statin is far lower with fenofibrate than with gemfibrozil [143]. Meanwhile, there are few concerns about the use of fenofibrate with statins in the setting of severely elevated TG levels, for which fenofibrate is generally first-line due to its excellent TG-lowering efficacy (often 50% or greater) and the presumption that it reduces the risk of pancreatitis [108].

Guidance for use of fenofibrate for lowering moderately elevated TG (the usual cutoff in the USA being a fasting level <500 mg/dL) is far more variable because the clinical focus is solely ASCVD risk reduction. Here, statins are always first-line, and the lack of definitive data for ASCVD reduction with the addition of fenofibrate weighs heavily. Specific to the focus of this chapter, the lack of convincing evidence from FIELD [107], and especially ACCORD-Lipid [108], that fenofibrate may enhance the ASCVD benefits from statin monotherapy in patients with DM-2 has resulted in a lack of uniform recommendations for its use for moderate TG elevations. For example, the 2021 American Diabetes Association guidelines recommend fenofibrate just for reducing pancreatitis risk, in patients with diabetes and TG >500 mg/dL (especially if >1000 mg/dL) but not for ASCVD reduction with TG <500 mg/dL [151]. Similarly, the 2018 American Heart Association/American College of Cardiology/Multisociety Cholesterol Guideline recommended fibrate therapy only if TG levels are persistently ≥500 mg/dL (for pancreatitis), but not otherwise [152]. In 2021, the American College of Cardiology published a consensus, endorsed by National Lipid Association, recommending fibrates to prevent acute pancreatitis in patients with TG >500 mg/dL, but again not for ASCVD risk reduction in patients with moderate HTG [153]. In contrast, some current guidance does suggest consideration of fenofibrate as a statin adjunct for potential ASCVD reduction when TG remains in the moderate range (elevated but <500 mg/dL) on statin monotherapy. For example, the 2020 AACE lipid algorithm [154] and the 2021 Canadian Cardiovascular Society Guidelines [155] recommend this use of fenofibrate (especially in the presence of low HDL-C levels). The European Society of Cardiology guidelines on ASCVD prevention published in 2021 also suggest consideration for use of fenofibrate or bezafibrate to reduce ASCVD in patients taking statins who are at LDL-C goal but with TG >2.3 mmol/L (>200 mg/dL) [156]. Meanwhile, in a consensus statement in the same year from a related but separate

group, the European Atherosclerosis Society does not suggest the use of fenofibrate for ASCVD prevention, instead pointing forward to "further insights … awaited from the PROMINENT trial" [14], as discussed below. Given the current lack of certainty for ASCVD benefit with fibrates when added to statins, the lack of consensus in guidance for fenofibrate use for TG <500 mg/dL is understandable.

Dosage of Fenofibrate, Differing by Formulation

Fenofibrate is provided in two sets of doses: the full dose, for use in patients with normal renal function (eGFR >60 mL/min per 1.73 m^2), and due to the predominant metabolism of fenofibrate by the kidney, and a one-third dose for patients with eGFR 30–60 mL/min per 1.73 m^2 (also suggested for use in elderly patients). Due to its renal clearance, fenofibrate is contraindicated for patients with eGFR <30 mL/min per 1.73 m^2. Unfortunately, fenofibrate is provided at many differing degrees of micronization, which result in many corresponding degrees of oral bioavailability, which determine many corresponding sizes of daily dose. Thus, the two sets of fenofibrate of dose, regular and "renal," actually each consist of a broad range of doses, determined inversely by the degree of micronization and bioavailability of the drug (Table 24.3) [157, 158]. Although these various doses appear to deliver approximately the same amount of fenofibrate to the body, the existence of so many different doses can make the prescribing of fenofibrate a rather cumbersome process.

Table 24.3 The available doses (tablet or capsule size in mg, all doses being one tablet or capsule daily) of fenofibrate by generic and brand name. Both the standard dose and the reduced dose (used primarily for renal insufficiency, eGFR 30–60 mL/min per 1.73 m^2) are shown as also the generic and brand name(s)

Dose (mg/day)		Product Name
Regular	Renal	Generic (Brand)
200	67	Fenofibrate (Lofibra®)
160	54/50	Fenofibrate (Lofibra®/Triglide®)
150	50	Fenofibrate (Lipofen®)
145	48	Fenofibrate (Tricor®)
135	45	Fenofibric Acid (Trilipix®)
130	43	Fenofibrate (Antara®)
120	40	Fenofibrate (Fenoglide®)
90	30	Fenofibrate (Antara®)

Data taken from Brinton EA. et al. (2015) [157] and Brinton EA. et al. (2016) [158]
The doses vary inversely with the bioavailability of the particular formulation such that the amount of fenofibrate absorbed is approximately the same for each formulation

Pemafibrate, Present and Future Perspective

Pemafibrate is a novel fibrate in a new subcategory of PPAR-alpha agonism, called "selective PPAR-alpha modulator" or SPPARM. Like the currently available PPAR-alpha agonists, pemafibrate has substantial TG-lowering efficacy, but in contrast, it also has higher selectivity, potency, and fewer adverse effects compared to fenofibrate [159–161]. Compared to placebo, pemafibrate 0.2 mg twice daily (0.4 mg/day) significantly decreases non-HDL-C, VLDL-C, RLPC (variable method of measurement), apo B48, and apo C-III and increases HDL-C, apo A-I, and apo A-II levels [159, 161, 162]. Importantly, pemafibrate 0.2 mg twice daily (0.4 mg/day) decreases VLDL-C and increases apo A-II levels significantly more than does fenofibrate [159–161]. Further, pemafibrate has been shown to dramatically reduce apo C-III levels by 35–38% in two related trials compared to placebo [162], substantially more than the 5% ± 1% (range 1–21%) apo C-III decrease shown in a meta-analysis of ten trials of fenofibrate [43].

As with fenofibrate, there can be a slight increase in LDL-C with pemafibrate compared to placebo, the magnitude of change of LDL-C from baseline being positively correlated with baseline TG and negatively correlated with baseline LDL-C [159]. Pemafibrate significantly increases fibroblast growth factor 21 levels compared to both placebo and fenofibrate [159, 160], and this may have anti-atherosclerotic effects independent of changes in lipids or other ASCVD risk factors. Compared to placebo, pemafibrate with or without statin lowered TG and improved atherogenic dyslipidemia without a significant increase in adverse events, even among patients on statin with renal dysfunction [163]. A phase 3 study evaluated the efficacy and safety of pemafibrate vs. placebo in 166 patients with DM-2 and HTG (TG ≥150 mg/dL and ≤1000 mg/dL) [164, 165]. Pemafibrate 0.2 mg twice daily significantly reduced TG by 35% (placebo corrected, $p < 0.001$) [164]. With regard to other lipid-related parameters, compared to placebo, pemafibrate showed significant reduction in RLPC (measured by "metaboLead," one of many competing methods for RLPC levels [Kyowa Medex Co., Ltd., Tokyo, Japan]), apo B48, and apo C-III levels and a significant increase in HDL-C and apo A-I levels [164] (the latter being uncommon with fenofibrate). Pemafibrate showed no significant change in apo B100 compared to placebo; however, high-performance liquid chromatography analyses showed decreased sdLDL and increased large LDL [164]. In an observational study, pemafibrate significantly reduced TG, VLDL, and sdLDL, but increased LDL-C in DM-2 patients with higher baseline TG and lower baseline LDL-C, thus showing improvement in LDL composition despite the fact that baseline LDL-C was in the normal range [166]. Pemafibrate increased cholesterol content in medium, small, and very small HDL and decreased cholesterol content in large HDL particles, probably evincing enhanced reverse cholesterol transport [164, 167]. The impact of pemafibrate on glycemic parameters in patients with DM-2 is neutral at worst, and in some ways is favorable. Pemafibrate 0.2 mg twice daily showed no significant difference in fasting glucose, fasting insulin, insulin resistance, and hemoglobin A1C compared to placebo [164]; however, post hoc repeated-measures

ANCOVA at weeks 4–24 showed significant reduction in fasting glucose, fasting insulin, and insulin resistance with pemafibrate compared to placebo [164]. In that same trial, the incidence of adverse events and adverse drug reactions was similar across pemafibrate and placebo groups in patients with DM-2 [164]. In a meta-analysis involving 1623 patients, insulin resistance was significantly lower with pemafibrate than placebo ($p < 0.001$), although that benefit did not reach statistical significance compared to fenofibrate [168]. In a small study, involving Japanese patients with DM-2, the statin-pemafibrate combination improved lipid metabolism without increasing the risk of hepatic dysfunction and muscle side effects [169]. A pooled analysis of 1253 subjects in six clinical trials reported that pemafibrate, at low to standard doses, improved glucose metabolism and reduced circulating levels of hepatic transaminases [170]. Among patients with DM-2, 33–44% of participants being on statin therapy, the incidence of adverse events was similar with or without statin therapy [162]. Although it does not reduce hepatic steatosis, pemafibrate appears to reduce hepatic steatohepatitis, or fat-related hepatic inflammation [171], and thus might be useful in treating the growing epidemic of nonalcoholic fatty liver disease (NAFLD) [172]. In patients with chronic kidney disease, pemafibrate showed good safety profile and lipid-lowering efficacy [173]. Finally, a recent review of the activity of PPAR-alpha receptors in the pathophysiology of diabetic cardiomyopathy suggests that pemafibrate may be safe and effective in treating this disorder, even as have the sodium-glucose co-transporter 2 inhibitors (SGLT2i) [174]. In light of these and other favorable safety and intermediate endpoint data [175], pemafibrate has been endorsed as having considerable potential for ASCVD prevention in a consensus statement from the International Atherosclerosis Society [175].

Finally, and most importantly, there is an ongoing ASCVD outcome trial, the pemafibrate to reduce cardiovascular outcomes by reducing TG in patients with diabetes (PROMINENT) study [NCT03071692] with 10,000 high-risk patients on statins with HTG (>200 and ≤500 mg/dL), HDL-C ≤40 mg/dL, and DM-2, with or without established ASCVD. Subjects are randomized to pemafibrate 0.2 mg twice daily or placebo, and median follow-up is to be about 4 years [176]. The primary endpoint is first occurrence of nonfatal MI, nonfatal ischemic stroke, hospitalization for unstable angina requiring unplanned coronary revascularization, or cardiovascular death. The last study visits are speculated to be completed in the very near future, as early as late 2022/early 2023, with results available in the months following. Pemafibrate should continue to show considerable safety and might prove to have substantial ASCVD efficacy in this trial, hopefully sufficient to earn FDA approval for ASCVD prevention. If so, despite its likely being an expensive branded product, it would be preferred over currently available generic fibrates (unproven for ASCVD). In PROMINENT, pemafibrate might potentially rise to match the current remarkable position of icosapent ethyl, with its robust data for ASCVD event reduction in patients at high risk for ASCVD with HTG persistent despite good LDL-C control with statin monotherapy [177, 178]. Pemafibrate might thus possibly achieve widespread endorsement in guideline recommendations as has icosapent ethyl [151, 154, 179–181], by helping fill what is otherwise a large unmet need for patients with the atherogenic dyslipidemia and DM-2.

Summary and Conclusions

Fibrates have been studied widely in clinical trials and used extensively in clinical practice for more than five decades. They are the most effective medication class for reducing elevated TG levels and are primarily used for TG lowering in severe HTG. They also raise low HDL-C levels and decrease in sdLDL particles. Since HTG, low HDL-C, and sdLDL (the atherogenic dyslipidemia) are common in patients with insulin resistance and DM-2, most current clinical use of fibrates is to treat these factors, with secondary consideration to reduce inflammatory markers and apo C-III levels, as well as to increase LPL activity and reverse cholesterol transport. In addition, fibrates lack the adverse glycemic effects seen with niacin and even with statins, and thus remain attractive in patients with metabolic syndrome and DM-2. Since statins are first-line treatment for ASCVD prevention, fenofibrate is far more useful than gemfibrozil (due to its greater safety in combination with statins), although it is no longer FDA approved in this setting. Despite these many actual and potential advantages, however, cardiovascular outcome trials with fenofibrate have failed to prove ASCVD benefit, and its clinical utility is sharply limited in the statin era.

Meanwhile, the novel, selective PPAR-alpha modulator (SPPARM) pemafibrate has greater potency and selectivity for PPAR-alpha agonism than fenofibrate or gemfibrozil, and its favorable results for intermediary markers and factors of atherogenesis suggest that pemafibrate might be safer and more effective for long-term prevention of ASCVD. If the ongoing PROMINENT trial manages to show that pemafibrate effectively and safely reduces ASCVD events, then it would become the fibrate of choice in most clinical settings. Pemafibrate would then warrant consensus approval as a valuable statin adjunct for ASCVD prevention in patients with DM-2 who have moderate HTG and low HDL-C despite statin monotherapy.

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Chapter 25
EPA and Mixed Omega-3 Fatty Acids: Impact on Dyslipidemia and Cardiovascular Events in Patients with Diabetes

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Introduction

The growing diabetes epidemic poses a substantial threat to public health worldwide, particularly with respect to the increased risk for atherosclerotic cardiovascular disease (ASCVD), which is two- to threefold higher in patients with diabetes compared with those without diabetes [1–3]. In the United States in 2016, 13% of all adults had type 2 diabetes mellitus (T2DM). Furthermore, according to the International Diabetes Federation, there were 537 million people globally with diabetes mellitus (DM) in 2021, and this number is projected to exceed 783 million by 2045 [4].

Dyslipidemia is a common feature of T2DM and is one of the key risk factors for major ASCVD events [5, 6]. In patients with T2DM and poorly controlled type 1 DM (T1DM), the combination of elevated triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels and diminished high-density lipoprotein

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cholesterol (HDL-C) levels is often apparent and significantly increases ASCVD risk [6]. Use of statins for dyslipidemia remains the cornerstone of therapy for lowering LDL-C levels and CV risk in patients with DM; however, even with well-controlled LDL-C levels, residual CV risk persists [7–9].

Hypertriglyceridemia contributes to this residual risk [10–12]. The 2007–2014 National Health and Nutrition Examination Survey of 1448 statin-treated and non-statin-treated patients with T2DM found that among statin users with LDL-C levels <70 mg/dL, prevalence of hypertriglyceridemia was 16.7%; among these patients, 40% had an estimated 10-year risk of ASCVD of at least 20% [11]. Further real-world evidence of residual risk attributed to elevated TG levels in the T2DM patient population was demonstrated in an observational longitudinal cohort study using electronic health records. Overall, data from 27,953 patients with T2DM showed that the incidence rates of nonfatal myocardial infarction (MI), nonfatal stroke, and coronary revascularization were 30% ($P = 0.006$), 23% ($P = 0.037$), and 21% ($P = 0.027$) higher, respectively, in patients with high TG levels (200–499 mg/dL) versus normal TG levels (<150 mg/dL), despite statin-controlled LDL-C levels [10]. In addition, results from the landmark meta-analyses from the Cholesterol Treatment Trialists' (CTT) Collaboration, which included 14 statin trials of 18,686 participants with DM, demonstrated a 21% reduction in major vascular events; nevertheless, while LDL-C levels were controlled, residual CV risk persisted, and elevated TG levels (baseline mean TG levels: 177.15 mg/dL) may have contributed to this residual CV risk [12].

Similarly, glucose-lowering therapies do not eliminate the risk of CV events in patients with DM [13]. Findings from the Intensified Multifactorial Intervention in Patients With Type 2 Diabetes and Microalbuminuria (STENO2) trial, in which 160 patients were randomized to conventional treatment or intensified treatment, showed that after 21 years of follow-up, patients randomized to intensive multifactorial therapy had a 62% reduction in death from CV causes, besides glucose control alone [14].

In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, which included over 10,000 patients with T2DM and ASCVD or CV risk factors, intensive glucose-lowering therapy (i.e., targeting a glycated hemoglobin level [HbA1c] below 6.0%) did not significantly reduce the composite endpoint of nonfatal MI, nonfatal stroke, or death from CV causes compared with standard therapy (i.e., targeting an HbA1c level of 7–7.9%), and in fact was associated with more deaths from any cause, primarily CV death [13]. The Veterans Affairs Diabetes Trial (VADT) and Action in Diabetes and Vascular Disease—Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial similarly showed that intensive glucose control did not meet CV endpoints [15, 16].

Treatment with omega-3 fatty acids (OM3FAs) has been shown to significantly reduce TG levels [17], and, until recently, prescription OM3FAs, including mixed formulations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as the highly purified EPA-only agent, icosapent ethyl (IPE), were approved to reduce TG levels [18–21].

However, in 2019, IPE became the only OM3FA approved as an adjunct to maximally tolerated statin therapy to reduce the risk of MI, stroke, coronary revascularization, and unstable angina requiring hospitalization in adult patients with elevated TG levels (≥150 mg/dL) and established CVD, or in patients with DM and two or

more additional risk factors for CVD [19]. This approval was based on findings from the pivotal Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT), a phase 3b, randomized, double-blind, placebo-controlled study in which 8179 patients were randomized to receive IPE 4 g daily or placebo [22]. This approval marked an important milestone for OM3FAs, especially in patients with T2DM, as IPE became the first OM3FA approved to reduce CV events not only in those with established ASCVD, but also in patients at high risk for CV events, such as patients with DM and additional risk factors [19].

This chapter reviews the effect of mixed OM3FAs and EPA alone on lipid and lipoprotein levels; the mechanism of action of OM3FAs with respect to lipid-lowering effects and reductions in CV risk; the effect of OM3FA on gut microbiota, albuminuria, and plaque regression and stabilization; the results of key clinical trials; and current guidelines for use of OM3FAs in patients with DM.

History of OM3FAs and Glucose Homeostasis

Glucose homeostasis and insulin resistance are central players in the pathophysiology of T2DM [23], and numerous studies have explored the effect of OM3FAs on glucose homeostasis and insulin resistance [24–31]. Early clinical studies often reported impairment in glucose homeostasis and rise in blood glucose levels with the use of OM3FAs [28–30]. Findings suggesting that OM3FAs compromise glucose homeostasis may be attributed, in part, to high doses of OM3FAs—in some of these earlier studies, doses of OM3FAs exceeded 7.5 g/day [29, 32, 33].

More recent clinical trials of OM3FAs have generally reported neutral or favorable effects on glucose homeostasis [34–38]. A 2019 double-blind placebo-controlled study of 200 patients with impaired glucose regulation reported significant improvement in glucose metabolism, including insulin resistance, with mixed OM3FAs [37].

Results with EPA-only formulations have demonstrated neutral effects on glucose metabolism. A sub-analysis of the Japan EPA Lipid Intervention Study (JELIS; a randomized, open-label trial in which 18,645 hypercholesterolemic patients received statin and 1.8 g EPA or statin alone) [39] included patients with impaired or normal glucose metabolism and reported that EPA did not significantly affect fasting plasma glucose or HbA1c levels [35]. Similar results were observed in two phase 3 double-blind studies, including the Multicenter, Placebo-controlled, Randomized, Double-blind, 12-week Study with an Open-label Extension (MARINE; $N = 229$) and Evaluation of the Effect of Two Doses of AMR101 (Ethyl Icosapentate) on Fasting Serum Triglyceride Levels in Patients With Persistent High Triglyceride Levels ($\geq$200 mg/dL and <500 mg/dL) Despite Statin Therapy (ANCHOR; $N = 702$), in which patients received IPE 4 g/day, 2 g/day, or placebo [34, 38]. Furthermore, no significant changes in glycemic control were noted in the sub-analysis of ANCHOR in patients with DM [40]. Most recently, no significant changes in HbA1c were observed with IPE treatment in patients with or without DM in the REDUCE-IT trial [36].

Findings from meta-analyses and systematic reviews investigating the effect of OM3FA on glucose metabolism have yielded mixed results. For example, one meta-analysis of 20 randomized clinical trials of OM3FAs (ranging from 0.52 to 3.89 g of EPA and 0 to 3.69 g of DHA) in patients with T2DM found no significant changes in HbA1c, fasting plasma glucose, postprandial plasma glucose, body mass index (BMI), or body weight overall; however, fasting plasma glucose levels in Asian patients with T2DM were significantly increased by 0.42 mmol/L ($P = 0.023$) [41]. Meanwhile, the same study also found that a high ratio of EPA:DHA was associated with greater reductions in plasma insulin, HbA1c, total cholesterol, TGs, and BMI [41].

In a systematic review and meta-analysis of 17 studies involving 672 participants (including healthy participants, patients with T2DM, and others with at least one component of metabolic disorders), OM3FA (ranging from 1 to 4 g/day) had no effects on insulin sensitivity compared with placebo [42]. Findings from a subgroup analysis showed that OM3FA was associated with a 47% reduced risk of insulin resistance in patients with metabolic disorders ($P < 0.001$); however, there were no effects on insulin sensitivity in healthy individuals or patients with T2DM [42]. Similarly, a meta-analysis of 11 randomized controlled studies showed that OM3FAs did not affect insulin sensitivity in patients with T2DM [43].

More encouraging results were observed in a recent pooled analysis of 20 prospective cohort studies including 65,147 patients without DM, which showed that OM3FAs were associated with a reduced risk for T2DM [44].

Heterogeneity in results from human studies may be attributed in part to variations in OM3FA dosage, composition, and formulation; population ethnicity; and study design [45, 46]. Findings from in vitro and animal studies, however, have generally generated positive results and suggest potential mechanisms for these favorable effects. An in vitro study by Kato et al. involving C57BL/6 islet cells treated with palmitate showed that EPA restored beta-cell function via inhibition of the nutritionally regulated lipid transcription factor SREBP-1c, which has been shown to impair insulin secretion and glucose tolerance [27].

Rodent studies showed that replacement of a high-fat diet with OM3FAs protected against the development of dyslipidemia, impaired glucose homeostasis, and insulin resistance [24–26, 31]. A study by Storlien and colleagues in which rats were fed OM3FAs demonstrated prevention of insulin resistance, predominantly in the liver and skeletal muscle [24]. Similarly, a study using rats fed a high-saturated-fat diet showed that 24-h replacement of 7% of dietary fats with OM3FAs reversed insulin hypersecretion [31]. Consistent with these results, a recent study explored the effect of IPE on glucose homeostasis in mice and similarly reported that IPE resulted in reduced insulin resistance, reduced fasting insulin and glucose, and improved glucose intolerance and beta-cell function [25]. The mechanisms responsible for these favorable effects of OM3FAs on insulin sensitivity may be attributed in part to reduced hepatic diacylglycerol accumulation, reduced triacylglycerol deposition in insulin-responsive tissues, and reduced low-grade inflammation of abdominal white adipose tissue [47].

OM3FAs and Effects on Lipid and Lipoprotein Levels

The effects of OM3FAs on lipid and lipoprotein levels depend largely on whether a mixed formulation of EPA and DHA is being used or the individual components [48]. Mixed OM3FAs have been associated with reduced TG levels, but also increased LDL-C levels. Multiple preclinical and clinical studies suggest that DHA may facilitate increased LDL-C levels via several pathways, including downregulation of the LDL receptor or increased conversion of very-low-density lipoprotein (VLDL) to LDL and increased LDL particle size [48]. Use of OM3FAs that contain DHA may necessitate additional monitoring in patients who require LDL-C control [49]. Conversely, treatment with EPA alone results in a minimal reduction or neutral effect on LDL-C levels [49, 50].

In patients with T2DM, OM3FAs reduce both TG levels and VLDL levels [51], and reductions in non-HDL-C, total cholesterol, and VLDL levels have been reported across all prescription OM3FAs [50]. DHA may also modestly increase HDL-C and lower TG levels to a greater degree than EPA [48].

Significant reductions in apolipoprotein (Apo B) and high-sensitivity C-reactive protein (hsCRP) have been reported with EPA compared with placebo [22, 34, 39], and the effects of OM3FAs containing DHA on hsCRP levels are inconsistent. In clinical studies of patients with very high TG levels (ranging from 500 to 2000 mg/dL, depending on the type of prescription OM3FA) who were given DHA-containing OM3FA prescription products, Apo B levels increased [52].

Mechanism of Lipid-Lowering Effects

Elevated plasma TG levels are attributed to TG-rich lipoproteins, including VLDLs, intermediate-density lipoproteins (or VLDL remnants), chylomicrons, or chylomicron remnants [53]. Several mechanisms have been proposed to explain the TG-lowering effects of OM3FAs. One proposed mechanism of action suggests that inhibition of acyl coA1, 2 diacylglycerol acyltransferase (DGAT) by OM3FAs reduces the hepatic synthesis of TGs. EPA and DHA are poor substrates for enzymes involved in TG synthesis, thereby preventing the esterification and release of other fatty acids [54, 55].

The second proposed mechanism of action suggests that OM3FAs have high affinity for peroxisome proliferator-activated receptor (PPAR) subtypes, resulting in increased hepatic peroxisomal β-oxidation and upregulation of fatty acid catabolism in the liver and, ultimately, reduction of the quantity of free fatty acids available for TG synthesis, reduced TG levels, and inhibited secretion of TG-rich VLDL [56]. In addition, OM3FAs may increase removal of TGs from circulating VLDL and chylomicron particles through increased hydrolysis by lipoprotein lipase [51, 56].

Effect of OM3FAs on CV Events

Mixed OM3FAs

Clinical trials investigating the effect of mixed OM3FAs on the risk of CV events have yielded inconsistent results. In the multicenter, open-label study by the Italian Group for the Study of the Survival of Myocardial Infarction (GISSI), GISSI-Prevenzione, 11,324 patients (15% of whom had DM) were randomly assigned to mixed OM3FA supplements (1 g/day), vitamin E, OM3FAs plus vitamin E, or no treatment. Treatment with OM3FAs, but not vitamin E, was associated with a relative decrease in risk for the primary combined efficacy endpoint (i.e., death, nonfatal MI, and stroke) of 15% ($P = 0.023$) and for CV death, nonfatal MI, and nonfatal stroke of 20% ($P = 0.008$). However, most patients were not on contemporary medical therapy, including statins [57].

Conversely, in A Study of Cardiovascular Events in Diabetes (ASCEND), which included 15,480 patients with DM, there was no difference in the occurrence of vascular events between patients receiving daily mixed OM3FAs (1 g capsules) and those receiving placebo over >7 years of follow-up [58]. Similarly, the Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction (OMEGA), Study of Omega-3 Fatty Acids and Coronary Mortality (Alpha Omega), Outcome Reduction with an Initial Glargine Intervention (ORIGIN), Risk and Prevention Study, Omega-3 Fatty Acids in Elderly with Myocardial Infarction (OMEMI), and Vitamin D and Omega-3 Trial (VITAL) trials, all of which included a proportion of patients with DM, did not meet their primary CV endpoints [59–64]. Furthermore, the Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova on High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH) trial, in which 70% of patients had DM, was stopped early because it failed to demonstrate the benefit of mixed OM3FAs (4 g/day) in reducing CV events [65]. Lack of positive results in those trials may be attributed, in part, to the low dose of mixed OM3FAs [65].

Purified EPA

JELIS

Studies using purified EPA have generated more encouraging data. In the open-label JELIS study of 18,645 Japanese patients receiving EPA 1.8 g/day plus statin or statin alone, EPA plus statin was associated with a 19% reduction in CV events versus statin alone [39]. A sub-analysis of JELIS that included patients with DM or impaired glucose metabolism ($N = 4565$) demonstrated a 22% decrease in the incidence of coronary artery disease (CAD) in patients with DM [35]. Significant benefit of EPA on CV events was especially evident in patients with high baseline TG

levels (≥150 mg/dL) and low HDL-C levels (<40 mg/dL), in whom EPA reduced CV events by 53% (95% confidence interval [CI] 0.23–0.98; $P = 0.043$) versus statin alone [66]. These findings helped lay the groundwork for the design of the REDUCE-IT clinical trial, which included statin-treated patients with TG levels 135–499 mg/dL [22].

REDUCE-IT

Similarly, the pivotal, phase 3b REDUCE-IT trial was the first double-blind, placebo-controlled trial of IPE to show significant reduction in CV outcomes and formed the basis of the United States Food and Drug Administration (FDA) approval of IPE for lowering the risk of CV events [19, 22]. A total of 8179 patients aged ≥45 years with established CVD or aged ≥50 years with DM and ≥1 additional CV risk factor were randomized to receive 4 g IPE (2 g twice daily with food) or mineral oil placebo. Patients had fasting TG levels of 135–499 mg/dL and LDL-C levels of 41–100 mg/dL and were receiving a stable dose of statin for at least 4 weeks [22]. The primary outcome was a composite endpoint of CV death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina [22]. The key secondary endpoint included the composite of CV death, nonfatal MI, or nonfatal stroke [22]. Other secondary endpoints included a composite of CV death or nonfatal MI; fatal or nonfatal MI; emergency or urgent revascularization; CV death; hospitalization for unstable angina; fatal or nonfatal stroke; a composite of death from any cause, nonfatal MI, or nonfatal stroke; and death from any cause [22].

A reduction of 18.3% from baseline TG levels was observed in IPE-treated patients at 1 year versus an increase of 2.2% in the placebo group; LDL-C levels increased 3.1% in the IPE group versus 10.2% in the placebo group [22]. IPE was associated with a 25% reduction in the primary outcome (95% CI 0.68–0.83; $P < 0.001$). The key secondary composite endpoint occurred in 11.2% of patients in the IPE group versus 14.8% of patients in the placebo group (95% CI 0.65–0.83; $P < 0.001$), translating to a 26% reduction with IPE versus placebo [22].

Except for deaths from any cause, patients in the IPE group had significantly lower relative risks of individual CV endpoints compared with placebo, including a 20% reduction in death due to CV causes (95% CI 0.66–0.98; $P = 0.03$), 31% reduction in MI (95% CI 0.58–0.81; $P < 0.001$), and 28% reduction in stroke (95% CI 0.55–0.93; $P = 0.01$) [22].

Overall, rates of adverse events (AEs) were similar in the two treatment groups, including incidence of serious AEs (SAEs) leading to treatment discontinuation [22]. Rates of bleeding-related SAEs, atrial fibrillation, and peripheral edema were higher in the IPE group than in the placebo group; none of the bleeding events were fatal [22].

Hospitalization for atrial fibrillation or flutter was significantly more frequent in the IPE group than in the placebo group (3.1% vs. 2.1%; $P = 0.004$) [22]; however, it should be noted that the incidence of atrial fibrillation was greater among patients with a history of atrial fibrillation or atrial flutter [67].

Prespecified analyses of REDUCE-IT further highlighted CV benefits of IPE, especially in patients with T2DM. The REDUCE-IT Diabetes analysis included approximately 4780 patients with T2DM, 91% of whom were on ≥1 T2DM medication, and 49.5% of whom were on ≥2 T2DM medications. IPE reduced the first and total primary composite endpoint of CV death, MI, stroke, coronary revascularization, and unstable angina by 23% each (P = 0.00005 and P = 0.0003, respectively) (Fig. 25.1) [36]. In addition, the first and total key secondary composite endpoints, respectively, of CV death, MI, and stroke were reduced by 29% (P = 0.00005) and 30% (P = 0.000003) [36]. In another sub-analysis investigating the impact of BMI on CV risk reduction in patients with or without DM, similar risk reductions were observed on the primary and secondary endpoints with IPE 4 g/day, regardless of BMI category or DM status (Fig. 25.2) [68].

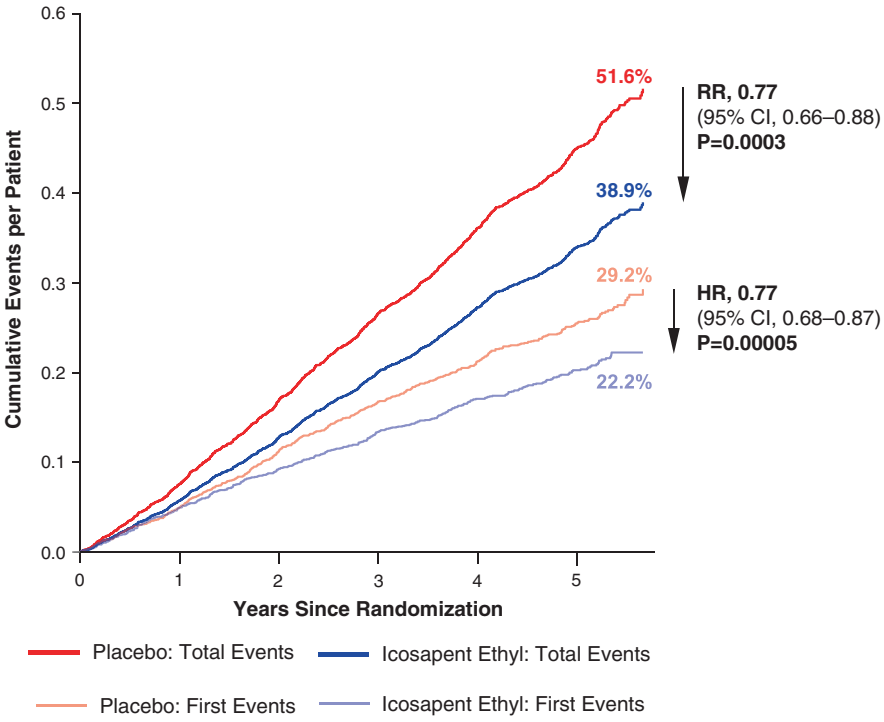

Fig. 25.1 REDUCE-IT diabetes: reduction in primary composite endpoint with IPE vs. placebo [36]. American Diabetes Association. Bhatt DL, Brinton EA, Miller M, Steg G, Jacobson TA, Ketchum SB, et al., editors. Icosapent ethyl provides consistent cardiovascular benefit in patients with diabetes in REDUCE-IT [presentation]. Annual Scientific Sessions of the American Diabetes Association; 2020 June 12–16, 2020. Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association. *CI* confidence interval; *HR* hazard ratio; *IPE* icosapent ethyl; *RR* relative risk reduction

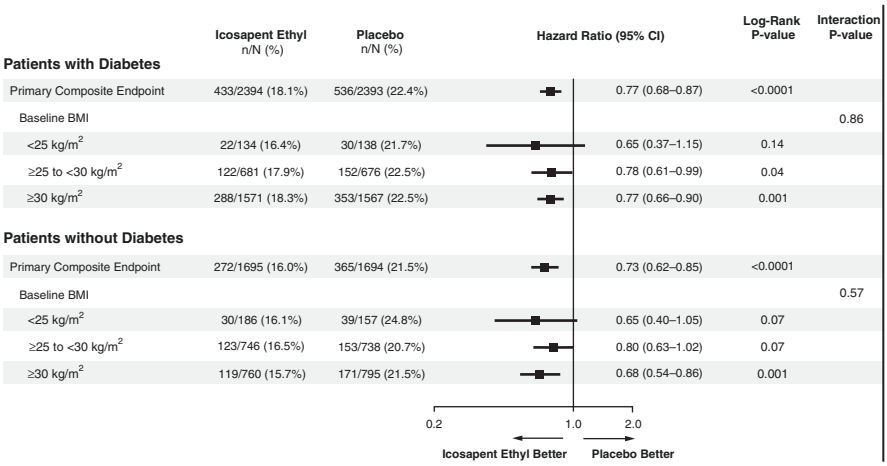

Fig. 25.2 REDUCE-IT body mass index (BMI) primary composite endpoint by baseline and BMI category [68]. American Diabetes Association. Bhatt DL, Brinton EA, Steg PG, Ketchum SB, Juliano RA, Jiao L, et al. Substantial cardiovascular risk reduction with icosapent ethyl regardless of diabetes status or BMI: REDUCE-IT BMI [abstract 256-OR]. Diabetes. 2021;70(Supplement 1):256-OR. Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association. *CI* confidence interval

RESPECT-EPA

Following the positive results of purified EPA treatment in REDUCE-IT, the Randomized Trial for Evaluating the Secondary Prevention Efficacy of Combination Therapy – Statin and EPA (RESPECT-EPA) investigated the effect of adding EPA to a statin in patients with established CVD [69]. Patients were randomized in a 1:1 ratio to purified EPA 1.8 g/day plus statin therapy or statin monotherapy. In total, 2460 Japanese patients treated with statins aged 20 to 79 years with chronic coronary artery disease and a low EPA-to-arachidonic acid ratio (<0.4) comprised the full analysis population. The primary endpoint was a composite of CV death, nonfatal MI, nonfatal cerebral infarction, unstable angina pectoris requiring emergency hospitalization and coronary revascularization procedure, and revascularization procedure. The secondary endpoint was a composite comprised of sudden cardiac death, MI, unstable angina, and coronary revascularization. Treatment with EPA added to a statin was associated with a borderline statistically significant reduction of 21.5% in CV risk in the primary endpoint ($P = 0.054$) and a significant reduction of 26.6% in the secondary composite endpoint ($P = 0.03$) versus statin monotherapy [69]. Consistent with findings from JELIS [70] and REDUCE-IT [71], benefit was more pronounced in study patients in RESPECT-EPA who achieved higher blood EPA levels from baseline.

Dietary Supplements

It should be noted that many patients use nonprescription formulations of DHA + EPA [72]. Important differences exist between prescription OM3FA and OM3FA dietary supplements: supplements are not subject to the same rigorous oversight review as prescription medications and do not have sufficient scientific evidence to support CV benefit [72, 73]. Furthermore, supplements are categorized as "foods" by the FDA and typically have lower amounts of OM3FAs than specified on the label, while exceeding international recommendations for oxidation markers. In addition, OM3FA dietary supplements may contain additional fats and oils that may increase CV risk [72, 74].

Pleiotropic Mechanisms of Action in Reducing the Risk of CV Events

In addition to TG-lowering properties, OM3FAs have been shown to exert other nonlipid effects that may confer cardioprotective benefits, including anti-inflammatory activity, antithrombotic effects, and improvements in vascular and coronary health (Fig. 25.3). Specifically, EPA has been shown to impact multiple atherosclerotic processes, including endothelial function, oxidative stress, foam cell formation, inflammation/cytokines, plaque formation/progression, platelet aggregation, thrombus formation, and plaque rupture [55, 75].

Moreover, EPA has been associated with the preservation of cell membrane structure and normal distribution of membrane cholesterol; increased EPA content in the vessel wall; increased circulating EPA:arachidonic acid (AA) ratio; and inhibited platelet aggregation [55]. Additional details concerning the effects of OM3FAs on specific components of CV risk are covered in the following sections.

Antioxidant Activity

Paraoxonase 1 (PON1) is a crucial antioxidant enzyme located in a subfraction of HDL and is responsible for protecting against lipoprotein oxidation as well as exerting anti-inflammatory effects. A 2011 study found that patients with T2DM with complications have significantly decreased HDL-C levels and PON1 activity [76]. The role of EPA in affecting PON1 activity has been reported in multiple studies [77, 78]. One randomized, double-blind, placebo-controlled clinical trial involving 36 patients with T2DM receiving 2 g/day EPA or placebo for 8 weeks found that EPA was associated with a significant ($P = 0.001$) increase in the serum levels and activity of PON1 [77]. Consistent with these results was a 2019 double-blind, placebo-controlled trial in which patients randomized to receive 2 g/day EPA

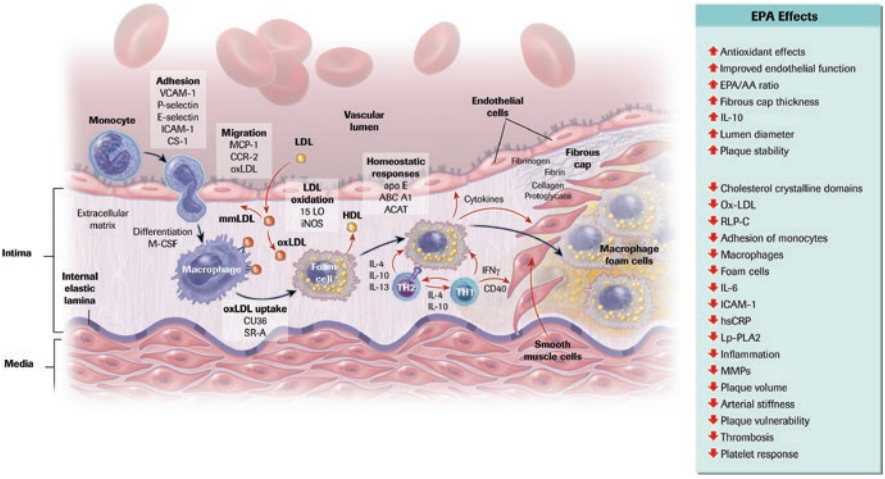

Fig. 25.3 Pleiotropic mechanisms of action of EPA [55]. *ACAT* acyl CoA:cholesterol acyltransferase; *Apo E* apolipoprotein E; *CCR* C-C chemokine receptor; *CD* clusters of differentiation; *CS* connecting segment; *EPA* eicosapentaenoic acid; *EPA/AA* eicosapentaenoic acid/arachidonic acid ratio; *HDL* high-density lipoprotein; *hsCRP* high-sensitivity C-reactive protein; *ICAM* intercellular adhesion molecule; *IFN* interferon; *IL* interleukin; *iNOS* inducible nitric oxide synthase; *LDL* low-density lipoprotein; *LO* lipoxygenase; *Lp-PLA2* lipoprotein-associated phospholipase A2; *MCP* monocyte chemotactic protein; *mm-LDL* minimally modified LDL; *MMP* matrix metalloproteinase; *ox-LDL* oxidized LDL; *RLP-C* remnant-like lipoparticle cholesterol; *SMC* smooth muscle cell; *Th* T helper; *VCAM* vascular cell adhesion molecule

showed a significant ($P = 0.027$) increase in the gene expression of PON2 versus placebo [78]. In the same study, EPA supplementation also significantly increased HDL-C levels and decreased fasting blood sugar compared with placebo.

The antioxidant effects of EPA were also demonstrated in a laboratory study that compared the effects of placebo, EPA, and DHA on rates of small-dense LDL, VLDL, and membrane oxidation [79]. EPA had potent antioxidant effects that were sustained over time versus DHA and placebo [79]

Effect on Albuminuria

Multiple studies have shown that OM3FAs reduce albuminuria, an established risk factor for CVD and marker of plaque destabilization [80–82]. Longitudinal data from the Diabetes Control and Complications Trial, including 1436 patients with T1DM, showed that dietary OM3FAs were associated with a slower deterioration of urinary albumin excretion rate in patients with HbA1c levels >7.7% [83]. In another study of 344 patients with DM and hypertriglyceridemia, OM3FAs were associated with a significant reduction in urine albumin:creatinine ratio (from 475.8 ± 1235.9 mg/g to 385.6 ± 1067.9 mg/g, $\Delta = -72.1 \pm 507.6$ mg/g; $P = 0.003$); the effects were dependent on the daily dose of OM3FAs [84].

Effect on Plaque Reduction and Stabilization

Plaque buildup is a hallmark feature of atherosclerosis and increases the risk for CV events, which occur predominantly by way of plaque rupture resulting from inflammation, oxidation, and a thin fibrous cap [85]. In patients with DM, plaques in the coronary arteries are characterized by larger necrotic cores and increased inflammation and calcification, leading to increased plaque buildup and vulnerability compared with individuals without DM [86]. This was demonstrated in the PARADIGM (Progression of Atherosclerotic Plaque Determined by Computed Tomographic Angiography Imaging) study, in which patients with DM were at an increased risk for plaque progression, low-attenuation plaque, and spotty calcification [87]. Indeed, in one study, patients with DM had a twofold increase in the progression of normalized total plaque volume versus patients without DM [88]. In another study of 322 patients with acute coronary syndrome, comparison of culprit plaque characteristics between patients with DM and those without DM showed that patients with DM had more vulnerable features in both culprit and nonculprit lesions, suggesting plaque instability [89].

Multiple clinical trials have demonstrated that the use of EPA affects plaque stabilization and regression, and even results in reversal of atherosclerosis in patients with DM [85]. A randomized trial in 81 patients with T2DM found that EPA 1.8 g/day for 2 years significantly decreased carotid intima-media thickness (CIMT) and improved brachial-ankle pulse wave velocity, suggesting a reduction in atherosclerosis and improved endothelial function; EPA was shown to be a significant and independent factor associated with reduction of CIMT [90]. Similarly, in another study of 10 patients with hypertriglyceridemia, including 4 with concurrent DM, treatment with EPA 1.8 g significantly reduced CIMT ($P < 0.05$), and the decrease in CIMT was significantly correlated with the EPA:AA ratio ($P < 0.05$) [91].

The Effect of Icosapent Ethyl on Progression of Coronary Atherosclerosis in Patients with Elevated Triglycerides on Statin Therapy (EVAPORATE) trial included 80 statin-treated patients with coronary atherosclerosis and elevated TG levels; 69% of patients had T2DM [92]. IPE 4 g/day reduced low-attenuation plaque volume by 17% versus an increase of 109% in the mineral oil placebo group ($P = 0.006$) (Fig. 25.4) [92]. There were significant differences in rates of progression between IPE and placebo, including total plaque (−9% vs. +11%, respectively; $P = 0.002$), total noncalcified plaque (−19% vs. +9%, respectively; $P = 0.0005$), and fibrofatty plaque (−34% vs. +32%, respectively; $P = 0.0002$) [92]. The reduced rate of plaque progression with IPE was not accompanied by significant reductions in lipid levels, including LDL-C or TG levels, suggesting that slowed progression of atherosclerosis through plaque regression may, in part, help to explain the favorable CV outcomes seen in REDUCE-IT [92].

Similar findings were observed in the prospective, randomized, nonblinded, multicenter Combination of Therapy of Eicosapentaenoic Acid and Pitavastatin for

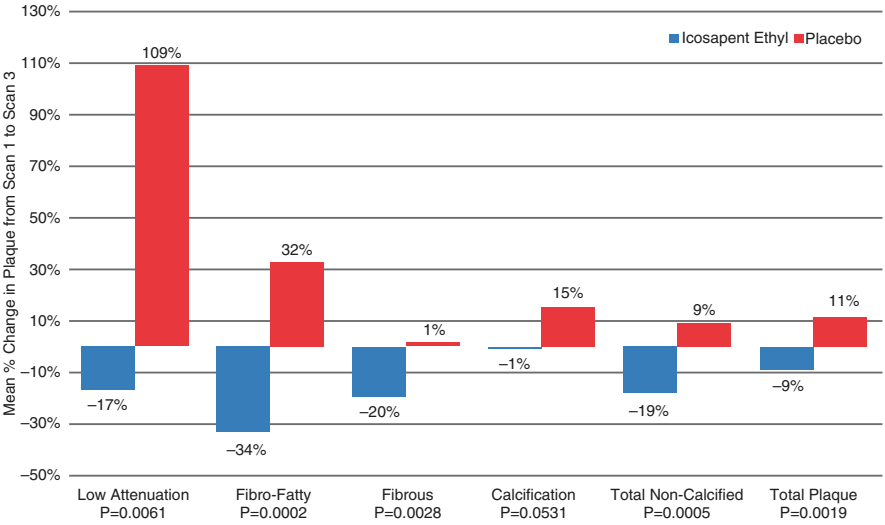

Fig. 25.4 EVAPORATE clinical trial: mean plaque progression with IPE vs. placebo [92]. From Budoff MJ, Bhatt DL, Kinninger A, Lakshmanan S, Muhlestein JB, Le VT, et al. Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial. Eur Heart J. 2020;41(40):3925–32, by permission of Oxford University Press. *IPE* icosapent ethyl

Coronary Plaque Regression Evaluated by Integrated Backscatter Intravascular Ultrasonography (CHERRY) trial, which included 193 patients with coronary heart disease who underwent percutaneous coronary intervention. Patients in CHERRY were randomized in a 1:1 ratio to receive 4 mg/day pitavastatin or pitavastatin 4 mg/day plus EPA 1.8 g/day; approximately 36% of patients had DM [93]. Addition of EPA to statin therapy resulted in significant plaque regression and stabilization versus statin therapy alone, as demonstrated by significantly reduced coronary plaque volume ($P < 0.0001$) (Fig. 25.5) [93]. There was a significant correlation between the EPA:AA ratio and percent change in lipid volume, adding to existing evidence that EPA is an important contributor to stabilizing coronary plaque [93].

A Japanese study of 95 dyslipidemic, statin-treated patients with stable angina pectoris investigated the effect of EPA on plaque regression and on inflammatory markers; approximately 50% of the patient population had DM [94]. A significant reduction in lipid volume (18.5 ± 1.3 to 15.0 ± 1.5 mm^3; $P = 0.007$) and a significant increase in fibrous volume (22.9 ± 0.8 to 25.6 ± 1.1 mm^3; $P = 0.01$) were reported in the EPA group, with no significant changes in the control group [94]. In addition, inflammatory cytokine PTX3 and MCP-1 levels were significantly reduced in the EPA versus placebo group, suggesting that anti-inflammatory effects of EPA were associated with its anti-atherosclerotic effects [94].

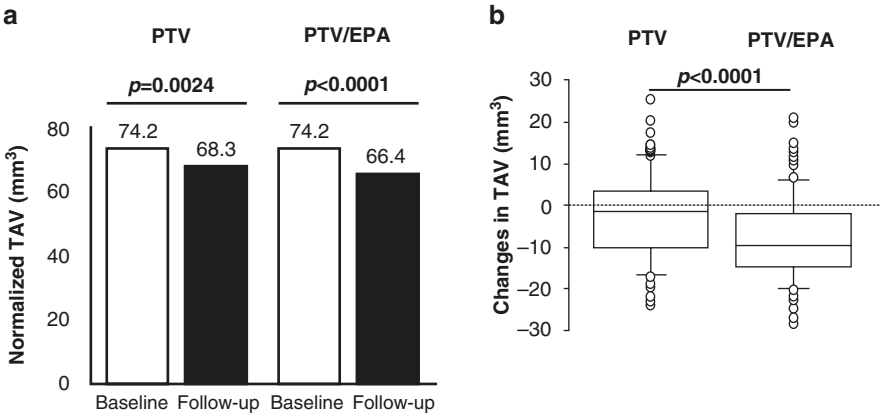

Fig. 25.5 CHERRY clinical trial: effect of EPA on total atheroma volume [93]. (**a**) Comparisons of total atheroma volume between baseline and follow-up in the PTV only and the PTV plus EPA groups. (**b**) Comparison of TAV reduction between the PTV only and PTV plus EPA group. Reprinted from J Cardiol. 70(6), Watanabe T, Ando K, Daidoji H, Otaki Y, Sugawara S, Matsui M, et al. A randomized controlled trial of eicosapentaenoic acid in patients with coronary heart disease on statins, 537–44, copyright 2017, with permission from Elsevier. *PTV* pitavastatin; *EPA* eicosapentaenoic acid; *TAV* total atheroma volume

Effect of OM3FAS on Microbiome

Gut microbiota is an important component of overall health, and it is well established that diet can influence the host-specific gut microbiota [95, 96]. The type and quantity of gut microbes can help maintain overall health, as well as trigger the development of diseases [97]. Addition of OM3FAs to diet is associated with myriad benefits on the gut microbiota [95–97]. OM3FAs have been shown to positively influence the gut microbiome in three main ways [97]. First, they modulate the type and abundance of gut microbes, such as decreasing the growth of deleterious Enterobacteria and increasing the growth of beneficial Bifidobacteria [97]. In addition, they alter the levels of pro-inflammatory mediators (e.g., endotoxins [lipopolysaccharides], interleukin-17, and tumor necrosis factor) and promote the production of anti-inflammatory mediators [97]. Finally, they regulate the levels of short-chain fatty acids or short-chain fatty acid salts [97].

OM3FAs, Gut Microbiome, and Glycemic Control

In patients with DM, the effects OM3FAs on the gut may have downstream benefits on glycemic control. Preclinical and clinical data demonstrate that altering the gut microbiome may favorably impact glucose homeostasis. One study showed that fecal microbiota transplantation from fat-1 mice to wild-type mice reversed weight gain and normalized glucose tolerance [98]. Most recently, a 2021 study assessed changes

in the gut microbiome and effect on glucose homeostasis in db/db mice after supplementation with 1% (w/w) EPA or DHA for 10 weeks. Supplementation with EPA or DHA attenuated hyperglycemia and insulin resistance, all of which were facilitated by changes in the gut microbiome, including abundance of certain bacteria, increased levels of propionate and butyrate, increased release of glucagon-like peptide-1, and lower serum lipopolysaccharide concentration. Overall, the therapeutic effect was more evident with EPA versus DHA [99]. The multicenter, randomized Pilchardus Study included treatment-naive patients with T2DM, with HbA1c levels between 6.0% and 8.0% [100]. Patients were randomly assigned to receive 100 g of sardines (translating to approximately 3 g of EPA and DHA) or standard diet for 5 days/week for 6 months. The OM3FA group had improved insulin resistance, and OM3FA supplementation was not associated with negative effects on glycemic control [100].

Guidelines

Encouraging data from REDUCE-IT on the use of IPE for reducing CV events has prompted several societies to update guidelines on reducing dyslipidemia and ASCVD, including in patients with DM (Table 25.1) [101–112].

The American Diabetes Association (ADA) recommends that IPE be considered in patients with ASCVD or other CV risk factors on a statin with controlled LDL-C levels but elevated triglycerides (135–499 mg/dL) [101]. In addition, according to a statement by the American Heart Association (AHA), "prescription n-3 fatty acids (EPA+DHA or EPA-only) at a dose of 4 g/d (>3 g/d total EPA+DHA) are an effective and safe option for reducing TGs as monotherapy or as an adjunct to other lipid-lowering agents" [17]. In addition, the AHA statement on the management of stable CAD in patients with T2DM recognizes the value of IPE with respect to reducing CV events, consequently recommending IPE as the first-line therapy for patients with T2DM and CAD whose TG levels remain elevated (>135 mg/dL) despite maximally tolerated statin and lifestyle changes [107].

A 2020 consensus statement by the American Association of Clinical Endocrinology (AACE)/American College of Endocrinology (ACE) on the management of dyslipidemia and prevention of CVD recommends IPE to be added to a statin in any patient with established ASCVD or DM with ≥2 ASCVD risk factors and TG levels between 135 and 499 mg/dL [108]. Similarly, the Endocrine Society (ENDO) 2020 Clinical Practice Guidelines for Lipid Management in Patients With Endocrine Disorders suggest IPE for the reduction of CV risk in adults on statins with controlled LDL-C but elevated TG levels (>150 mg/dL) and either ASCVD or DM plus two additional risk factors [109].

The American College of Cardiology supports the use of IPE for ASCVD risk reduction for adults aged ≥50 years with diabetes mellitus, at least one additional ASCVD risk factor, and fasting TG levels ≥150 and <500 mg/dL [104]. Finally, the Canadian Cardiovascular Society recommends IPE to lower the risk of CV events in patients with ASCVD, or with DM and ≥1 CVD risk factors, who have an elevated fasting TG level of 135–499 mg/dL despite treatment with maximally tolerated statin therapy [112].

Table 25.1 Guideline recommendation for use of EPA

Medical society guideline	Year	Recommendation
American Diabetes Association [102]	2021	In patients with ASCVD or other cardiovascular risk factors on a statin with controlled LDL-C but elevated TG levels (1.5–5.6 mmol/L [135–499 mg/dL]), the addition of IPE can be considered to reduce cardiovascular risk
European Society of Cardiology/European Association for the Study of Diabetes [103]	2019	For patients with diabetes mellitus who are statin intolerant and have high TG levels (≥2.3 mmol/L [≥200 mg/dL]), if TG levels are not controlled by statins or fibrates, high-dose n-3 PUFA (4 g/day) of IPE may be used
American College of Cardiology [104]	2021	For patients with ASCVD and LDL-C <1.8 mmol/L (<70 mg/dL) and with persistent fasting TG ≥1.7 and <5.6 mmol/L (≥150 and <500 mg/dL) who are on maximally tolerated statin therapy, readdress lifestyle and medication adherence and reconsider possible secondary causes of hypertriglyceridemia; in the absence of these factors, it may be reasonable to add IPE as the next step[a]
National Lipid Association [105]	2019	For patients aged 45 years or older with clinical ASCVD, or 50 years or older with diabetes mellitus requiring medication and ≥1 additional risk factor, with fasting TG levels 1.5–5.6 mmol/L (135–499 mg/dL) on high-intensity or maximally tolerated statin, with or without ezetimibe, treatment with IPE is recommended for ASCVD risk reduction
American Association of Clinical Endocrinologists/ American College of Endocrinology [106]	2020	For patients with TG levels 1.5–5.6 mmol/L (135–499 mg/dL) and a high ASCVD risk on a maximally tolerated statin, add IPE 4 g/day
American Heart Association [107]	2020	In patients with stable CAD whose atherogenic abnormalities include HTG, low HDL-C, and small, dense LDL particles, consider IPE for further cardiovascular risk reduction when TG levels remain elevated (>1.5 mmol/L [135 mg/dL]) despite maximally tolerated statin
American Association of Clinical Endocrinologists/ American College of Endocrinology [108]	2020	For patients treated with maximally tolerated statins who have established ASCVD or diabetes with ≥2 ASCVD risk factors and TG between 1.5 and 5.6 mmol/L (135 and 499 mg/dL), IPE should be added
Endocrine Society [109]	2020	In adults who are on statins and still have moderately elevated TG levels (>1.7 mmol/L [>150 mg/dL]), and who have either ASCVD or diabetes plus two additional risk factors, suggest adding EPA ethyl ester to reduce the risk of CVD In adults with T2DM on a statin at LDL-C goal with residual TG levels >1.7 mmol/L (>150 mg/dL) and with two additional traditional risk factors or risk-enhancing factors, suggest adding EPA ethyl ester to reduce cardiovascular risk
American Heart Association/American Stroke Association [110]	2021	In patients with ischemic stroke or TIA, with fasting TG levels 1.5–5.6 mmol/L (135–499 mg/dL) and LDL-C of 1.1–2.6 mmol/L (41–100 mg/dL) on moderate- or high-intensity statin therapy, with A1C <10%, and with no history of pancreatitis, AF, or severe heart failure, treatment with IPE 2 g BID is reasonable to reduce the risk of recurrent stroke

Table 25.1 (continued)

Medical society guideline	Year	Recommendation
European Society of Cardiology/European Atherosclerosis Society [111]	2019	In high-risk (or above) patients with TG levels 1.5–5.6 mmol/L (135–499 mg/dL) despite statin treatment, n-3 PUFAs (IPE 2 × 2 g/day) should be considered in combination with a statin
Canadian Cardiovascular Society [112]	2021	Recommend the use of IPE to lower the risk of cardiovascular events in patients with ASCVD, or with diabetes and ≥1 CVD risk factors, who have an elevated fasting TG level of 1.5–5.6 mmol/L despite treatment with maximally tolerated statin therapy

ASCVD atherosclerotic cardiovascular disease; *AF* atrial fibrillation; *CAD* coronary artery disease; *CVD* cardiovascular disease; *EPA* eicosapentaenoic acid; *HDL-C* high-density lipoprotein cholesterol; *HTG* hypertriglyceridemia; *IPE* icosapent ethyl; *LDL* low-density lipoprotein; *LDL-C* low-density lipoprotein cholesterol; *n-3* omega-3; *PUFA* polyunsaturated fatty acid; *T2DM* type 2 diabetes mellitus; *TG* triglycerides; *TIA* transient ischemic attack

[a] In patients with a history of paroxysmal AF or at high risk for AF, discuss the potential net benefit of IPE based on the 1% increase in hospitalization for AF or atrial flutter in REDUCE-IT [104]

Conclusions

Residual CV risk in patients with DM and statin-controlled LDL-C levels may be attributed, in part, to elevated TG levels [10–12]. Prescription OM3FAs containing DHA + EPA and the EPA-only formulation, IPE, are approved to treat elevated TG levels [18–21]. Until recently, clinical trial data showing robust effect of OM3FAs on CV outcomes were limited and weak. Mixed OM3FAs in CV outcome trials have yielded contradictory results—few have shown reductions in CV events while the majority have not [59–62]. However, recent findings from the JELIS and REDUCE-IT trials of EPA-only formulations showed significant reduction of CV events. Based on findings from the pivotal REDUCE-IT trial, IPE became the only OM3FA and the first non-LDL-C-lowering drug approved for reducing CV events in statin-treated patients with established CVD or with DM and other risk factors [19]. The effect of OM3FAs on glucose homeostasis continues to be a topic of ongoing research, but more recent clinical trials provide evidence that OM3FAs preserve glucose control and may even reduce the risk of DM. Meanwhile, EPA/IPE in CV outcome trials has shown significant reduction in primary and secondary composite CV endpoints [22, 35, 39]. In addition, EPA formulations have shown plaque regression and stabilization [90–94]. Current US guidelines, as well as those from international societies, support the use of IPE in patients with established ASCVD or in those with DM and other risk factors [10, 17, 101, 107–109, 111, 112].

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Chapter 26
Cholesterol Absorption Inhibitors (Ezetimibe) and Bile Acid-Binding Resins (Colesevelam HCl) as Therapy for Dyslipidemia in Patients with Diabetes Mellitus

Harold Edward Bays

Diabetes Mellitus and Risk for Cardiovascular Disease

General Considerations

Earlier stages of type 2 diabetes mellitus (T2DM) most often involve adiposopathic insulin resistance and (compensatory) hyperinsulinemia [1, 2]. Hyperglycemia emerges when the pancreatic insulin secretory response wanes as patients get older or becomes insufficient to overcome increasing insulin resistance. Hyperglycemia and insulin resistance are sentinel components of the pathogenic potential of diabetes mellitus to contribute to microvascular disease (e.g., retinopathy, nephropathy, neuropathy) and macrovascular disease [e.g., atherosclerotic cardiovascular disease (ASCVD)]. Adverse vascular effects of hyperglycemia include endothelial dysfunction, oxidative stress, heightened systemic inflammation, activation of receptors of advanced glycosylated end products, increased low-density lipoprotein oxidation, endothelial nitric oxide synthase (eNOS) dysfunction, and platelet hyperactivity [3]. T2DM doubles the risk for death and contributes to a tenfold increase in hospitalizations for ASCVD [4]. Given that diabetes mellitus is a major risk factor for CVD [5], patients with diabetes mellitus are best treated aggressively for common CVD risk factors (e.g., overweight or obesity, high blood pressure, dyslipidemia, cigarette smoking) [3, 6].

Among the priorities of ASCVD risk reduction among patients with diabetes mellitus is aggressive management of dyslipidemia. Patients with diabetes mellitus

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have lower lipid thresholds to implement lipid-lowering therapy and have more aggressive lipid treatment goals regarding the level of low-density lipoprotein cholesterol (LDL-C) that might best be achieved. For example, it is recommended that patients with diabetes mellitus 40–75 years of age be administered moderate- to high-intensity statin therapy, regardless of the estimated ASCVD risk [3, 5]. It is recommended that patients with diabetes mellitus who have experienced a CVD event, or who have multiple CVD risk factors, be administered high-intensity statins, with an LDL-C goal of <70 mg/dL. In more severe cases, the LDL-C treatment goals for patients with diabetes mellitus may be <55 mg/dL or <40 mg/dL [5, 7].

Unfortunately, not all patients with diabetes mellitus achieve LDL-C treatment recommendations. This is often due to insufficient efficacy of statin alone to achieve the more aggressive LDL-C goals, or because of intolerance or lack of adherence to statin therapy. As many as 60% of patients with diabetes mellitus do not achieve an LDL-C level of <100 mg/dL [8]. Regarding more aggressive LDL-C treatment goals, even among specialty clinics, <20% of patients with diabetes mellitus achieve LDL-C levels less than 70 or 55 mg/dL, which is a rate that drops to <10% for patients not treated with statins [9]. The lack of lipid goal attainment suggests the need for additional lipid-lowering therapies either as add-on to statins for further lower LDL-C levels or, in some cases, as an alternative to statins (i.e., for patients with statin intolerance who are unable to take statins).

Clinical Relevance of Intestinal Cholesterol

Among patients with T2DM, dyslipidemia is a modifiable risk factor. This is especially important given that patients with T2DM are at high risk for ASCVD [10]. Statins are the first treatment of choice to lower cholesterol levels in patients with T2DM. However, when statins are not tolerated, or if statin therapy alone is not sufficient in achieving LDL-C treatment goals, then a cholesterol absorption inhibitor (i.e., ezetimibe) is another lipid-lowering drug treatment option.

In peripheral tissues and the liver, the major precursor for cholesterol synthesis is acetyl coenzyme A (acetyl-CoA), which gives rise to hydroxy-methylglutaryl coenzyme A (HMG-CoA). HMG-CoA reductase is the enzyme that converts HMG-CoA to mevalonic acid and is the rate-limiting step in cholesterol biosynthesis. Statins inhibit HMG-CoA reductase. Clinically, statins are the most commonly used drug to treat high cholesterol and were originally termed HMG CoA reductase inhibitors, reflecting their mechanism of action as inhibiting the rate-limiting step of cholesterol production.

Textbook descriptions differ in describing the origin of bodily cholesterol production. Strictly speaking, primate studies suggest that the greatest amount of cholesterol produced in the body is derived from non-hepatic tissues, such as skin, muscle, and intestine, with the greatest amount of cholesterol produced per gram of tissue being endocrine organs, such as the adrenal gland and sex organs [11]. This

is because cholesterol is required for cell membranes, cellular functions, and especially steroidogenesis. However, what is most clinically relevant regarding dyslipidemia and ASCVD is not where most total body cholesterol is produced, but rather the hepatic origin of circulating cholesterol-carrying lipoproteins that are exposed to the vasculature and that potentially contribute to the atherosclerotic process.

Cholesterol is a waxy substance first described in gallstones. The Greek derivation of the term "cholesterol" refers to "chole" for bile and "stereos" for solid. Because cholesterol (a lipid) is insoluble in water, it must be packaged and carried in the blood by polar protein-containing biochemical particles, known as lipoproteins. Especially among patients with overweight or obesity, the greatest stores of cholesterol and triglyceride are in adipose tissue [12]. However, most of the *circulating* cholesterol is hepatic/gastrointestinal in origin (i.e., cholesterol carried by lipoproteins originates from the liver or intestine).

Regarding intestinal cholesterol, dietary sterols variably contribute to circulating cholesterol and other sterol blood levels, with circulating lipoprotein cholesterol levels increased during times of high cholesterol consumption—especially in patients who are hyperabsorbers of intestinal cholesterol [13]. Typically, approximately three-quarters of the cholesterol delivered to the intestine is derived from biliary cholesterol excretion from the liver, with the other one-quarter from dietary consumption [11]. Once in the intestinal lumen, both biliary and dietary cholesterol (and other lipids) interact with bile acids allowing for micelle formation, which enhances transport of cholesterol through the jejunal brush border membranes into intestinal epithelial cells. Once in intestinal cells, free cholesterol is typically returned to the intestinal lumen through a heterodimer of adenosine triphosphate (ATP)-binding cassette (ABC) transporters G5 and G8, or esterified and then eventually packaged into chylomicron particles which then deliver intestinal cholesterol to peripheral tissues and the liver. Thus, hepatic cholesterol synthesis, intestinal cholesterol absorption, and cholesterol carried by circulating lipoproteins secreted by the liver are all interrelated.

Ezetimibe

Ezetimibe Mechanism of Action

Ezetimibe is a lipid-lowering drug indicated to lower LDL-C levels [11]. Its "mibe" suffix reflects its discovery during the evaluation of the clinical utility of various acyl-CoA cholesterol acyltransferase (ACAT) inhibitors [i.e., "mibe" is the designated name for this group of agents (e.g., avasimibe, pactimibe)]. Curiously, most ACAT inhibitors do not have clinically meaningful effects upon intestinal cholesterol absorption. It is perhaps even more curious that at approved doses, ezetimibe has no clinically meaningful ACAT activity [14]. When ezetimibe was approved for clinical use in 2002, the molecular target was unknown and was classified as a "cholesterol absorption inhibitor." Subsequently, ezetimibe was found to competitively

inhibit the Niemann–Pick C1-like 1 protein (NPC1L1), a sterol transporter located on the brush border membrane of intestinal epithelial cells and at sites in the liver [14]. Likely because ezetimibe had already been approved and marketed before this discovery, it was never reclassified as a "cholesterol transport inhibitor." Rather, it maintained its classification as a "cholesterol absorption inhibitor." Through inhibiting intestinal cholesterol transport, ezetimibe reduces cholesterol entering the enterocyte, reduces the cholesterol packaged into chylomicrons, and decreases the amount of cholesterol delivered to the liver. While reports are not always consistent [15], most publications report ezetimibe as reducing circulating LDL-C levels via impairment of intestinal cholesterol transport, increased fecal cholesterol excretion, reduced cholesterol delivered to the liver, reduced hepatic secretion of cholesterol-containing lipoproteins, and upregulation of hepatic LDL receptors [16].

As before, the NPC1L1 protein promotes cholesterol transport through the enterocyte brush border membrane of the proximal small intestine [17]. In humans, mutations of the NPC1L1 gene expression are associated with a 12 mg/dL reduction in LDL-C levels, and a 53% reduction in the risk of ASCVD [18]. If applicable beyond genetic predictions, then it is possible that the large reduction in ASCVD risk with NPC1L1 impairment, despite only a modest reduction in LDL-C levels, might represent lifelong benefits in LDL-C level reduction. The clinical benefits of lipid lowering are related to both the degree of LDL-C lowering and duration of LDL lowering [19].

In addition to being located in the small intestine, NPC1L1 is also found in the liver. Ezetimibe appears to mediate the reuptake of cholesterol from the biliary system into the liver [20]. Overexpression of NPC1L1 in mice results in reduced biliary excretion of cholesterol [21] and increased hepatic cholesterol stores that potentially contribute to increased hepatic secretion and/or reduced hepatic reuptake of cholesterol-containing lipoproteins. Ezetimibe administration may increase hepatic excretion of cholesterol, impair cholesterol uptake in the small intestine, and increase fecal excretion of cholesterol [16]. Interestingly, despite increased biliary excretion of cholesterol, meta-analyses do not support an increased risk of cholesterol gallstones with ezetimibe [22].

Because the mechanism of action of ezetimibe and statins differs, ezetimibe is complementary to statins regarding LDL-C lowering. The combination of ezetimibe and statins is sometimes described as representing "dual inhibition." Statins inhibit cholesterol production, and ezetimibe inhibits intestinal cholesterol absorption. While they differ in their mechanism, most reports suggest that both statins and ezetimibe share some end results, such as potentially increasing hepatic LDL receptor activity resulting in enhanced clearance of LDL particles. By utilization of this "dual inhibition" approach, cholesterol blood levels are reduced more with the combination of ezetimibe and statin than compared to either agent alone. Given that the LDL receptor activity may also increase the clearance of other apolipoprotein B-containing lipoproteins, such as triglyceride-rich lipoproteins (e.g., "remnant" lipoprotein particles, intermediate-density lipoproteins, and some very-low-density lipoprotein particles) [23, 24], this may help account for why statins moderately, and ezetimibe modestly, lower triglyceride blood levels.

Further Complementary Mechanism of Action and Potential Benefits of Ezetimibe and Statin "Dual Inhibition"

Circulating LDL-C levels are determined by genetics, dietary intake, physical activity, concurrent drugs, and illnesses such as diabetes mellitus. Circulating LDL levels are dependent upon hepatic synthesis, gastrointestinal absorption of dietary cholesterol, and biliary metabolism. Hepatic cholesterol is a substrate for bile acid synthesis; hepatic cholesterol is excreted in the bile. Individuals vary in the degree of cholesterol hepatic synthesis versus the degree of cholesterol gastrointestinal absorption. Hyperabsorbers of intestinal cholesterol may have reduced LDL-C lowering in response to statin therapy. In other words, while the data are not always consistent [25, 26], some reports suggest that hyper-responders to statins are patients with higher baseline cholesterol synthesis markers (e.g., lathosterol and desmosterol). Conversely, statin hypo-responders may be those with increased markers of cholesterol absorption (e.g., campesterol, sitosterol, stigmasterol, and cholestanol) [27–29]. Some reports suggest that administration of statin therapy promotes a compensatory increase in intestinal cholesterol absorption [30]. Other reports suggest that inhibition of intestinal cholesterol absorption with ezetimibe may increase cholesterol synthesis [16]. This potential increase in intestinal cholesterol absorption with statins, and the potential increase in cholesterol synthesis with ezetimibe, further supports the potential benefits of "dual inhibition" via the complementary use of statin and ezetimibe.

Pharmacokinetics, Safety, and Drug Interactions

Oral ezetimibe is extensively metabolized (>80%) to active ezetimibe-glucuronide, where it undergoes extensive enterohepatic circulation. As a result, the half-life of ezetimibe and ezetimibe-glucuronide is ~22 h, allowing for a once-a-day dose of only 10 mg [31]. Approximately 78% of ezetimibe is excreted in the feces (predominantly as ezetimibe), and 11% in the urine (mainly as ezetimibe-glucuronide). In contrast to many statins, ezetimibe is not metabolized by common cytochrome P450 isoenzymes to a clinically meaningful degree [31]. Concomitant administration of ezetimibe with statins does not significantly affect statin levels (or most other drugs); the concomitant administration of statins does not alter the bioavailability of ezetimibe [31]. Ezetimibe and cyclosporin coadministration increases the circulating levels of both agents (Zetia prescribing information: https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021445s018lbl.pdf). Potentially relevant to some patients with diabetes mellitus, the increase in cyclosporin exposure may be greater in patients with severe renal insufficiency. When patients are treated with cyclosporine and ezetimibe, cyclosporin levels should be carefully monitored.

Many patients with diabetes develop chronic kidney disease. Ezetimibe is not significantly excreted by the kidneys and thus does not require adjustment in patients

with renal disease. (Zetia prescribing information: https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021445s018lbl.pdf). While statin dosing adjustment is often recommended among patients with renal insufficiency, clinical trials support the combined use of ezetimibe and statins (at the appropriate statin dose adjusted for the degree of renal insufficiency) in patients with chronic kidney disease to improve the lipid profile and to reduce major adverse cardiovascular events and all-cause deaths [32].

Ezetimibe in Special Patient Populations

Many patients with T2DM have overweight, obesity, or metabolic syndrome, all associated with reduced intestinal cholesterol absorption [33]. It is therefore relevant to have an understanding of the clinical implications of intestinal cholesterol absorption.

Beta-sitosterolemia is a rare autosomal recessive disorder caused by mutations in ATP-binding cassette (ABC) subfamily G5 or G8. Normally, 50–60% of dietary cholesterol is absorbed in the intestine, while <5% of the xenosterols are absorbed [34]. When more plant sterols (the major type of xenosterols) are ingested, they compete with the bulk cholesterol for solubilization and transport, thereby reducing dietary absorption of cholesterol and lowering plasma cholesterol [34]. This is the basis why increased plant sterols are sometimes recommended as a dietary means to lower blood cholesterol [35]. The majority of xenosterols that enter enterocytes are immediately excreted via ABCG5/G8 back into the intestinal lumen [34].

Patients with beta-sitosterolemia have impaired function of ABCG5 and/or ABCG8, and clinically manifest with variable lipid levels dependent upon dietary cholesterol intake, and signs and symptoms similar to heterozygous familial hypercholesterolemia [36, 37]. Sitosterolemia is characterized by hyperabsorption and accumulation of plant sterols and cholesterol with tendinous and cutaneous xanthomas, arthritis or arthralgia, and premature ASCVD [38]. Patients with sitosterolemia may be hypo-responders to statins, potentially because endogenous cholesterol synthesis is already inhibited by increased intestinal sterol absorption [36, 37]. Ezetimibe is the only pharmacotherapy approved for treatment of sitosterolemia, which reduces intestinal sterol absorption and thus reduces sterol xanthomas [36, 37].

Beyond sitosterolemia, the potential clinical importance of intestinal cholesterol absorption among patients with T2DM is illustrated by the implementation of ketogenic diets. A ketogenic diet is a medical nutrition therapy involving a very-low-carbohydrate, proportionately higher fat diet, which is most often prescribed for short-term weight loss. In most cases, the ketogenic diet is associated with an overall modest increase in LDL-C levels [39]. In some individuals, a ketogenic diet may result in marked increases in LDL-C levels [40]. This may be due to the dietary intake of saturated fats and cholesterol, as well as an increase in intestinal

cholesterol absorption prompted by weight loss (i.e., intestinal cholesterol absorption is decreased with obesity and metabolic syndrome) [33]. In general, reduced dietary saturated fats and reduced dietary cholesterol are less effective in improving the lipid profile in individuals with obesity and/or metabolic syndrome, while lean persons are more responsive to reductions in dietary saturated fats and cholesterol [33]. If a ketogenic diet is implemented in a patient with T2DM having overweight or obesity, and if enhanced intestinal cholesterol absorption is diagnosed or suspected due to (1) increased dietary saturated fat intake, (2) increased dietary cholesterol intake, and (3) increased intestinal cholesterol absorption due to weight loss, then treatment approaches may include limiting dietary cholesterol and saturated fats and implementation of ezetimibe (and statin) [12].

Ezetimibe in Patients with Diabetes Mellitus

Lowering LDL-C remains the primary lipid treatment target for most patients with T2DM [5]. While ezetimibe's main clinical use is lowering LDL-C, ezetimibe has additional effects applicable to dyslipidemias often found in patients with T2DM. This has clinical relevance because ASCVD risk reduction is best achieved by modification of multiple ASCVD risk factors [3], including modification of multiple lipid and inflammatory ASCVD risk factors.

Non-HDL Cholesterol

Non-HDL cholesterol (non-HDL-C) is the sum of cholesterol carried by all atherogenic lipoproteins such as LDLs, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), remnant lipoproteins (RLP), lipoprotein(a) [Lp(a)], and chylomicrons. Non-HDL-C is calculated as total cholesterol minus HDL-C. Given that non-HDL-C is more inclusive in assessing the cholesterol carried by atherogenic lipoproteins, it may not be surprising that clinical trial data suggests that non-HDL-C may be a better predictor of ASCVD risk than LDL-C levels alone [41]. In recognition of the clinical importance of non-HDL-C, decades ago, the National Cholesterol Education Program Adult Treatment Panel III recommended the non-HDL-C treatment goals be set at levels 30 mg/dL above the respective LDL-C treatment goals, as a secondary goal for high-risk patients with triglyceride of more than 200 mg/dL [42]. Non-HDL-C assessment in patients with metabolic syndrome and T2DM may be particularly relevant, because many such patients have increased levels of triglyceride-rich lipoproteins [43]. The cholesterol carried by triglyceride-rich lipoproteins is reflected in measures of non-HDL-C, but may not be adequately reflected by measuring LDL-C alone. In statin-treated patients, ezetimibe significantly reduces non-HDL-C approximately 15–20% in those with metabolic syndrome and reduces non-HDL-C approximately 20–25% in patients with T2DM [44, 45].

Apolipoprotein B

As with non-HDL-C, apolipoprotein B may be a better predictor of ASCVD risk than LDL-C alone [46] and is a lipid measurement supported by international lipid guidelines [47]. Apolipoprotein B levels less than 90 mg/dL may be considered an alternative secondary target for patients at high ASCVD risk [48]. Unlike non-HDL-C, apolipoprotein B provides a direct assessment of atherogenic particle number, which is thought to be potentially the most important lipid determinant of atherogenic burden and ASCVD risk [47]. Chylomicron particles contain one molecule of ApoB-48, while each atherogenic lipoprotein particle such as LDL, VLDL, IDL, and other triglyceride-rich lipoproteins contains one molecule of ApoB-100. Some assays used in clinical practice may measure both apolipoprotein B48 and 100. Other apolipoprotein assays only assess apolipoprotein B100. Either way, apolipoprotein B blood levels represent the concentration of atherogenic lipoproteins, which is a measurement of atherogenic risk beyond measuring the cholesterol carried by LDL alone (as reflected by LDL-C levels) [47]. This may be especially important among patients with metabolic syndrome and T2DM who often have elevated triglyceride-rich lipoproteins [43]. Adding ezetimibe to ongoing statin therapy significantly reduces ApoB levels approximately 13% in patients with metabolic syndrome and approximately 18% among those with T2DM [44].

Triglycerides

Hypertriglyceridemia is generally considered a ASCVD risk factor, as elevated triglycerides usually represent increased levels of atherogenic triglyceride-rich lipoproteins. Elevated triglyceride levels are often found in patients with metabolic syndrome and T2DM [43]. If triglyceride levels remain ≥200 mg/dL after LDL-C goals are attained, then non-HDL-C may be assessed, with treatment to non-HDL-C treatment goals [42]. Although support via clinical trial outcome data is lacking, among patients with T2DM, achieving a triglyceride level of <150 mg/dL is considered desirable [49]. When added to statin therapy, ezetimibe modestly, but significantly, reduces triglyceride levels in patients with metabolic syndrome by approximately 5–10% and approximately 10% in patients with T2DM [44, 45].

HDL Cholesterol (HDL-C)

Low HDL-C levels correlate to increased ASCVD risk. It is unclear that pharmacotherapy to raise HDL-C reduces ASCVD events. HDL-C levels may not only correlate to the prognosis of patients with T2DM (i.e., low HDL-C levels are associated with increased ASCVD risk), but may also reflect the pathogenesis of T2DM. Reduced HDL-C levels and impaired HDL functionality may adversely affect pancreatic and skeletal muscle glucose homeostatic processes. While not all HDL disturbances are causatively associated with the development and progression of T2DM, a

bidirectional correlation may exist in some cases [50]. When added to statin therapy, ezetimibe modestly, but significantly, increases HDL-C levels in patients with metabolic syndrome and T2DM by approximately 3% [44, 45].

Remnant-Like Lipoproteins

Elevated levels of triglyceride-rich lipoproteins and their remnants are associated with an increased ASCVD risk [43, 51, 52]. The cholesterol carried by remnant triglyceride-rich lipoproteins is included in measurements of non-HDL-C levels, and the number of total atherogenic lipoprotein particles (i.e., including remnant lipoproteins) is reflected in measurements of apolipoprotein B. Ezetimibe reduces remnant lipoprotein cholesterol levels approximately 10–20% [53].

Lipoprotein Particle Size

Both metabolic syndrome and T2DM are often associated with a disproportionate baseline number of smaller LDL particles, which is often described as increasing ASCVD risk. However, while baseline lipoprotein particle size may have some utility in predicting ASCVD risk, no evidence suggests that the assessment of lipoprotein particle size is useful in determining the efficacy of lipid-altering intervention. In fact, in some circumstances, posttreatment lipoprotein particle size analyses may be misleading [54].

Mechanistically, smaller, more dense LDL particles may (1) have decreased affinity for tissue and liver LDL receptors, thus prolonging LDL particle presence in the blood; (2) have increased permeability through the arterial endothelium with preferential retention in the arterial wall; and (3) be more readily oxidized, further increasing their atherogenic potential [54]. However, while the lipoprotein particle size effects of a lipid-altering intervention may be scientifically intriguing, the vast majority of scientific data supports LDL-C reduction, non-HDL-C reduction, and atherogenic lipoprotein particle number reduction (as reflected by a reduction in apolipoprotein B) as most clinically relevant.

A challenge among some clinicians who advocate “advanced lipid testing” arises when administration of cholesterol-lowering drugs (such as statins or ezetimibe) lowers LDL-C, lowers non-HDL-C, and lowers apolipoprotein B levels, but increases the proportion of remaining LDL particles that are more small and dense [54]. Anecdotally, the increased proportion of smaller, more dense LDL particles has prompted some clinicians to discontinue statin and/or ezetimibe therapy. Physiologically, reduced LDL clearance due to impaired LDL receptor binding is one of the proposed reasons why smaller, more dense LDL particles are potentially more atherogenic. It might therefore be expected that when hepatic LDL receptors are upregulated through therapies such as statins and/or ezetimibe, then the larger circulating LDL particles are preferentially cleared. This leaves a disproportionate amount of smaller, more dense LDL particles. That said, what is most clinically

relevant is that both small and large LDL particles are atherogenic. Both statins and ezetimibe reduce the number of the total of large and small LDL particles. Both statins and ezetimibe reduce apolipoprotein B, reduce LDL-C, and reduce non-HDL-C levels, which are the lipid parameters of most clinical relevance when assessing posttreatment lipid-altering efficacy and reducing ASCVD risk [54]. While lipoprotein particle size may be helpful in assessing baseline ASCVD risk, no evidence exists that such measurements are helpful to assess the efficacy of lipid-altering pharmacotherapy [55].

High-Sensitivity C-Reactive Protein

Atherosclerosis is promoted by inflammation. C-reactive protein is an acute-phase reactant and biomarker of inflammation whose increase is associated with increased ASCVD risk. The reduced progression of ASCVD associated with intensive statin treatment correlates with reductions in hsCRP levels [56]. While ezetimibe monotherapy may reduce hsCRP compared to placebo, these modest differences are generally not statistically significant [57]. However, when ezetimibe is added to ongoing statin therapy, then hsCRP may be more consistently and significantly reduced [57, 58].

Clinical Trials of Ezetimibe in Patients with Diabetes Mellitus

In a post hoc assessment of patients with metabolic syndrome or T2DM treated with ongoing statin therapy, adding ezetimibe significantly lowered LDL-C, non-HDL-C, total cholesterol, apolipoprotein B, and triglyceride levels, irrespective of the presence of metabolic syndrome or T2DM [44]. In a pooled post hoc analysis of 27 clinical trials (n = 6541 with T2DM; n = 15,253 without T2DM), ezetimibe combined with statin was more effective than statin monotherapy in improving LDL-C, total cholesterol, HDL-C, triglyceride, non-HDL-cholesterol, apolipoprotein B, and high-sensitivity C-reactive protein in the overall population, as well as both subgroups with and without T2DM. The safety profile was also similar between groups. This analysis also suggested that ezetimibe combined with statin may lower LDL-C, total cholesterol, and non-HDL-C more among those with T2DM, compared to those without T2DM [59]. In a study of 1229 hypercholesterolemic patients with T2DM comparing ezetimibe 10 mg/simvastatin 20 mg/day versus atorvastatin 10 or 20 mg/day, or ezetimibe 10 mg/simvastatin 40 mg/day versus atorvastatin 40 mg/day, ezetimibe/simvastatin generally provided additional improvements over atorvastatin with regard to LDL-C, total cholesterol, HDL-C, non–HDL-C, triglyceride, and high-sensitivity C-reactive protein, although these findings were not statistically significant at all dose comparisons. Ezetimibe/simvastatin was also superior to

atorvastatin in allowing patients with T2DM to attain LDL-C levels less than 70 mg/dL ($P < 0.001$ for all dose comparisons) [60].

Ezetimibe can be prescribed as monotherapy or combined in a single pill with statins (e.g., combined with simvastatin, atorvastatin, or rosuvastatin). Compared to simvastatin alone, a subgroup analysis of three similarly designed, randomized, double-blind, placebo-controlled studies in patients with primary hypercholesterolemia revealed that ezetimibe plus simvastatin significantly reduced LDL-C, non-HDL-C, apolipoprotein B, triglyceride, and C-reactive protein. These effects were similar among those with and without metabolic syndrome [61]. When compared to doubling of the atorvastatin dose in hypercholesterolemic patients at high ASCVD risk, T2DM, and metabolic syndrome, a post hoc analysis of a double-blind, parallel group trial of hypercholesterolemia at high ASCVD risk demonstrated that atorvastatin plus ezetimibe resulted in greater reductions in LDL-C, triglyceride, apolipoprotein B, non-HDL-C, total cholesterol, and lipid ratios in the T2DM, metabolic syndrome, and neither groups [62]. When ezetimibe plus simvastatin was compared to atorvastatin or rosuvastatin in patients with metabolic syndrome or T2DM, subgroup analyses supported ezetimibe plus simvastatin as providing greater improvements than atorvastatin or rosuvastatin in LDL-C, total cholesterol, HDL-C (versus atorvastatin only), non-HDL-cholesterol, LDL-C:HDL-C ratio, TC:HDL-C ratio, and apolipoprotein B in all subgroups. A greater percentage of patients receiving ezetimibe plus simvastatin attained LDL cholesterol goals of <100 mg/dL or LDL-C <70 mg/dL [42, 63], as well as non-HDL-C treatment goal, again, regardless of subgroup [64].

Because patients with metabolic syndrome and T2DM are at higher ASCVD risk, attainment of LDL-C treatment goals may be especially challenging, because the LDL-C goals are likely to be lower than those without metabolic syndrome and T2DM. Greater LDL-C reduction is usually required to achieve desired lipid targets among patients with metabolic syndrome and/or T2DM. In an analysis of a study of patients with metabolic syndrome and T2DM wherein ezetimibe was added on to statin therapy, LDL-C was significantly reduced by a placebo-corrected 23% among those with metabolic syndrome, and 25% among those with T2DM. In both groups, approximately 70% of patients receiving ezetimibe added to statins achieved LDL-C goal versus about 20% who had placebo added to statins [45].

In order to better achieve lipid treatment goals, multiple lipid-altering drugs are often required. In a long-term efficacy and safety subgroup analysis of a 64-week trial of 1220 patients with metabolic syndrome, T2DM, or neither, who were administered ezetimibe plus simvastatin, versus ezetimibe plus simvastatin and niacin, the triple combination was significantly better than either alone in lowering LDL-C and raising HDL-C compared to ezetimibe plus simvastatin. As expected, the niacin-treated groups had greater flushing and increases in glucose levels with the greatest increases in new-onset T2DM being among those with metabolic syndrome, and the greatest glucose rises among those with T2DM [65]. Mixed dyslipidemia is another clinical situation requiring multiple lipid-altering drug therapies, because statin

monotherapy is frequently inadequate for normalizing simultaneous derangements in multiple lipid parameters. In a study of patients with metabolic syndrome having mixed dyslipidemia, ezetimibe plus simvastatin, as well as ezetimibe plus simvastatin and fenofibrate, significantly reduced LDL-C better than fenofibrate alone, in patients with or without metabolic syndrome. Similarly, improvements in total cholesterol, triglyceride, non-HDL-C, apolipoprotein B, HDL-C, apolipoprotein A1, and hsCRP were greater with ezetimibe plus simvastatin or ezetimibe plus simvastatin and fenofibrate compared to fenofibrate alone. These effects appeared to be consistent in patients with or without metabolic syndrome [66].

Recent Clinical Research of Ezetimibe in Patients with Diabetes Mellitus

Table 26.1 summarizes illustrative research data from 2016 to 2021 regarding ezetimibe in patients with diabetes mellitus. General principles suggested from these publications include:

- Potential glucose lowering of ezetimibe in rodents is not something found or reported in numerous human clinical trials.
- If similar LDL-C lowering is achieved, then ezetimibe plus lower potency statin or ezetimibe plus lower dose statin may provide similar ASCVD benefits as higher potency/dose statin.
- In patients with T2DM and hypercholesterolemia not receiving statins or other lipid-lowering drugs, bempedoic acid plus ezetimibe in a fixed-dose combination significantly lowers LDL-C levels ~40% and CRP and is generally well tolerated.
- Compared to rosuvastatin monotherapy, the combination of rosuvastatin/ezetimibe in patients with T2DM results in greater reduction in total cholesterol, non-HDL-C, LDL-C, and apoB levels; greater proportion of patients achieving >50% reduction in LDL-C levels; greater achievement of lipid goals (e.g., LDL-C <70 mg/dL, non-HDL-cholesterol [non-HDL-C] <100 mg/dL, and apoB <80 mg/dL); and greater improvement in the apoB/A1 ratio.
- While not definitively demonstrated by a dedicated cardiovascular outcome trial, analyses suggest that ezetimibe-statin combination therapy may have greater cardiovascular benefits in patients with diabetes mellitus than in those without diabetes mellitus—possibly due to reduced aggravation of vascular endothelial dysfunction after high-fat diet loading.
- Given that high-intensity statin is associated with a higher risk of incident diabetes in individuals with pre-diabetes, an alternative consideration for patients having this concern is lower dose statin combined with ezetimibe, with ezetimibe having a neutral effect on glucose metabolism.

Table 26.1 Summary of illustrative publications regarding ezetimibe and diabetes mellitus published in 2016–2021[a]

Year	Summary	Reference
2021	Ezetimibe exhibited anti-diabetes and reno-protective properties in rats with diabetes mellitus	[67]
2021	Combination ezetimibe and statin treatment was not associated with significantly different risk of T2DM and CVD compared with statin monotherapy in Korean adults with impaired fasting glucose	[68]
2021	In patients with T2DM having acute coronary syndrome or acute ischemic stroke, treatment with atorvastatin 40 mg or ezetimibe 10 mg/simvastatin 20 mg resulted in similar major cardiovascular outcomes	[69]
2021	ACS patients with diabetes mellitus showed weaker coronary plaque regression than counterparts; more intensive lipid-lowering therapy may be required in ACS patients with diabetes mellitus	[70]
2021	The data from IMPROVE-IT provide reassurance regarding longer term safety and efficacy of the intensification of lipid-lowering and very low LDL-C levels therapy in very-high-risk patients (including patients with T2DM)	[71]
2021	In patients with T2DM and hypercholesterolemia who were not receiving statins or other lipid-lowering drugs, bempedoic acid plus ezetimibe in a fixed-dose combination significantly lowered LDL-C levels and was generally well tolerated	[72]
2020	In older patients with T2DM, prediction of the degree of LDL-C lowering may be more related to baseline LDL-C levels compared to predictions based upon age	[73]
2020	In patients with T2DM, the apoB/A1 ratio was significantly reduced in patients receiving combination therapy with ezetimibe and rosuvastatin compared to those receiving rosuvastatin monotherapy. Both treatments were well tolerated. The proportion of patients achieving >50% reduction in low-density lipoprotein cholesterol (LDL-C) and in the comprehensive lipid target (LDL-C <70 mg/dL, non-HDL-cholesterol [non-HDL-C] <100 mg/dL, and apoB <80 mg/dL) was significantly different between the two groups (76.5% and 73.5% in the rosuvastatin/ezetimibe group and 47.1% and 45.6% in the rosuvastatin group, respectively; $P < 0.001$). The reduction in total cholesterol, non-HDL-C, LDL-C, and apoB was greater in the rosuvastatin/ezetimibe group than in the rosuvastatin monotherapy group	[74]
2020	The use of PCSK9i and ezetimibe does not appear to impact the risk of incident diabetes mellitus when added to guideline-directed medical therapy	[75]
2019	Statin-ezetimibe co-therapy is more efficacious than statin monotherapy in reducing the incidence of CVD with no significant difference between patients with diabetes versus patients without diabetes mellitus	[76]
2019	In patients with T2DM, combination therapy of low-dose rosuvastatin and ezetimibe (5 mg/10 mg/day) reduced LDL-C, apoB, and apoB/A1 ratio comparable to higher dose rosuvastatin monotherapy (20 mg/day). Triglyceride and free fatty acid reductions were greater with the combination therapy than with rosuvastatin monotherapy	[77]
2019	The lipid efficacy of atorvastatin + ezetimibe in treating patients with T2DM accompanied with ACS was significantly improved compared to atorvastatin alone	[78]

(continued)

Table 26.1 (continued)

Year	Summary	Reference
2019	In patients with T2DM with ASCVD and LDL >70 mg/dL, ezetimibe was a cost-saving strategy compared with evolocumab, except when evolocumab price was significantly reduced and the branded ezetimibe was used	[79]
2018	The reduction of major adverse cardiac event risk with ezetimibe plus simvastatin, relative to simvastatin alone, may be greater in patients with diabetes mellitus than in patients without diabetes mellitus	[80]
2018	Ezetimibe-statin combination therapy was associated with greater cardiovascular benefits in patients with diabetes mellitus than in those without diabetes mellitus. The conclusion was that ezetimibe-statin combination therapy might be a useful strategy in patients with diabetes mellitus at a residual risk of major adverse cardiac events	[81]
2018	In IMPROVE-IT, the benefit of adding ezetimibe to statin was enhanced in patients with diabetes mellitus and in high-risk patients without diabetes mellitus	[82]
2017	The combined use of atorvastatin and ezetimibe was better than atorvastatin alone in reducing blood lipid levels and improving plague stability in patients with diabetes mellitus with ASCVD	[83]
2017	Ezetimibe could hold promise as an adjunctive, host-directed therapy for tuberculosis in patients with diabetes mellitus	[84]
2017	Among patients with T2DM, compared with the treatment with statins, the treatment with the combination of fenofibrate and ezetimibe effectively controlled LDL-C and triglyceride levels, increased HDL-C levels, and improved vascular function	[85]
2017	Among patients with T2DM, ezetimibe add-on therapy lowering LDL-C levels and improved attainment of LDL-C goals compared with the doubling of statin dose (i.e., atorvastatin 10 mg or pitavastatin 1 mg)	[86]
2016	Among patients with T2DM, progression of atherosclerosis is due to abnormalities in postprandial lipid metabolism; ezetimibe can potentially inhibit the aggravation of vascular endothelial dysfunction after high-fat diet loading	[87]
2016	High-intensity statin treatment is associated with a higher risk of incident diabetes in individuals with pre-diabetes; the addition of ezetimibe to statin therapy has a neutral effect on glucose metabolism	[88]

ACS acute coronary syndrome, *CVD* cardiovascular disease, *IMPROVE-IT* IMProved Reduction of Outcomes: Vytorin Efficacy International Trial, *PCSK9i* proprotein convertase subtilisin kexin 9 inhibitor, *T2DM* type 2 diabetes mellitus

[a] Derived from articles in a PubMed search of "diabetes" AND "ezetimibe" in the publication title from 2016 to 2021

Bile Acid Sequestrants

General Considerations

In addition to intestinal cholesterol absorption inhibitors such as ezetimibe, another class of gastrointestinal lipid-altering drugs are bile acid sequestrants (BAS), sometimes referred to as bile acid "resins." The most direct mechanism of action of BAS

Table 26.2 Summary of illustrative publications regarding colesevelam HCl and diabetes mellitus published in 2015–2021[a]

Year	Summary	Reference
2021	Colesevelam HCl possesses anti-glycemic properties, which could potentiate sulfonylurea or insulin-induced hypoglycemia	[90]
2020	Colesevelam HCl is a bile acid sequestrant, approved for the management of both dyslipidemia and type 2 diabetes, with limitations and precautions regarding its use	[91]
2018	Colesevelam HCl offers a clinically relevant combination of glucose and LDL lowering that in selected patients may be relevant as an add-on treatment to other glucose-lowering drugs and statins. Potential patients include those with renal impairment, and patients that are close to reaching their lipid and glycemic treatment goals but would benefit from further LDL-C and hemoglobin A1c reductions	[92]
2015	Among patients with T2DM, colesevelam HCl lowers hemoglobin A1c and LDL-C levels, although specific cardiovascular outcome studies are lacking with colesevelam HCl. Mechanisms regarding colesevelam's glucose-lowering effect in T2DM include increasing insulin sensitivity and secretion, incretin effects, changes in bile acid composition, and splanchnic sequestration of mealtime glucose. Colesevelam HCl reduces HbA1c in patients with T2DM ranging from 0.32 to 1.1% points. Colesevelam is generally well tolerated. Comparisons with cholestyramine suggest that it is better tolerated and has fewer gastrointestinal symptoms	[93]

[a] Derived from articles in a PubMed search of "diabetes" AND "ezetimibe" in the title of the publication title from 2015 to 2021

(e.g., cholestyramine, colestipol, and colesevelam HCl) is the binding of bile acids in the intestine. Because this effect is restricted to the gastrointestinal (GI) tract, BAS are considered nonsystemic agents, although they have metabolic effects beyond the GI tract [89]. BAS hold a special place in lipid-altering drug history; they were among the first lipid-lowering drugs that reduced cholesterol and improved ASCVD outcomes (Table 26.3) [89]. In fact, in 1988, before statins and other therapies became more established, the initial Expert Panel of the National Cholesterol Education Program listed BAS as a first treatment of choice for hypercholesterolemia (along with niacin). This was because BAS were generally safe with long-term use, and because studies that began in the 1970s supported BAS as reducing ASCVD risk [94].

One of the illustrative studies listed in Table 26.3 was the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT), which was a primary prevention trial evaluating cholestyramine administered over 7 years in 3806 men. In this study, cholestyramine reduced total cholesterol by 13%, reduced LDL-C by 20%, and reduced ASCVD death or nonfatal myocardial infarction by 19%. Unfortunately, 68% of study participants experienced adverse gastrointestinal experiences, with the average cholestyramine dose actually taken being 14 g/day (the study called for a dose of 24 g/day). The LRC-CPPT study was a landmark study in that it was one of the first ASCVD outcome studies to support the "cholesterol hypothesis," in that not only was an elevated cholesterol level contributive

Table 26.3 Examples of cardiovascular disease outcome trials of bile acid sequestrants

Clinical trial (year published)	Demographics	Duration (years)	Intervention	Lipid effect[a]	Results
ASCVD outcome study					
LRC-CPPT (1984) [95]	3806 men w/o ASCVD	7.4	Cholestyramine 24 g/day	LDL-C: −20.3% HDL-C: +1.6%	19% reduction in fatal and nonfatal MI in treated group
Angiographic studies					
NHLBI (1984) [96]	116 men and women with ASCVD	5	Cholestyramine 24 g/day	LDL-C: −26% HDL-C: +8%	Significant decreased progression in coronary artery lesions >50% stenosis at baseline
CLAS I (1987) [97]	162 men with CABG	2	Colestipol 30 g/day and niacin 4.3 g/day	LDL-C: −43% HDL-C: +37%	Significant regression, and decreased progression in treated group than placebo group
CLAS II (1990) [98]	103 men with CABG	4	Colestipol 30 g/day and niacin 4.2 g/day	LDL-C: −40% HDL-C: +37%	Significant regression, and decreased progression in treated group than placebo group
FATS (1990) [99]	38 men with coronary atherosclerosis and family history of CVD	2.5	Colestipol 30 g/day and lovastatin 40 mg/day	LDL-C: −46% HDL-C: +15%	Significant regression, decreased progression, and decreased ASCVD events compared to conventional therapy
FATS (1990) [99]	36 men with coronary atherosclerosis and family history of CVD	2.5	Colestipol 30 g/day and niacin 4 g/day	LDL-C: −32% HDL-C: +43%	Significant regression, decreased progression, and decreased ASCVD events compared to conventional therapy
UCSF-SCOR (1990) [100]	72 men and women with familial hypercholesterolemia	2	Colestipol, niacin, and lovastatin	LDL-C: −39% HDL-C: +26%	Mean within-patient change in percent area stenosis was significantly greater in diet than drug intervention group with the treatment group demonstrating mean regression and the diet group demonstrating mean progression

(continued)

Table 26.3 (continued)

Clinical trial (year published)	Demographics	Duration (years)	Intervention	Lipid effect[a]	Results
STARS (1992) [101]	90 men with ASCVD	3	Cholestyramine 16 g/day	LDL-C: −35.7% HDL-C: +4%	Change in mean absolute width of the coronary segment (MAWS) was decreased with dietary and dietary + cholestyramine intervention compared to control group. The change in MAWS was independently and significantly correlated with LDL-C levels. Both diet and diet + cholestyramine groups had significant regression, decreased progression, and decreased ASCVD events compared to "usual care" therapy

Recreated from Ref. [102]
CABG coronary artery bypass graft, *ASCVD* atherosclerotic cardiovascular heart disease, *CVD* cardiovascular disease, *CLAS* cholesterol-lowering atherosclerosis study, *FATS* familial atherosclerosis treatment study, *HDL-C* high-density lipoprotein cholesterol, *LCAS* lipoproteins in coronary atherosclerosis study, *LDL-C* low-density lipoprotein cholesterol, *LRC-CPPT* lipid research clinics-coronary primary prevention trial, *MI* myocardial infarction; *NHLBI* National Heart and Lung Blood Institute; *STARS* St. Thomas Arteriosclerosis Regression Study; *UCSF-SCOR* University of California, San Francisco Specialized Center of Research
[a] Values compared to baseline

to ASCVD, but also the LRC-CPPT demonstrated that a reduction of cholesterol could reduce ASCVD events. In retrospect, the poor compliance during the study was predictive of the challenges of its future use in clinical practice. BAS were poorly tolerated from a gastrointestinal standpoint (e.g., nausea, constipation, and other GI adverse experiences), high potential for drug interactions, and predominant administration via multiple daily scoops of sandy-textured drug. Thus, once statins were introduced, the use of BAS declined, representing a small fraction of the drugs utilized for the treatment of hypercholesterolemia. In fact, in some clinical practices, the most common current use for bile acid resins such as cholestyramine is for treatment of noninfectious chronic diarrhea, attributable to bile acid malabsorption [103, 104].

Colesevelam HCl

In the 1990s, colesevelam hydrochloride (HCl)was developed as a BAS with a unique polymer structure specifically designed to maintain lipid efficacy but improve BAS tolerability. Regarding efficacy and compared to placebo, early monotherapy trials demonstrated that six 625 mg colesevelam HCl tablets per day significantly reduced LDL-C levels 15–21%, increased HDL-C levels 3–9%, and increased triglyceride levels 2–16% [89]. Similarly, when added to statins, six 625 mg tablets/day of colesevelam HCl per day significantly reduced LDL-C 10–16%, increased HDL-C 3–7%, and increased triglyceride levels 5–23% compared with statin alone [89]. Regarding tolerability, in addition to the first-introduced tablet formulation, a subsequent "sugar-free" (phenylalanine) colesevelam HCl powder was developed wherein, as opposed to multiple scoops of sandy-textured drug (as was typical of cholestyramine), colesevelam HCl powder could be administered via one small 3.75 g packet of drug, once a day. In a BAS acceptability trial, 71% of study participants reported taste as being important for long-term compliance. In this controlled comparison study, those participating in the study found that the colesevelam HCl powder tasted significantly better than generic cholestyramine [105].

Pharmacokinetics, Safety, and Drug Interactions

Colesevelam hydrochloride is a hydrophilic, water-insoluble polymer. It is not hydrolyzed by digestive enzymes and is not absorbed with its distribution limited to the gastrointestinal tract. Colesevelam hydrochloride does not undergo systemic metabolism and does not interfere with systemic drug-metabolizing enzymes such as cytochrome P-450 (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022362s007lbl.pdf).

Colesevelam HCl is contraindicated in patients with a history of bowel obstruction, patients with triglyceride levels >500 mg/dL, and patients with a history of hypertriglyceridemia-induced pancreatitis (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022362s007lbl.pdf).

Bile acid sequestrants such as colesevelam HCl may decrease intestinal absorption of fat-soluble vitamins and should be used with caution in patients susceptible to fat-soluble vitamin deficiency; routine vitamin supplementation should be given at least 4 h before administration of colesevelam HCl. Colesevelam HCl is not recommended in patients at risk of bowel obstruction. With specific respect to diabetes mellitus, colesevelam HCL is not recommended in patients with gastroparesis or other gastrointestinal motility disorders (or a history of major gastrointestinal surgery). While colesevelam HCl likely has reduced potential for drug interactions compared to other bile acid resins such as cholestyramine and colestipol, it is still recommended drugs with a known interaction with colesevelam be administered at least 4 h prior to colesevelam HCl. If fact, because only a small minority of drugs

have undergone drug interaction studies with colesevelam HCl, it is probably wise to administer all concomitant drugs 4 h before colesevelam HCl (e.g., including but not limited to oral contraceptives and glyburide). Drugs having narrow therapeutic index should have drug levels monitored [e.g., phenytoin, International Normalized Ratio (INR) in patients receiving warfarin, thyroid-stimulating hormone (TSH)] in patients receiving thyroid hormone therapy, and cyclosporine levels] (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022362s007lbl.pdf).

The most common adverse reactions with colesevelam HCl are constipation, dyspepsia, and nausea. In diabetes mellitus trials, the overall incidence of hypoglycemia was 3.0% in colesevelam HCl-treated patients and 2.3% in placebo-treated patients (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022362s007lbl.pdf).

Colesevelam HCl: Cholesterol Lowering of Bile Acid Sequestration

While colesevelam HCl and ezetimibe are both gastrointestinal lipid-altering drugs, they are different drugs with different mechanisms of actions. Ezetimibe is administered as a single pill, while colesevelam HCl is administered as six pills per day, or one packet of colesevelam HCl powder per day. Ezetimibe inhibits cholesterol transporters, while colesevelam HCl binds bile acids. Ezetimibe is technically a systemic drug, in that it undergoes enterohepatic circulation, while colesevelam HCl is nonsystemic, in that the colesevelam HCl drug remains limited to the intestine, without systemic exposure [89]. But while these two agents do have differences, much of the physiology of the GI tract in lipid metabolism previously described with ezetimibe is similar and applicable to colesevelam HCl.

Cholesterol is converted into bile acids in the liver via the enzyme 7-alpha-hydroxylase. Bile acids are secreted into the biliary system, and then into the intestine, predominantly for the purpose of micelle formation and fat digestion. Over 95% of bile acids are transported to the terminal ileum, where they are then returned to the liver via enterohepatic recirculation. Once these bile acids are bound by BAS such as colesevelam HCl, these bile acids are excreted in the feces and do not undergo enterohepatic recirculation. The decreased bile acid return to the liver upregulates enzymes that increase the cholesterol catabolism to bile acids, resulting in a compensatory increase in hepatic LDL receptor activity, clearing LDL from the circulation, and reducing LDL-C levels [89]. Thus, the increase in LDL receptor activity is a mechanism shared by colesevelam HCl and ezetimibe (as well as other lipid-lowering drugs such as statins, proprotein convertase subtilisin kexin 9 inhibitors, and bempedoic acid). Finally, another similarity to ezetimibe is that colesevelam HCl was the first BAS to report reductions in C-reactive protein when added to statins, which is an effect most consistently reported in well-controlled trials of combination lipid-altering drug trials with statins [106].

Colesevelam HCl: Glucose Lowering of Bile Acid Sequestration

Another important difference between BAS such as colesevelam HCl and ezetimibe is that while ezetimibe has no effects upon blood glucose levels in humans, colesevelam HCl is a lowering agent for treatment of T2DM. In the years spanning the 1990s and 2000s, some smaller pilot studies consistently suggested that BAS may lower glucose and hemoglobin A1c levels in patients with T2DM (Table 26.4). Among patients with T2DM, colesevelam HCl lowers hemoglobin A1c and LDL-C levels, although specific cardiovascular outcome studies are lacking with colesevelam HCl. Subsequently, colesevelam HCl underwent a development program for the intent of being the first BAS to obtain an approval and indicated use as an anti-diabetes mellitus agent. This development program involved three pivotal trials as summarized in Table 26.5. Each of these trials evaluated colesevelam HCl added to a specified anti-diabetes drug regimen, which included metformin, insulin, or sulfonylurea-based therapies, either alone or in combination with other anti-diabetes mellitus drugs. These clinical trials demonstrated that colesevelam HCl consistently reduced fasting glucose levels approximately 13–15%, reduced hemoglobin A1c approximately 0.5–0.54%, and reduced LDL-C 12–17%. Regarding tolerability and safety, the only noteworthy differences in reported adverse experiences between placebo and colesevelam HCl were numerical increases in constipation and dyspepsia.

The manner by which BAS reduce glucose levels is largely unknown. Mechanisms include increasing insulin sensitivity and secretion, incretin effects, and splanchnic sequestration of mealtime glucose [93]. One might imagine that the most likely mechanism is related to the direct action of bile acid sequestrants, which is binding of bile acids and the complex interplay of nuclear receptors and other influencers of glucose metabolism [102].

Ezetimibe and Colesevelam HCl

One of the challenges for clinicians in achieving acceptable lipid treatment goals involves the care of patients with statin intolerance, with the most common reported statin intolerance being myalgias defined as muscle pain with or without elevated muscle enzymes [113]. In most clinical trials, statin-induced myalgias are reported in only about 5% of study participants. However, in other trials and clinical practice surveys, the reports of myalgias are widely variable, ranging between 0.3 and 33% [113]. Thus, having non-statin lipid-altering drug options is often useful in clinical practice.

In a multicenter, randomized, double-blind, parallel group study of patients with primary hypercholesterolemia, the colesevelam HCl plus ezetimibe combination (i.e., without statins) significantly reduced LDL-C levels by 32.3%. This

Table 26.4 Examples of clinical trials evaluating the effects of bile acid sequestrants upon glucose levels

Clinical trial	Demographics	Duration	Intervention	Lipid effect	Baseline HbA1c	Results at study end[b]
Garg A, Grundy SM [107] (1994)	20 men and 1 woman with T2DM	Crossover study; 6 weeks for each period	Cholestyramine 16 g a day	LDL-C = −28% HDL-C = no change TG = +13.5%	Not reported	FPG = −13% HbA1c = −0.5% (NS)
Yamakawa T, et al. [108] (2007)	70 men and women with T2DM	3 months	Colestimide 6 g per day or pravastatin 10 mg per day	LDL-C = −23%[a] HDL-C = −0.06% (NS) TG = +14%(NS)	7.7%	FPG = −8% HbA1c = −0.9%
Zieve FJ, Kalin MF, Schwartz SL et al. (GLOWS trial) [109] (2007)	65 men and women with T2DM	12 weeks	Colesevelam 3.75 mg/day[c]	LDL-C: −11.7% HDL-C: −1.5% (NS) TG: +7.8% (NS)	7.9%	HbA1c: −0.5% FPG: −14 mg/dL (NS) PPG: −31.5 mg/dL

Recreated from Ref. [102]

ASCVD atherosclerotic cardiovascular disease, *FPG* fasting plasma glucose, *GLOWS* glucose-lowering effects of WelChol Study, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *NS* not a statistically significant change, *OAD* oral anti-diabetes drugs, *PPG* two-hour postprandial glucose, *T2DM* type 2 diabetes mellitus, *TG* triglycerides

[a] Lipid and glucose values were colestimide-treated subjects compared to baseline

[b] FPG values represent percent change in glucose levels; HbA1c value reduction in HbA1c percent

[c] In addition to other OAD

Table 26.5 Prospective phase III clinical trials investigating the effects of colesevelam HCl upon glucose levels

Clinical trial	Demographics of total study participants	Duration	Intervention	Lipid effect	Baseline HbA1c	Results at study end[a]
Bays HE, et al. [110] (metformin ± OAD)	316 men and women with T2DM	26 weeks	Colesevelam HCl 3.75 mg/day[b]	LDL-C: −15.9% HDL-C: +0.9 TG: +4.7% (NS)	8.2%	HbA1c: −0.54% FPG: −13.9 mg/dL
Goldberg RB, Truitt K [111] (insulin ± OAD)	287 men and women with T2DM	16 weeks	Colesevelam HCl 3.75 mg/day[c]	LDL-C: −12.8% HDL-C: −0.9% (NS) TG: +21.5%	8.3%	HbA1c: −0.50% FPG: −14.6 mg/dL (NS)
Fonseca V, et al. [112] (sulfonylurea ± OAD)	461 men and women with T2DM	26 weeks	Colesevelam HCl 3.75 mg/day[d]	LDL-C: −16.7% HDL-C: +0.1% (NS) TG: +17.7%	8.2%	HbA1c: −0.54% FPG: −13.5 mg/dL

Recreated from Ref. [102]

FPG fasting plasma glucose, *NS* not a statistically significant change, *OAD* oral anti-diabetes drugs, *PPG* two-hour postprandial glucose, *T2DM* type 2 diabetes mellitus.

[a] FPG values represent percent change in glucose levels; HbA1c value reduction in HbA1c percent

[b] Study medication mean percent compliance was 93.3% in the colesevelam HCl group and 91.9% in the placebo

[c] Study medication mean percent compliance was 92.7% in the colesevelam HCl group and 94.5% in the placebo group

[d] Study medication mean percent compliance was 92.7% in the colesevelam HCl group and 90.8% in the placebo group

was in contrast to a reduction of 21.4% with ezetimibe alone. Also compared to ezetimibe monotherapy, colesevelam HCl plus ezetimibe significantly reduced total cholesterol, non-HDL-C, and apolipoprotein B and increased apolipoprotein A-I levels. Neither treatment regimen significantly increased median triglyceride levels compared with baseline, and both regimens were safe and generally well tolerated. The conclusion was that colesevelam HCl plus ezetimibe combination therapy significantly improved important lipid parameters compared to ezetimibe alone. Combining colesevelam HCl with ezetimibe may be a therapeutic option in hypercholesterolemic patients, such as those in whom statins are contraindicated and/or who may have intolerances to statin therapy [114].

Updated Clinical Research of Bile Acid Resins in Patients with Diabetes Mellitus

Table 26.2 summarizes illustrative research data from 2015 to 2021 regarding colesevelam HCl in patients with diabetes mellitus. General principles suggested from these studies include:

- Colesevelam HCl may increase the risk of hypoglycemia in patients treated with insulin or sulfonylureas.
- Among patients with T2DM, colesevelam HCl lowers hemoglobin A1c and lowers LDL-C levels. Colesevelam HCL cardiovascular outcome studies are lacking. Mechanisms regarding colesevelam's glucose-lowering effect in T2DM remain elusive, with potential applicable effects being increased insulin sensitivity and secretion, incretin effects, changes in bile acid composition, and splanchnic sequestration of mealtime glucose.
- Among patients with T2DM, colesevelam HCl generally reduces HbA1c about 0.5%, with a general range of 0.32–1.1%. Colesevelam HCl is generally well tolerated, and better tolerated than cholestyramine—especially regarding gastrointestinal side effects.

Conclusion

- Diabetes mellitus and/or metabolic syndrome are often associated with elevated triglyceride, very-low-density lipoprotein [and other triglyceride-rich lipoproteins and their remnants], small dense low-density lipoproteins (LDL), and increased apolipoprotein B, as well as decreased levels of high-density lipoprotein (HDL) and apolipoprotein A1 levels.
- Ezetimibe is a cholesterol absorption inhibitor, which primarily lowers LDL-C levels, which is the primary lipid treatment target to reduce ASCVD risk.
- Colesevelam HCl is a BAS that not only lowers LDL-C levels, but also reduces glucose levels in patients with type 2 diabetes mellitus.

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Chapter 27
Clinical Efficacy of Proprotein Convertase Synthase Kexin Type 9 Inhibition in Persons with Diabetes Mellitus

Peter P. Toth, Manfredi Rizzo, and Maciej Banach

Introduction

Type 2 diabetes mellitus (DM) is a widely prevalent metabolic disorder whose prevalence continues to rise throughout the world [1]. The insulin resistance underlying DM drives the development of a number of risk factors, such as atherogenic dyslipidemia, endothelial dysfunction and hypertension, hyperglycemia and activation of receptors of advanced glycated end products, increased central sympathetic outflow, as well as heightened systemic inflammatory, oxidative, and thrombotic tones [2]. The concerted action of these risk factors promotes progressive arterial injury and accelerated atherogenesis [3]. It is widely recognized that diabetic patients have significantly greater risk for developing atherosclerotic cardiovascular disease (ASCVD) than nondiabetic patients [4].

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Given the large number of clinical trials that support their use, the statins are designated as first-line agents for reducing the risk in patients with DM in guidelines promulgated around the world [5–8]. The statins reduce the risk for ASCVD-related events equally well in both diabetic and nondiabetic patients [9, 10]. Unfortunately, success rates among diabetic patients achieving their risk-stratified low-density lipoprotein cholesterol (LDL-C) goals are relatively poor despite the known safety and efficacy of the statins [11, 12]. Patients unable to attain their LDL-C goal can be treated with a variety of lipid-lowering agents that can significantly increase LDL-C goal attainment rates. These adjuvant therapies can reduce absorption of dietary and biliary cholesterol (ezetimibe) [13], bind bile acids (cholestyramine, colesevelam) [14], inhibit ATP citrate lyase (an enzyme that integrates fatty acid and carbohydrate metabolism) [15], or reduce the availability of proprotein convertase subtilisin/kexin type 9 (PCSK9) [16, 17]. This chapter is focused on PCSK9 and will explore its function, therapeutic approaches to inhibiting its activity, and its impact on atherogenic lipoprotein burden and ASCVD-related outcomes in patients with DM.

Proprotein Convertase Subtilisin/Kexin Type 9

A proprotein convertase proteolytically converts an inactive precursor enzyme to an active one by cleaving a region that either blocks the active site or allows for a change in three-dimensional conformation. This occurs, for example, when a proenzyme or zymogen is converted into a catalytically active enzyme ready to perform a specific function in intermediary metabolism. The PCSK family is comprised of 9 serine proteases, 8 of which are catalytically active immediately after production and convert receptors, transcription factors, hormones, and enzymes into their active species. The 9th member of the PCSK family has been only recently discovered and plays a critical role in lipoprotein metabolism. Once formed in the endoplasmic reticulum, its signal peptide is hydrolyzed to produce the zymogen proPCSK9 (Fig. 27.1). This zymogen exits the ER and enters the cytosol by catalyzing the autocleavage of its prosegment. This step eliminates any capacity for catalytic activity; the prosegment remains associated with PCSK9 and causes steric hindrance of PCSK9's active site. Hence, the only catalytic target of PCSK9 is its own prosegment.

Low-density lipoprotein particles (LDL-P) are predominately cleared from the circulation by LDL-R [19]. LDL receptors are expressed along the hepatocyte surface and are concentrated in clathrin-coated pits within cell membranes. Once an LDL-R binds an LDL-P, it is configured within the clathrin-coated pit by LDLR adaptor protein 1 (aka clathrin-associated sorting protein), though there is evidence that the disabled homolog adaptor protein 2 (dab-2) can also perform this role [20, 21]. An endosome forms and is covered with a clathrin polyhedral lattice

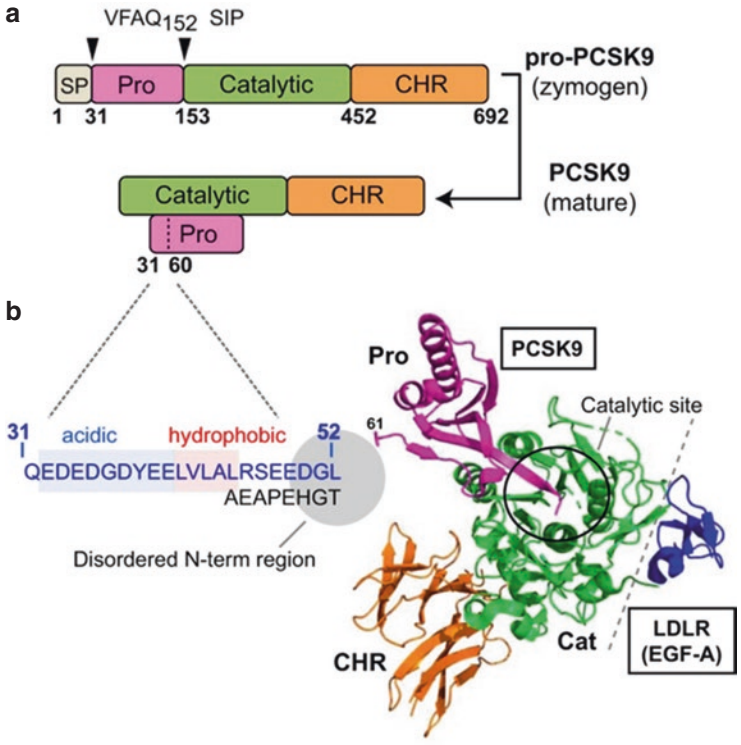

Fig. 27.1 PCSK9 structure with emphasis on disordered N-terminal region of the prodomain. (**a**) Following removal of a signal peptide (SP; aa 1–30; gray), human pro-PCSK9 undergoes autocatalytic cleavage after Gln-152, resulting in mature PCSK9 consisting of a prodomain (aa 31–152; magenta), catalytic domain (aa 153–451; green), and C-terminal CHR domain (aa 452–692; orange). (**b**) Crystal structure of PCSK9 in complex with the EGF-A domain of LDLR. The C-terminal end of the cleaved prodomain blocks the catalytic site (black circle), which is >20 Å from the binding interface with EGF-A (gray dashed line). An IDR in the N-terminus of the prodomain (aa 31–60) (shaded circle) is structurally disordered and unobserved in all PDB-deposited crystal structures of PCSK9. Highlighted in blue is the amino acid sequence of an N-terminal region (aa 31–52) required for binding to LDL particles. Sequences of interest within this region are a highly acidic tract (shaded blue) and adjacent hydrophobic region (shaded red). Figure and legend reproduced with permission from Sarkar et al. [18]. (This is an open-access article distributed under the terms of the Creative Commons CC-BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited)

[22] (Fig. 27.2). The endosome is released into the cytosol, the clathrin dissociates, and the internal milieu of the endosome is acidified. The drop in pH potentiates the dissociation of the LDLR from LDL-P. Through a mechanism that is yet to be defined, the LDL-P is specifically translocated into the lysosome for destruction by cathepsins and lipases, though it may also be rerouted for biliary clearance

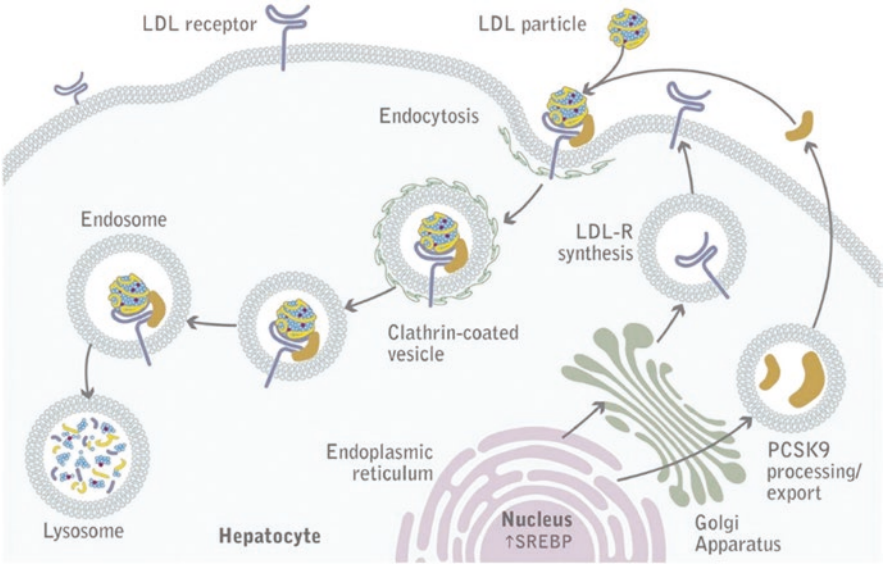

Fig. 27.2 PCSK9-mediated degradation of LDLR. A complex of LDL-C, LDLR, and PCSK9 is internalized into hepatocytes into clathrin-coated pits and subsequently undergoes lysosomal degradation. Figure and legend reproduced with permission from Lambert et al. [23]. (This is an open-access article distributed under the terms of the Creative Commons CC-BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited)

or conversion to bile salts via 7-α-hydroxylase. The LDLR is routed back to the hepatocyte cell surface to initiate another round of LDL-P uptake and catabolism (Fig. 27.3).

PCSK9 regulates the expression of LDLR on the surface of hepatocytes (Fig. 27.3). After secretion into the extracellular milieu, PCSK9 binds to the epidermal growth factor-like repeat A domain of LDLR [25]. LDLR bound to both an LDL-P and PCSK9 is concentrated in clathrin-coated endosomes. The endosomes dissociate from the cell membrane and carry LDLR-PCSK9-LDL-P complexes into the cytosol. The PCSK9 holds the LDLR and LDL-P tightly together, and they do not dissociate as the intra-endosomal pH decreases [26]. The PCSK9 chaperones the LDLR-LDL-P complex into the lysosome for proteolytic destruction. This results in fewer LDLRs circulating back to the cell membrane and reduced cellular capacity to engage in systemic LDL-P clearance.

Hepatocyte lipoprotein receptor physiology is complex. In addition to LDLR, PCSK9 also regulates cell surface expression of a number of other lipoprotein receptors, thereby impacting serum levels of multiple lipoproteins and their subfractions. PCSK9 regulates the expression of the very-low-density lipoprotein receptor (VLDLR), the apolipoprotein E2 receptor, and the LDL receptor-related protein-1, all of which participate in the clearance of various apo B-containing lipoproteins [27–30]. In addition, PCSK9 regulates the expression of cluster of differentiation 36

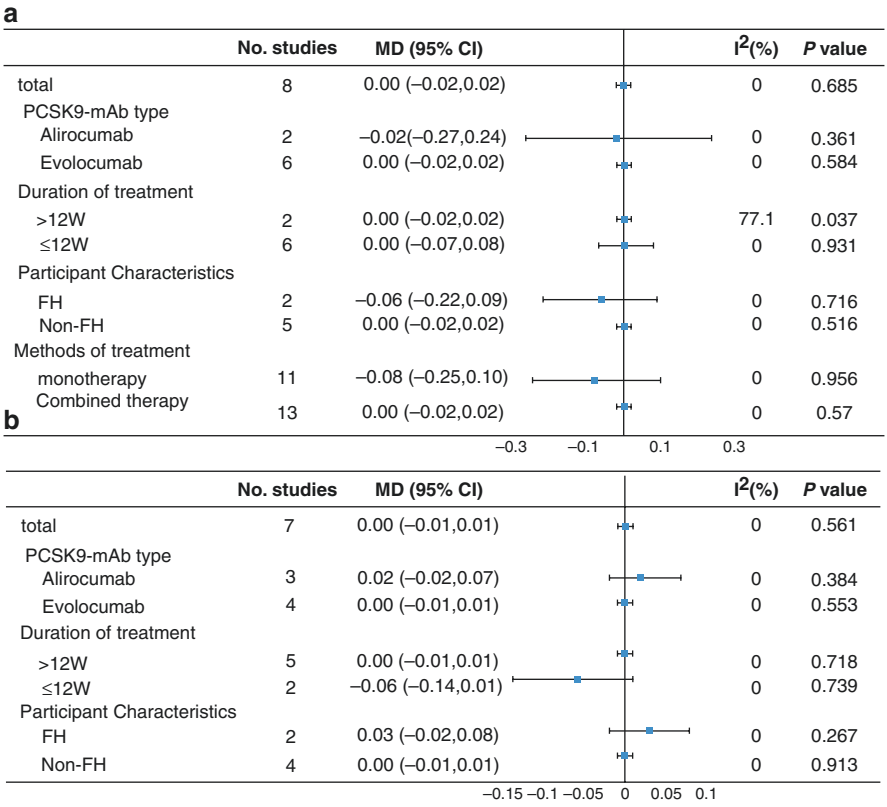

Fig. 27.3 Pooled analysis of effect of PCSK9 mAbs on (**a**) fasting plasma glucose and (**b**) glycated hemoglobin. Figure and legend reproduced with permission from Cao et al. [24]

(CD 36), a fatty acid translocator in hepatocytes and adipocytes [31]. The D374Y gain-of-function mutation suggests that PCSK9 upregulates Nieman-Pick C1-like protein (a cell membrane-based sterol translocator), without impacting the expression of SR-BI or ATP-binding membrane cassette transport proteins G5/G8 (ABCG5/G8) [32].

Therapeutic Approaches to Inhibiting PCSK9

Monoclonal Antibodies

A monoclonal antibody is a highly specific antibody directed toward a single molecular target (antigen) [33]. Since PCSK9 is a secreted protein and is active in the extracellular milieu, it can be targeted by a monoclonal antibody (mAb) in order to

neutralize its activity. Two fully human mAbs (evolocumab and alirocumab) targeting PCSK9 have been developed for treating hyperlipidemia. Making them fully human reduces the risk of both autoimmune responses and tachyphylaxis. These agents can be used independent of baseline hepatic and renal function because they have no dependence on hepatic uptake and metabolism for activation, and they do not require renal elimination for their clearance [34–36]. The antibody complexes formed between these agents and extracellular PCSK9 are removed from serum by the reticuloendothelial system (Kupffer cells in the liver, spleen, lymph nodes, bone marrow). The PCSK9 mAbs do not promote any drug interactions since they do not influence the activity of organic anion transport proteins, cytochrome P450 isozymes, or glucuronidation.

PCSK9 and Risk for Diabetes Mellitus

After many years of use, the statins were found to be modestly diabetogenic [37]. In the Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, rosuvastatin therapy was associated with a rise in risk in incident DM [38]. However, when compared to placebo, rosuvastatin therapy accelerated time to new-onset DM by only 5.5 weeks. In one meta-analysis, one would have to treat 1000 patients per year to see one new case of DM with low-dose statin therapy and 500 patients per year to observe one new case of DM with moderate- to high-dose statin therapy [39]. Risk for DM among statin-treated patients also has a strong dependence on the number of components of the metabolic syndrome a given patient has (i.e., the greater the number of components, the higher the risk) and whether or not they are already prediabetic [40]. A recent retrospective matched cohort study suggested that statin therapy was also associated with more rapid progression of diabetes (rising glucose, requiring greater number of drugs, progressing to the need for exogenous insulin) [37].

Given these observations, it has been of interest to investigate whether or not the inhibition of PCSK9 and the concomitant large reduction in LDL-C were associated with new-onset DM. In a Mendelian randomization study that included over 550,000 persons, the impact of *PCSK9* variants (rs11583680, rs11591147, rs2479409, and rs11206510) scaled to 1 mmol/L lower LDL-C showed associations with increased fasting glucose (0.09 mmol/L, 95% CI 0.02–0.15), body weight (1.03 kg, 0.24–1.82), waist-to-hip ratio (0.006, 0.003–0.010), and an odds ratio for type diabetes of 1.29 (1.11–1.50). Clearly, the association with diabetes is substantial.

A number of studies have been performed on patients treated with PCSK9 mAbs in an effort to discern if a signal between treatment with these agents and new-onset DM is detectable. In a pooled analysis of ten clinical trials performed with alirocumab including 4974 participants, the hazard ratio (HR) associated with transition from prediabetes to new-onset DM was 0.90 (0.63–1.29) vs. placebo and 1.10 (0.57–2.12) vs. ezetimibe. Mean change in fasting plasma glucose and HgbA1c

showed no difference between treatment groups in patients without diabetes over the follow-up period [41]. Among 4802 patients treated with either evolocumab or standard of care for 1 year, there was no difference in fasting plasma glucose or HgbA1c values between groups. In the ODYSSEY Outcomes trial, when measured over a median follow-up period of 2.8 years, alirocumab did not increase the risk of new-onset DM (HR 1.00, 95% CI 0.89–1.11). Among patients without diabetes at baseline, 676 (10.1%) developed diabetes treated with placebo, compared with 648 (9.6%) treated with alirocumab [42]. In the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) trial, evolocumab therapy did not increase the risk for new-onset DM compared to placebo among participants with established ASCVD [43]. In addition, among participants with established metabolic syndrome in the FOURIER trial, evolocumab did not potentiate the transition to new-onset DM [44]. Representative data for evolocumab are presented in Table 27.1. Evolocumab does not cause disturbances in fasting plasma glucose, HgbA1c, C-peptide, serum insulin, HOMA-B cell function, or HOMA-Insulin Resistance in study participants who were normoglycemic or had metabolic syndrome and impaired fasting glucose or were diabetic [45]. Similar data are available for alirocumab [46].

Table 27.1 Impact of evolocumab on glycemia-related parameters after 52 weeks of therapy. Reproduced with permission from [45]

	Type 2 diabetes Pbo (N = 43) vs EvoMab (N = 77)	IFG Pbo (N = 99) vs EvoMab (N = 194)	MetS Pbo (N = 107) vs EvoMab (N = 182)	No Dysglycaemia or MetS Pbo (N = 119) vs EvoMab (N = 274)
Glycaemic Parameters				
HbA1c				
Tx difference vs Pbo, median change from baseline (SE)[a]	0.10 (0.10)	0.00 (0.05)	0.00 (0.03)	0.00 (0.03)
FPG				
Tx difference vs Pbo, median change from baseline (SE)[a]	−0.11 (0.21)	0.00 (0.07)	−0.06 (0.06)	0.06 (0.06)
Insulin				
Tx difference vs Pbo, median change from baseline (SE)[a]	−7.2 (14.6)	0.0 (7.3)	0.0 (5.5)	−7.2 (5.5)
C-Peptide				
Tx difference vs Pbo, median change from baseline (SE)[a]	0.1 (0.1)	0.0 (0.04)	0.0 (0.04)	0.0 (0.03)
HOMA_%B				
Tx difference vs Pbo, median change from baseline (SE)[a]	5.5 (5.7)	−1.3 (3.5)	−1.7 (3.9)	−2.3 (4.2)
HOMA_IR				
Tx difference vs Pbo, median change from baseline (SE)[a]	−0.2 (0.3)	0.0 (0.1)	−0.1 (0.1)	−0.1 (0.1)

Abbreviations: *EvoMab* **evolocumab;** *FPG* **fasting plasma glucose;** *HbA1c* **glycated hemoglobin; HOMA_%B (β-cell function) and HOMA_IR (insulin resistance) calculated using the HOMA2 model;** *IFG* **impaired fasting glucose;** *LS* **least squares;** *MetS* **metabolic syndrome;** *Pbo* **placebo;** *SE* **standard error;** *tx* **treatment;** *LDL-C* **ultracentrifugation low-density lipoprotein cholesterol**

[a] Difference in median and SE calculated using Hodges-Lehmann method. *P* values were calculated using the Wilcoxon rank-sum test. All *P* values for all glycemic parameters were nonsignificant ($P > 0.05$)

[b] $P \leq 0.001$ vs. placebo

[c] $P \leq 0.05$ vs. placebo

Cao et al. performed a meta-analysis of 18 studies which included 26,123 participants without diabetes treated with either evolocumab or alirocumab. No significant difference was observed in the PCSK9-mAb treatment groups including new-onset DM (RR 1.05, 95% CI 0.95–1.16), fasting plasma glucose (MD 0.00 mmol/L, 95% CI −0.02 to 0.02), or HbA1c (MD 0.00% [0 mmol/L], 95% CI −0.01 to 0.01) compared with control groups (Fig. 27.3). In addition, neither treatment duration nor magnitude of LDL-C reduction correlated with a rise in risk for new-onset DM. Sensitivity analyses did not change the results. Another meta-analysis by de Carvalho et al. included 20 studies (68,123 participants) of patients treated with mAbs over a median follow-up of 78 weeks. PCSK9i increased fasting blood glucose (weighted mean difference 1.88 mg/dL [95% CI 0.91–2.68]; $P < 0.001$) and HbA1c (0.032% [0.011–0.050]; $P < 0.001$) when compared with placebo. These changes in glucose and HbA1c were not large enough to increase the incidence of diabetes (RR 1.04 [0.96–1.13]; $P = 0.427$). It is possible that it will require very large numbers of patients to detect a signal for heightened hazard for new-onset DM with the PCSK9 mAbs. Surveillance of this issue continues.

Both of the PCSK9 mAbs have the capacity to reduce serum levels of lipoprotein(a) [Lp(a)] by about 20–30% [47, 48]. The reduction in Lp(a) with the PCSK9 mAbs is large enough to contribute to overall risk reduction noted in their respective cardiovascular outcome trials [49, 50]. Prospective longitudinal cohorts and some clinical trials have demonstrated a relationship between low levels of Lp(a) and risk of developing DM [51–53]. The precise mechanistic basis for this remains unknown. In the ODYSSEY Outcomes trial, alirocumab decreased Lp(a) by a median of 23.2% with greater absolute reductions at higher baseline levels and no effect on incident DM (hazard ratio 0.95, 95% CI 0.85–1.05) [54]. At low baseline Lp(a) levels, alirocumab exhibited a trend for reducing incident DM; in contrast, with high baseline Lp(a), alirocumab was associated with a trend for increasing incident DM compared to placebo (treatment-baseline Lp(a) interaction $P = 0.006$). In the alirocumab treatment group, a 10 mg/dL reduction in Lp(a) from baseline correlated with an HR of 1.07 (95% CI 1.03–1.12; $P = 0.0002$) for incident DM. Although reductions in Lp(a) correlated with decreases in risk for major acute cardiovascular events, it may also correlate with increased risk for incident DM. We clearly require longer term outcome data to quantify the risk of new-onset DM more precisely with Lp(a) reduction when using PCSK9 mAbs.

Impact of PCSK9 mAbs on Serum Lipids in Persons with Diabetes Mellitus

The PCSK9 mAbs induce substantial reductions in atherogenic lipoprotein burden in serum. When evaluating the efficacy of alirocumab for reducing LDL-C, non-HDL-C, and apo B, the reductions in these species were comparable between diabetic patients who were and were not insulin dependent [55] (Fig. 27.4). The addition

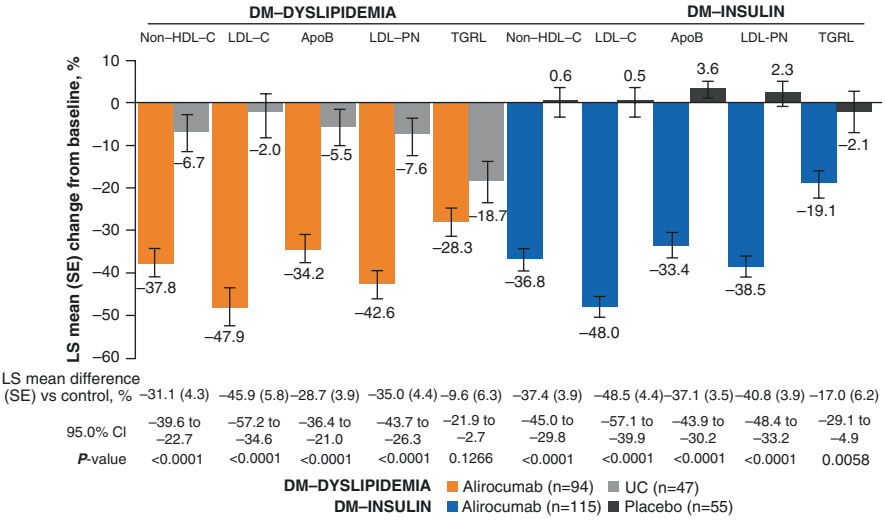

Fig. 27.4 Percentage change from baseline to week 24 in non-HDL-C, LDL-C, apo B, LDL-PN (ITT). *Apo* apolipoprotein, *HDL-C* high-density lipoprotein cholesterol, *ITT* intent to treat, *LDL-C* low-density lipoprotein cholesterol, *LDL-PN* low-density lipoprotein particle number, *LS* least squares, *SE* standard error, *UC* usual care. Figure and legend reproduced with permission from Ray et al. [55]. (**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided that you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated)

of alirocumab to standard-of-care therapy for dyslipidemia in diabetic patients increases goal attainment rates by two- to threefold for LDL-C, LDL particle number, non-HDL-C, and apo B in diabetic patients who do and do not require exogenous insulin [55] (Fig. 27.5). Both alirocumab and evolocumab induce nearly identical changes in LDL-C, non-HDL-C, apo B, and Lp(a) when comparing diabetic patients to nondiabetic patients [56] (Table 27.2). Incremental reduction of atherogenic lipoprotein burden is robust, safe, and sustained in both T1DM and T2DM.

The PCSK9 mAbs Reduce the Risk for ASCVD Events in Patients with Diabetes Mellitus

In the FOURIER trial, patients with a prior history of myocardial infarction (MI), stroke, or peripheral arterial disease were randomized to either evolocumab or placebo in addition to statin background therapy for a median of 2.2 years. There were

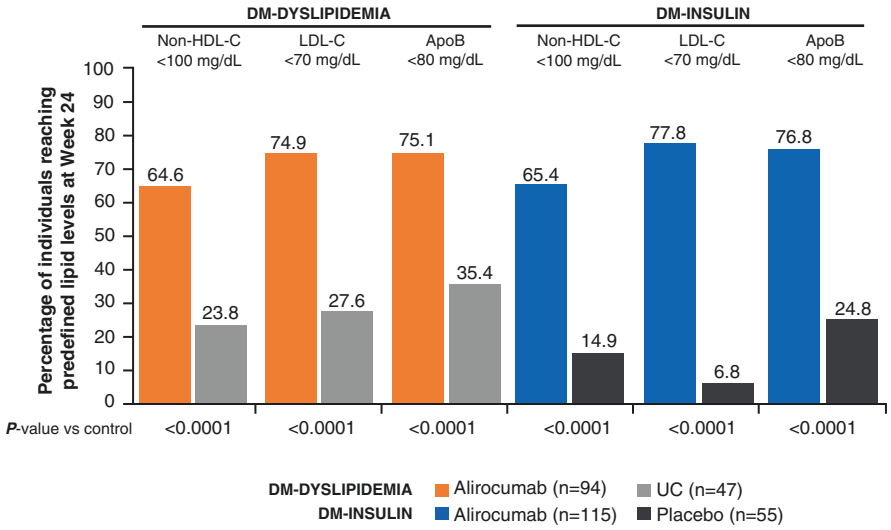

Fig. 27.5 Percentage of individuals achieving non-HDL-C, LDL-C, and apo B targets at week 24 (ITT). Non-HDL-C: 100 mg/dL = 2.59 mmol/L; LDL-C: 70 mg/dL = 1.81 mmol/L. *Apo* apolipoprotein, *HDL-C* high-density lipoprotein cholesterol, *ITT* intent to treat, *LDL-C* low-density lipoprotein cholesterol, *UC* usual care. Figure and legend reproduced from Ray et al. [55]. (**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided that you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated)

11,031 patients (40%) with DM and 16,533 without DM [65]. Among the patients without DM at baseline, 10,344 had prediabetes and 6189 were normoglycemic. The primary endpoint was a composite of cardiovascular death, MI, stroke, hospital admission for unstable angina, and coronary revascularization. The secondary endpoint was a composite of cardiovascular death, MI, and stroke. Hazard ratios for the primary endpoint were 0.83 (95% CI 0.75–0.93; $P = 0.0008$) for participants with DM and 0.87 (0.79–0.96; $P = 0.0052$) for participants without DM (P for interaction = 0.60) (Fig. 27.6). Hazard ratios for the secondary endpoint were 0.82 (0.72–0.93; $P = 0.0021$) for those with DM and 0.78 (0.69–0.89; $P = 0.0002$) for those without DM (P for interaction = 0.65). Evolocumab did not potentiate the risk of new-onset DM in participants without DM at baseline (HR 1.05, 0.94–1.17) nor in those with prediabetes (HR 1.00, 0.89–1.13).

The ODYSSEY Outcomes trial evaluated the efficacy and diabetogenicity of alirocumab in over 18,000 patients with a history of an acute coronary syndrome [42]. The primary endpoint was a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospital admission. Among patients without DM at baseline, 676 (10.1%)

Table 27.2 Impact of PCSK9 therapies on serum levels of atherogenic proteins. Extracted with permission from [56]

Clinical trial [1]	PCSK9 treatment	Number of patients	Follow-up (weeks)	LDL-C (%)	Non-HDL-C (%)	Apo B (%)	Lp(a) (%)
ODYSSEY-DM-INSULIN [57]	Alirocumab	429 T2DM	24	−48.2	−37.9	−33.4	−19
ODYSSEY COMBO II [58]	Alirocumab	225 T1DM and T2DM	24	−49.1	−40.8	−39.1	−28.1
		495 Normoglycemic		−51.2	−42.7	−41.4	−27.7
POOLED ANALYSIS OF 5	Alirocumab	836 T2DM	24	59.9	−49.1	−49.6	−28.5
Phase 3 ODYSSEY TRIALS [59]		1500 Normoglycemic		−60.6	−52.2	−53.5	−29.3
ODYSSEY JAPAN [60]	Alirocumab	148 DM	24	−63.1	−54.6	−53.3	−42.1
		68 Normoglycemic		−60.8	−54.7	−56.1	−42.7
POOLED ANALYSIS OF 9	Alirocumab	522 T1DM AND T2DM	24	−61.5	−50.8	−51.1	−30.1
PHASE 3 ODYSSEY TRIALS [61]		With ASCVD					
ODYSSEY-DM-DYSLIPIDEMIA [62]	Alirocumab	409 T2DM with dyslipidemia	24	−41.6	−37.3	−33.8	−23.7
ODYSSEY LONG-TERM [63]	Alirocumab	403 T2DM with dyslipidemia	24	−56	−49.2	−50.2	−27.9
		409 T2DM without dyslipidemia		−53.9	−47.8	−49.1	−27.9
POOLED ANALYSIS OF 3	Evolocumab	304 T2DM	12	−60	−54		−31
PROFICIO TRIALS [64]		1700 Normoglycemic		−66	−58		−29
DESCARTES [45]	Evolocumab	120 T2DM	52	−50.8		−38	−18.5
		393 Normoglycemic		−58.1		−45	−24.6
FOURIER [65]	Evolocumab	11,301 DM	48	−57	−49.5	−44.8	−26.9
		16,533 Non-DM		−60	−52.5	−47	−26.9
ORION-1 [66]	Inclisiran	67 DM	24	−48.3	−41.6	−37.8	−26.1
		415 Non-DM		−43.6	−37.9	−34	−15.6

Abbreviations: *ASCVD* atherosclerotic cardiovascular disease; *ODYSSEY* phase 3 clinical trial development program for alirocumab; *PROFICIO* program to reduce LDL-C and cardiovascular outcomes following inhibition of PCSK9 in different populations; *T1DM* type 1 diabetes mellitus; *T2DM* type 2 diabetes mellitus

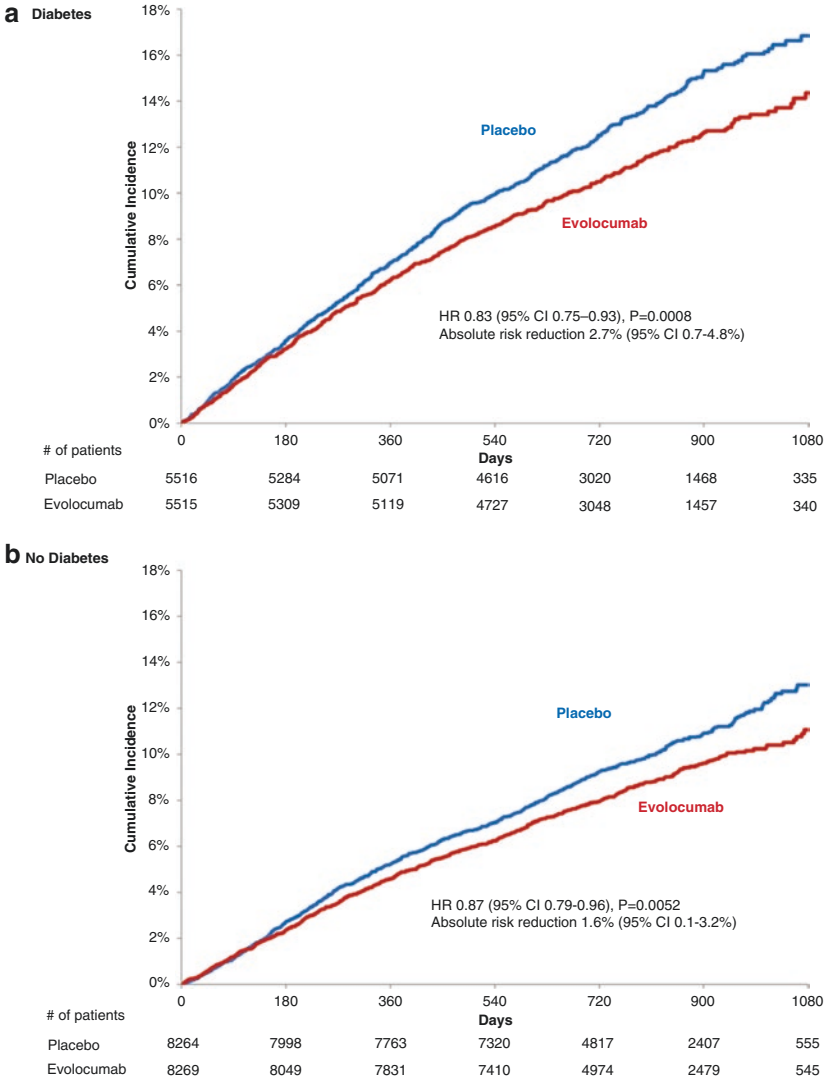

Fig. 27.6 Cumulative event rates for the primary endpoint (the composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization) in the evolocumab and placebo treatment arms, for patients with (panel **a**) and without diabetes (panel **b**). *P* values were calculated using log-rank tests. Hazard ratios and 95% CI are from a Cox model. Figure and legend reproduced with permission from Sabatine et al. [65]

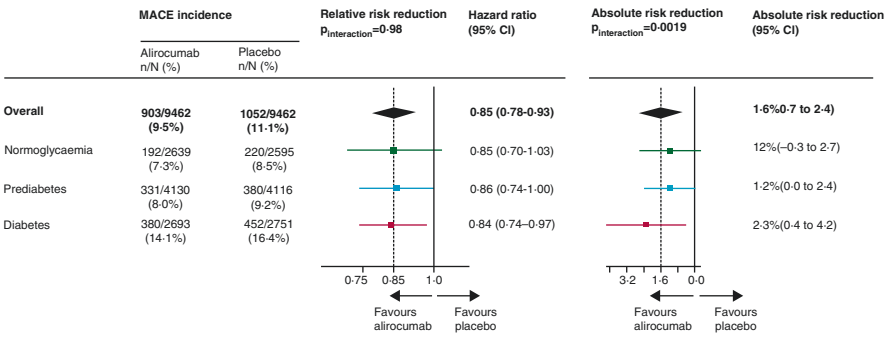

Fig. 27.7 Relative and absolute risk reduction with alirocumab, by baseline glycemic status. Median follow-up was 2.8 years (IQR 2.3–3.4). *MACE* major adverse cardiovascular events. Figure and legend reproduced with permission from Ray et al. [42]

developed DM in the placebo group, compared with 648 (9.6%) in the alirocumab group. Alirocumab did not increase the risk of new-onset diabetes (HR 1.00, 95% CI 0.89–1.11). There was uniform benefit across participants who were normoglycemic, had prediabetes, or had DM with an approximately 15% relative risk reduction in the primary composite endpoint over a median of 2.8 years of follow-up (Fig. 27.7).

Gene Silencing and PCSK9

A rapidly evolving field of highly innovative pharmacologic therapeutic agents are single-stranded and double-stranded ribonucleic acid (ssRNA and dsRNA, respectively) oligonucleotides that inhibit or silence the translation of specific gene products. RNA silencing is an ancient protective mechanism widely adopted by both prokaryotes and eukaryotes to defend against parasitic RNA sequences introduced into cells [67]. This highly evolved and conserved pathway is being harnessed with modified oligonucleotides to inhibit the translation of specific messenger RNAs (mRNA) into their respective gene products. Mipomersen is an example of a ssRNA oligonucleotide [68]. Mipomersen enters hepatocytes and binds to a complementary nucleotide sequence according to Watson-Crick base pairing with the mRNA for apo B. The dsRNA is hydrolyzed and inactivated by an RNase. This interrupts mRNA translation along the ribosome and leads to reduced apo B production [69]. Inclisiran is an example of a dsRNA interfering or "silencing" RNA (siRNA) [70].

DNA replication and transcription are precisely regulated processes within the nucleus of a cell. However, it is clear that gene expression is also regulated by microRNAs and siRNAs that inhibit or silence gene/mRNA expression post-transcriptionally [71]. Interfering RNAs are 20–30 nucleotides long and are comprised of both an antisense strand that is complementary to a target sequence in the

mRNA for a specific gene and a passenger strand [72]. The antisense strand is used to inhibit mRNA translation [73]. This, however, requires complex molecular machinery. The antisense strand is incorporated into the RNA-induced silencing complex (RISC). The RISC is a molecular complex used by cells to silence the expression of virtually any gene by three different mechanisms: (1) interrupting mRNA translation, (2) promoting mRNA degradation, and (3) promoting the formation of heterochromatin or even inducing DNA elimination [71]. The antisense strand binds to an Argonaute protein, which aligns the antisense strand with a target mRNA so that it can form a complementary Watson-Crick double helix. Glycine-tryptophan protein of 182 kDa (GW182) promotes both translational suppression and recruitment of CCR4–NOT deadenylase complex 4 which, in tandem with the Argonaute protein, hydrolyzes the RNA complex by RNase activity [73].

Inclisiran is a novel gene silencing technology that inhibits the translation of PCSK9 mRNA leading to the reduction of PCSK9 in both the intra- and extracellular compartments of the hepatocyte. Inclisiran is specifically targeted to hepatocytes by being covalently bound to triantennary *N*-acetylgalactosamine [74]. This conjugation promotes high-affinity binding of inclisiran to asialoglycoprotein receptors on the hepatocyte surface [75]. In the Trial to Evaluate the Effect of Inclisiran Treatment on Low Density Lipoprotein Cholesterol (LDL-C) (ORION-1) trial, inclisiran induced a dose-dependent reduction in serum LDL-C. Inclisiran dosed at 300 mg SQ on days 1 and 90 induced the following reductions by day 180 compared to baseline and placebo: LDL-C 52.6% ($P < 0.001$), non-HDL-C 46% ($P < 0.001$), triglycerides 14.2% ($P < 0.05$), VLDL 16% ($P < 0.01$), apo B 40.9% ($P < 0.001$), Lp(a) 25.6%, and PCSK9 69% ($P < 0.001$) [76]. On this regimen, 48% of participants achieved an LDL-C <50 mg/dL and 66% achieved an LDL-C <70 mg/dL.

Because of its pharmacokinetic profile and mechanism of action, inclisiran can be dosed every 6 months and induce stable reductions in LDL-C [77]. Inclisiran provides identical levels of LDL-C-reducing capacity in both diabetics and nondiabetics [66]. The clinical efficacy for reducing cardiovascular events by inclisiran is being evaluated in the ORION-4 trial, which includes approximately 15,000 patients 55 years of age or older with established ASCVD [78].

Conclusions

1. PCSK9 is an important regulator of LDLR as well as LDL particle uptake and catabolism.
2. PCSK9 impacts serum levels of multiple lipoprotein species and their subfractions by impacting the expression of multiple members of the LDLR family.
3. PCSK9 monoclonal antibodies (evolocumab and alirocumab) reduce LDL-C increase risk-stratified goal attainment rates for LDL-C, apo B, and non-HDL-C.
4. The PCSK9 mAbs have an excellent safety profile and are well tolerated.

5. The PCSK9 mAbs impact the risk for CV events significantly when used in combination with statins. The risk for MI, stroke, and need for revascularization are all significantly reduced. There is no increase in risk for hemorrhagic stroke with these agents.
6. The reduction in Lp(a) by the PCSK9 mAbs contributes to ASCVD risk reduction.
7. Inclisiran suppresses the translation of PCSK9 mRNA and provides substantial capacity for reducing LDL-C as well as VLDL, apo B, and non-HDL-C. Its unique mechanism of action allows for dosing this medication twice per year.
8. Inclisiran therapy is safe.
9. Neither the PCSK9 mAbs nor inclisiran are diabetogenic. Both the PCSK9 mAbs and inclisiran reduce atherogenic lipoprotein burden in serum to equivalent degrees when comparing persons with and without DM.

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Chapter 28
Clinical Care of Lipids in People with Type 1 Diabetes

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Background

Globally, the incidence and prevalence of type 1 diabetes (T1DM) vary widely, but this currently incurable organ-specific autoimmune condition exists in all countries, accounting for approximately 5–10% of all people with diabetes in high-incidence regions [1]. Whilst commonly regarded as a condition of childhood onset, T1DM can commence at any age. It is estimated that up to one half of people with T1DM will develop it as an adult [2]. Relative to childhood-onset T1DM, adult-onset T1DM is associated with lower HLA-associated risk, lower genetic risk scores, fewer diabetes-associated autoantibodies (though anti-GAD antibodies are prominent at all ages of onset), slower progression, higher residual C-peptide at diagnosis, and less frequent diabetic ketoacidosis (DKA) [2]. Nevertheless, all people with T1DM are at risk of macrovascular and microvascular complications and acute

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metabolic disturbances, including fluid and electrolyte imbalances, DKA, hypoglycemia, and dyslipidemia. The prognosis of T1DM varies greatly depending on the level of knowledge and experience, resources, and skills available to support the person living with T1DM. Many people in advantaged regions live a full life span, with 50, 60, 70, or more years of T1DM, yet irrespective of their age of T1DM onset, the commonest cause of death in adults with T1DM is atherosclerotic cardiovascular disease (ASCVD) [3].

People with T1DM have a significantly increased risk of morbidity and mortality from ASCVD, defined as coronary heart disease, cerebrovascular disease, or peripheral arterial disease [4–7]. Younger age at diagnosis of T1DM is associated with a significantly increased lifetime risk of ASCVD, where people diagnosed before the age of 10 years have a 30-fold increased risk of coronary heart disease and loss of approximately 16 years of life [8]. In addition, ASCVD disproportionally affects women with T1DM, who have approximately 40% greater excess risk of mortality and twice the excess risk of ASCVD events compared with men with T1DM [9]. Intensive glycemic control can significantly reduce the risk of microvascular disease (retinopathy, nephropathy, and neuropathy) and of ASCVD, based on data from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study [10–14]. Metabolic memory exists for glycemic control and likely also for lipid control [15], whereby the body continues to respond to good or suboptimal risk factor levels for many years after the risk factor levels have worsened or improved. However, an excess risk of death from ASCVD remains even despite optimal glycemic control, and thus early risk assessment and aggressive risk factor control are essential for reducing the burden of ASCVD (and other chronic complications) in people with T1DM [16–21]. It is important to address multiple risk factors to reduce complications, including glycemia, smoking, hypertension, adiposity, physical inactivity, and dyslipidemia.

Dyslipidemia is a modifiable ASCVD risk factor contributing to atherosclerosis, which begins earlier and progresses faster in people with T1DM [22–24]. Lipoprotein profiles in people with well-controlled T1DM can appear similar to people without diabetes, whilst poor glycemic control is associated with abnormal profiles [25–27]. The typical lipoprotein profile of well-controlled T1DM, relative to nondiabetic subjects, is characterized by low triglycerides and elevated high-density lipoprotein cholesterol (HDL-C) due to increased lipoprotein lipase activity from hyperinsulinemia related to subcutaneous insulin administration [28, 29]. However, chronic hyperglycemia, and nephropathy and insulin resistance, which are exacerbated by obesity, can result in pro-atherogenic lipoprotein profiles such as smaller and denser low-density lipoprotein (LDL) particles, elevated triglyceride-rich lipoproteins, and dysfunctional HDL particles [27, 30–32]. In addition, qualitative changes such as nonenzymatic glycation, oxidation, glycoxidation, and immune-complex formation may also aggravate lipoprotein pathogenicity in T1DM. Elevated HDL-C in people with T1DM has been considered cardioprotective; however, increasing evidence indicates deleterious effects on the vasculature due to qualitative/functional changes [31, 33, 34]. Furthermore, although improved glycemic control can improve the lipoprotein profile and reduce the risk of ASCVD

in people with T1DM, further lipid lowering such as by lifestyle and by lipid-lowering drugs may still be required as the risk of mortality and ASCVD is still more than twice that of the general population [35–37], and even more so in those who develop T1DM below 10 years of age [5].

The Cholesterol Treatment Trialists Collaboration demonstrated in a meta-analysis of 14 randomized controlled trials that reducing LDL-C with statin therapy can effectively and safely reduce the risk of ASCVD in people with diabetes in both the primary and secondary prevention setting [38]. Of 18,686 people with diabetes included in the meta-analysis, the reduction in major vascular events for the 1466 people with T1DM was similar to that for people with type 2 diabetes mellitus (T2DM) [38]. For every 1 mmol/L (40 mg/dL) reduction in LDL-C, the risk of major vascular events was reduced by an estimated 21% [38]. Importantly, people with diabetes derive greater absolute benefits from LDL-C lowering owing to their higher absolute baseline risk of ASCVD compared with people without diabetes [39].

Although observational data in people with T1DM demonstrate that LDL-C is a significant predictor of ASCVD and mortality, and that lipid-lowering therapy is associated with a reduction in the risk of ASCVD, there remains a relative paucity of T1DM-specific clinical trial data to inform guidelines [40–42]. As such, determining when to initiate statin or other lipid-lowering therapy remains a challenge, especially in younger adults with T1DM [20, 43]. It is unlikely that many large T1DM-specific studies will be conducted for many lipid drugs, but information may be gained from T1DM subgroups of trials and from analyses of "real-world" data such as registries.

Guideline Recommendations for Lipid Lowering

Recommendations from international guidelines for lipid lowering in diabetes are predominantly extrapolated from randomized trials in people with T2DM and expert opinion [39]. However, despite differences in underlying pathophysiology and ASCVD risk factors, a similar approach to LDL-C lowering in people with T2DM is suggested for T1DM [44]. Recommendations from select international guidelines for lipid screening are summarized in Table 28.1 [44–48]. Healthy lifestyle changes focusing on medical nutrition therapy, physical activity and weight loss, abstinence from excessive alcohol and from smoking, exclusion of secondary causes of dyslipidemia, and optimizing glycemia are fundamental in lipid management [39].

Table 28.2 summarizes recommendations from select international guidelines that focus on thresholds for initiating LDL-C-lowering therapies [44–47, 49]. High-intensity statin therapy (Table 28.3) is indicated for people with T1DM and established ASCVD, irrespective of lipid levels [44, 49]. Additional therapies such as ezetimibe or proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibitors may be required to further reduce LDL-C in people with established ASCVD who are

Table 28.1 Summary of recommendations from select guidelines for lipid screening in people with type 1 diabetes

Guideline	Screening	Ongoing assessment
ADA 2021	In adults with T1DM not on lipid-lowering therapy, it is reasonable to obtain a lipid profile at the time of diabetes diagnosis or at initial medical evaluation	• Obtain a lipid profile every 5 years if not on lipid-lowering therapy and age <40 years, or more frequently if indicated • In younger people with T1DM and longer duration of diabetes, more frequent lipid profiles may be reasonable • Obtain a lipid profile before initiation of lipid-lowering therapy, 4–12 weeks after initiation or a change in dose, and annually thereafter
	In children with T1DM, lipid testing should be performed when glycemic control has been achieved and age is ≥2 years	• If initial LDL-C is ≤2.6 mmol/L (100 mg/dL), subsequent testing should be performed at 9–11 years of age and then repeated every 3 years thereafter
AHA 2019 (Pediatrics)	Once diagnosed with T1DM, screen yearly for lipid disorders with non-fasting non-HDL-C, followed by fasting lipid profile if total cholesterol >5.2 mmol/L (200 mg/dL), HDL-C <1.2 mmol/L (45 mg/dL), or non-HDL-C >3.8 mmol/L (145 mg/dL)	–
ISPAD 2018 (Pediatrics)	Screen for dyslipidemia in the non-fasting state soon after T1DM diagnosis (when glycemia is stabilized) in all children age ≥11 years. If there is a family history of hypercholesterolemia or early ASCVD, or if family history is unknown, screening at age 2 years	• If normal results are obtained, lipid profiles should be repeated every 5 years
AACE/ACE 2017	Annually screen all adult individuals with T1DM for dyslipidemia with fasting lipid profiles	• Reassess lipids 6 weeks after initiating therapy and again at 6-week intervals until the treatment goal is achieved • Whilst on stable lipid-lowering therapy, test lipids at 6–12-month intervals, or more frequently if required[a]

Adapted from Ref. [44–48]

Abbreviations: *AACE* American Association of Clinical Endocrinologists; *ACE* American College of Endocrinology; *ADA* American Diabetes Association; *AHA* American Heart Association; *ASCVD* atherosclerotic cardiovascular disease; *ISPAD* International Society for Pediatric and Adolescent Diabetes; *LDL-C* low-density lipoprotein cholesterol; *T1DM* type 1 diabetes mellitus

[a] Situations where more frequent lipid status testing may be required include concern regarding adherence, unstable lipid profile, deterioration of diabetes control, progression of ASCVD or development of new ASCVD risk factor, considerable weight gain, or unexpected adverse change in any lipid parameter

Table 28.2 Summary of recommendations from select guidelines with thresholds for initiating LDL-C lowering therapy

Guideline	Risk category	Statin therapy	Non-statin LDL-C lowering therapies
ADA 2021	T1DM and established ASCVD[a]	• Use high-intensity statin	• Consider adding ezetimibe (preferred due to lower cost) or PCSK9 inhibitor if LDL-C ≥1.8 mmol/L (70 mg/dL) and very high risk[b]
	T1DM and age 40–75 years[a] without ASCVD	• Use moderate-intensity statin • It is reasonable to use high-intensity statin if other ASCVD risk factors present, age 50–70 years, or 10-year ASCVD risk ≥20%	• It may be reasonable to add ezetimibe to reduce LDL-C by ≥50% if 10-year ASCVD risk is ≥20%
	T1DM and age 20–39 years with additional ASCVD risk factors	• It may be reasonable to use moderate-intensity statin[c]	–
	T1DM and age >10 years with either: • LDL-C >4.1 mmol/L (160 mg/dL) • LDL-C >3.4 mmol/L (130 mg/dL) and another ASCVD risk factor Despite medical nutrition therapy and lifestyle changes	• Statin therapy may be considered with a LDL-C goal of <2.6 mmol/L (100 mg/dL)	–
AHA 2019 (Pediatrics)	T1DM (considered a high-risk disease) and LDL-C ≥3.4 mmol/L (130 mg/dL)	• Initiate statin and therapeutic lifestyle change simultaneously	• Add ezetimibe if LDL-C ≥2.6 mmol/L (100 mg/dL)
ISPAD 2018 (Pediatrics)	T1DM and age >10 years with LDL-C ≥3.4 mmol/L (130 mg/dL) despite improved glycemic control, dietary changes, and increased exercise	• Statin therapy should be considered with a LDL-C goal of <2.6 mmol/L (100 mg/dL)	–

(continued)

Table 28.2 (continued)

Guideline	Risk category	Statin therapy	Non-statin LDL-C lowering therapies
AHA/ACC 2018	T1DM and established ASCVD[a]	• Use high-intensity statin to reduce LDL-C by ≥50%	• It is reasonable to add ezetimibe if very high risk[b] and LDL-C ≥1.8 mmol/L (70 mg/dL) • It is reasonable to add PCSK9 inhibitor to statin and ezetimibe if very high risk[b] and LDL-C ≥1.8 mmol/L (70 mg/dL) or non-HDL-C ≥2.6 mmol/L (100 mg/dL)
	T1DM and age 40–75 years[a] without ASCVD	• Use moderate-intensity statin • It is reasonable to use high-intensity statin to reduce LDL-C by ≥50% if other ASCVD risk factors present	• It may be reasonable to add ezetimibe to reduce LDL-C by ≥50% if 10-year ASCVD risk is ≥20%
	T1DM and age 20–39 years with a "risk-enhancing factor": • Duration of T1DM ≥20 years • Albuminuria (≥30 mcg of albumin/mg creatinine) • eGFR <60 mL/min/1.73 m^2 • Retinopathy • Neuropathy • ABI <0.9	• It may be reasonable to use moderate-intensity statin[c]	–

Adapted from Ref. [44–47, 49]

Abbreviations: *ABI* ankle-brachial index; *ACC* American College of Cardiology; *ADA* American Diabetes Association; *AHA* American Heart Association; *ASCVD* atherosclerotic cardiovascular disease; *eGFR* estimated glomerular filtration rate; *HDL-C* high-density lipoprotein cholesterol; *ISPAD* International Society for Pediatric and Adolescent Diabetes; *LDL-C* low-density lipoprotein cholesterol; *PCSK9* proprotein convertase subtilisin–kexin type 9; *T1DM* type 1 diabetes mellitus

[a] In people aged >75 years, it is reasonable to start a statin after discussing potential benefits and risks. If age is >75 years and already on a statin, it is reasonable to continue treatment

[b] Very high risk is defined as a history of multiple ASCVD events or one major ASCVD event with multiple high-risk conditions

[c] Statin therapy is contraindicated in pregnancy and should also be avoided if pregnancy is being planned

Table 28.3 Summary of statins according to intensity

Intensity of statin	LDL-C reduction	Name and dose (once daily)
High intensity	≥50%	Atorvastatin 40–80 mg Rosuvastatin 20–40 mg
Moderate intensity	30–49%	Atorvastatin 10–20 mg Rosuvastatin 5–10 mg Simvastatin 20–40 mg Pravastatin 40–80 mg Lovastatin 40 mg Fluvastatin XL 80 mg Pitavastatin 1–4 mg

Adapted from Ref. [44]
Abbreviations: *LDL-C* low-density lipoprotein cholesterol, *XL* extended release

considered very high risk and have LDL-C above the threshold of 1.8 mmol/L (70 mg/dL) [44, 49]. For primary prevention, moderate-intensity statin therapy is recommended in people with T1DM and aged between 40 and 75 years without calculating 10-year ASCVD risk and irrespective of lipid levels. This is because statin trials demonstrated significant reductions in ASCVD events in people with diabetes (predominantly T2DM) in this age range [38, 44, 49]. In higher risk people with T1DM, based on age and ASCVD risk factors, high-intensity statins and ezetimibe may be considered for primary prevention [44, 49]. Low-intensity statins are not usually recommended for lipid management in adults with diabetes [44, 49].

The American College of Cardiology and American Heart Association guidelines include diabetes-specific "risk-enhancing factors" for improving ASCVD risk stratification independent of other ASCVD risk factors [49]. Risk-enhancing factors include long duration of T1DM (≥20 years), nephropathy with albuminuria (≥30 mcg of albumin/mg creatinine) or estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, retinopathy, neuropathy, and an ankle-brachial index <0.9, which reflects peripheral vascular disease [49]. The presence or absence of these factors can be used in the decision-making process around initiating or intensifying statin therapy (i.e., from moderate to high intensity). For example, in people with T1DM and age between 20 and 39 years with a risk-enhancing factor, it may be reasonable to initiate a moderate-intensity statin therapy after discussing the risks and benefits with the person with diabetes [49].

Table 28.4 summarizes the recommendations from select international guidelines that focus on lipid and lipoprotein targets [50–52]. According to the European Society of Cardiology and European Atherosclerosis Society guidelines, people with T1DM can be stratified as very high risk, high risk, or moderate risk depending on factors such as established ASCVD, target organ damage (microalbuminuria, retinopathy, or neuropathy), additional ASCVD risk factors, duration of diabetes, and age [52]. No person with diabetes is considered low risk based on this method of risk stratification [52]. In addition, the LDL-C, non-HDL-C, and apolipoprotein B goals are lower than what was recommended by previous European guidelines, reflecting a more intensive approach to lipid lowering in contemporary clinical care [52]. This is due to the results of the PCSK9 inhibitor trials, where very low levels

of LDL-C (the primary target) were safely attained with large reductions in ASCVD risk [53, 54]. Non-HDL-C and apolipoprotein B are measures of remnant lipoproteins and are considered complementary rather than competitive indices to LDL-C in people with T1DM [55]. The use of lipid and lipoprotein targets not only can guide statin dose titration and use of additional lipid-lowering therapies when goals are not attained, but may also facilitate patient-clinician communication, adherence to therapy, and goal setting [52].

As it can be seen, there are multiple guidelines available, with differing recommendations for lipid screening, ASCVD risk stratification, thresholds for initiating lipid lowering, and use of lipid and lipoprotein targets in people with T1DM. Such variation in guidelines can also exist for people with T2DM and for the general population. In T1DM, this is because ASCVD risk stratification in people with T1DM is complex, and further research in this area is needed. In young people with T1DM, 10-year ASCVD risk may be low, but lifetime risk is high [56]. Additional methods of risk stratification may need to be considered for people in whom the decision to initiate statin therapy remains unclear; however, even then, the evidence for these methods is not as strong as that for the general population or for people with T2DM.

Table 28.4 Summary of recommendations from select guidelines with lipid and lipoprotein targets

Guideline	Risk category	Lipid targets		
		LDL-C[a]	Non-HDL-C	ApoB
CCS 2021	T1DM and established ASCVD	<1.8 mmol/L (70 mg/dL) and LDL-C reduction of ≥50% from baseline	<2.4 mmol/L (93 mg/dL)	<70 mg/dL
	Statin indicated conditions: • T1DM and age ≥40 years • Duration of T1DM >15 years and age ≥30 years • T1DM and microvascular disease	<2.0 mmol/L (77 mg/dL)	<2.6 mmol/L (100 mg/dL)	<80 mg/dL
AACE/ACE 2020	Extreme risk • T1DM and established ASCVD	<1.4 mmol/L (55 mg/dL)	<2.1 mmol/L (80 mg/dL)	<70 mg/dL
	Very high risk • T1DM and ≥1 other ASCVD risk factor	<1.8 mmol/L (70 mg/dL)	<2.6 mmol/L (100 mg/dL)	<80 mg/dL
	High risk • T1DM with no other ASCVD risk factors	<2.6 mmol/L (100 mg/dL)	<3.4 mmol/L (130 mg/dL)	<90 mg/dL

(continued)

Table 28.4 (continued)

Guideline	Risk category	Lipid targets		
		LDL-C[a]	Non-HDL-C	ApoB
ESC/EAS 2019	Very high risk • T1DM and established ASCVD[b] • T1DM and target organ damage (microalbuminuria, retinopathy, or neuropathy) • T1DM and ≥3 other major ASCVD risk factors • Duration of T1DM >20 years	<1.4 mmol/L (55 mg/dL) and LDL-C reduction of ≥50% from baseline	<2.2 mmol/L (85 mg/dL)	<65 mg/dL
	High risk • Duration of T1DM >10 years without target organ damage • T1DM and another major ASCVD risk factor without target organ damage	<1.8 mmol/L (70 mg/dL) and LDL-C reduction of ≥50% from baseline	<2.6 mmol/L (100 mg/dL)	<80 mg/dL
	Moderate risk • Age <35 years and duration of T1DM <10 years with no other ASCVD risk factors[c]	<2.6 mmol/L (100 mg/dL)	<3.4 mmol/L (130 mg/dL)	<100 mg/dL

Adapted from Ref. [50–52]

Abbreviations: *AACE* American Association of Clinical Endocrinologists; *ACE* American College of Endocrinology; *ApoB* apolipoprotein B; *ASCVD* atherosclerotic cardiovascular disease; *CCS* Canadian Cardiovascular Society; *HDL-C* high-density lipoprotein cholesterol; *EAS* European Atherosclerosis Society; *ESC* European Society of Cardiology; *LDL-C* low-density lipoprotein cholesterol; *T1DM* type 1 diabetes mellitus

[a] LDL-C is usually used as the primary target and non-HDL-C and ApoB as secondary targets

[b] LDL-C <1.0 mmol/L (40 mg/dL), non-HDL-C <1.8 mmol/L (70 mg/dL), and ApoB <65 mg/dL goals may be considered in people with recurrent ASCVD events

[c] Statin therapy is contraindicated in pregnancy and should also be avoided if pregnancy is being planned

ASCVD Risk Calculators

People with diabetes are often considered to be at high or very high ASCVD risk and would therefore require intensive lipid lowering to achieve guideline-recommended LDL-C goals. However, diabetes is a heterogeneous condition and some young people with T1DM could be at moderate or potentially even low ASCVD risk, which is contrary to guidelines [57, 58]. ASCVD risk calculators are often recommended to estimate future ASCVD event risk in people without a

previous ASCVD event (i.e., primary prevention) and can assist therapeutic decision-making and act as educational tools. However, primary prevention risk calculators for ASCVD that are commonly used such as the *Pooled Cohort Equation*, the *Systematic Coronary Risk Evaluation* (SCORE), and the *Framingham Heart Study Risk Score* (FRS) are not generally recommended for people with T1DM, as they may underestimate risk [59–61]. This is because these risk calculators were validated in the general population rather than for people with T1DM and may not incorporate diabetes-specific variables. Such factors include diabetes duration, diabetes type, glycemic control, mode of insulin delivery (continuous insulin infusion (pump) therapy is associated with ≈40% less cardiovascular mortality than multiple daily injections even for the same HbA1c [62]), and presence of microvascular complications, particularly nephropathy and retinopathy, which are important predictors of ASCVD and mortality [43, 62–68]. As such, several ASCVD risk calculators specific to people with T1DM have been developed but are not yet widely used [69–72].

One example, the *Steno Type 1 Risk Engine,* estimates both 5-year and 10-year risk of first fatal or nonfatal ASCVD event [72]. It is derived from a comprehensive study of 4306 people with T1DM and a median follow-up of 6.8 years, during which 793 people (18.4%) experienced an ASCVD event [72]. The final prediction model was externally validated in 2119 people with T1DM and demonstrated excellent performance in both the derivation and validation cohorts, which were both predominantly of Danish ancestry [72]. Included in the final prediction model are the factors of age, sex, diabetes duration, glycated hemoglobin, systolic blood pressure, LDL-C, albuminuria, eGFR, smoking, and exercise [72]. The *Steno Type 1 Risk Engine* is available online as an interactive calculator (www.sdcc.dk/T1riskengine). Ideally, further external validation is required in cohorts with different ethnicities before widespread clinical implementation of the ASCVD risk calculator for people with T1DM [72–75].

Other Methods of ASCVD Risk Stratification

There are several methods that could be used for the detection or prediction of ASCVD, including carotid artery ultrasonography for measurement of intima-media thickness, measures of arterial stiffness, cardiac imaging and cardiac stress testing, glycemic variability, and biomarkers of inflammation, thrombosis, and oxidative stress [20, 76]. However, many methods of risk stratification are evolving research tools [76]. In this chapter, coronary artery calcium (CAC) scoring in asymptomatic people and measurement of lipoprotein(a) [Lp(a)] will be discussed further in the context of T1DM, as these methods are becoming more widely used in clinical practice, particularly for people in whom the decision to initiate lipid-lowering drugs, such as a statin, remains uncertain.

The CAC scan is a noninvasive test, utilizing electrocardiogram-gated non-contrast computed tomography, which detects CAC, a surrogate marker of

atherosclerosis [77]. CAC scores are superior to risk stratification using traditional ASCVD risk factors in the general population and in people with T2DM and can aid in reclassifying people into lower or higher risk categories [78, 79]. The Multi-Ethnic Study of Atherosclerosis (MESA) has incorporated CAC scores into the MESA 10-year coronary heart disease risk calculator for the general population [78]. In general, people with a CAC score of 0 are at very low risk, and statin therapy can be delayed until reassessment in approximately 5 years' time, whilst those with scores of ≥100 Agatston units or ≥75th percentile for age and sex are at higher risk and should be considered for statin therapy [49, 52]. The results of the CAC scan can motivate people to make lifestyle changes to reduce the risk of ASCVD, including initiation or continuation of pharmacological therapies such as statins [80]. However, CAC scoring is more expensive than traditional risk stratification methods, the score increases with statin use, the scan cannot detect noncalcified plaque (which is more vulnerable to rupture than calcified plaque), and it requires exposure to ionizing radiation, although at very low doses [77].

In people with T1DM, CAC scores are on average higher than that for age- and sex-matched controls, as demonstrated in the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study [24]. Intensive glycemic control is associated with significantly lower CAC scores based on data from 1205 people with T1DM and CAC scans from the DCCT/EDIC study [81]. More importantly, the DCCT/EDIC study also demonstrated that increasing CAC scores, particularly a score >100 Agatston units, is significantly associated with increasing risk of ASCVD, whilst scores of zero were associated with very low ASCVD event rates after 10–13 years' follow-up [82]. Data from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study demonstrated that the CAC score adds prognostic value to traditional risk factors in people with T1DM and should be incorporated into ASCVD risk calculators [83]. Overall, CAC scoring is an emerging tool for personalizing preventative therapies, such as lipid-lowering therapy. Another means of assessing coronary atheroma is coronary CT angiography (CCTA), which assesses both calcified and non-calcified plaque, but as yet there is insufficient evidence to support its inclusion in risk equations. Like CAC, it can be used to stratify risk and plan medical and potentially cardiac interventions. In a cross-sectional study, 88 patients with ≥45 years T1DM and 60 nondiabetic subjects had CCTA for evaluation of coronary artery plaque volume (total, calcified, or mixed/soft), CAC score, and epicardial fat tissue (EAT) [84]. Plaques were present in 85% of T1DM and 47% of nondiabetic subjects, $p < 0.01$; median (interquartile range) plaque volume (mm^3) in T1DM vs. controls was 21.0 (1.0–66.0) vs. 0.2 (0.0–7.1), $p < 0.01$, for calcified plaque; 0.0 (0.0–8.7) vs. 0.0 (0.0–0.0), $p < 0.01$, for soft/mixed; and 29.5 (3.9–95.8) vs. 0.4 (0.0–7.4), $p < 0.01$, for total plaque volume. Median CAC was 128 (13–671) vs. 1 (0.0–39.0), $p < 0.01$, in T1DM vs. controls. Median EAT volume did not differ between groups. Plaque volume and CAC and EAT were not correlated. Lower LDL-C and HbA1c levels were associated with less severe atheroma. Low time-weighted LDL-C and HbA1c for 30 years were associated with less plaque volume <25th percentile, OR (95% CI) 0.18 (0.05–0.70), $p = 0.01$, for LDL-C and 0.45 (0.20–1.00), $p < 0.05$, for HbA1c. Time-weighted LDL-C was linearly associated

with CAC (beta 0.82 (95% CI 0.03–1.62), $p = 0.04$) and total plaque volume (beta 0.77 (95% CI 0.19–1.36), $p = 0.01$) [84]. Equitable access to such advanced imaging and further studies as to their use in risk equations and as surrogate endpoints in T1DM trials are desirable.

Lp(a) is an LDL-C like lipoprotein that has an apolipoprotein(a) covalently bound to apolipoprotein B and is largely genetically determined. Lp(a) is pro-atherogenic, pro-inflammatory, and pro-thrombotic, and elevated levels significantly increase the risk of ASCVD in the general population and in people with T2DM [85–89]. However, the relationship between T1DM and Lp(a) remains unclear, and Lp(a) levels may be related to glycemic control and insulin administration [90]. As discussed in the book chapter by Dr. K. Kostner on Lp(a), diabetic nephropathy may increase Lp(a) levels in people with T1DM. In a study of 429 people with T1DM, Lp(a) levels >30 mg/dL were found to be an independent predictor of ASCVD [91]. In addition, another study of 1860 people with T1DM found Lp(a) to be a significant risk factor for ASCVD, albuminuria, and calcific aortic valve disease [92]. Plasma Lp(a) levels ≥50 mg/dL are considered a risk-enhancing factor for ASCVD according to guidelines [49, 93]. Thus, where the decision to initiate a statin or other lipid drug is uncertain, Lp(a) measurement may assist risk stratification, as people with elevated levels and diabetes could benefit more from early and aggressive ASCVD risk factor control [93]. Some lipid drugs, such as PCSK9 inhibitors, can also substantially lower Lp(a) levels, as discussed in other book chapters herein, such as that by Dr. P Toth on PCSK9 inhibitors.

Evidence for Non-statin Lipid-Lowering Therapies

In addition to statin therapy, *cholesterol absorption inhibitors* (i.e., ezetimibe) and *PCSK9 inhibitors* (i.e., evolocumab and alirocumab) are currently available medications that can reduce LDL-C by 20–30% and 50–60%, respectively, on top of statin therapy. Although landmark randomized controlled trials for ezetimibe (IMPROVE-IT), evolocumab (FOURIER), and alirocumab (ODYSSEY OUTCOMES) enrolled people with diabetes, this was predominantly people with T2DM [53, 54, 94]. Prespecified subgroup analyses of these trials demonstrated that people with diabetes at baseline derived greater absolute benefit from these medications compared with people without diabetes [95–97]. Interestingly, people with T1DM may have higher cholesterol absorption and PCSK9 levels and could theoretically derive more benefits from lipid lowering [98–100]. The FOURIER trial included 27,564 people, of whom 11,031 (40.0%) had diabetes and 286 of these had T1DM [96]. In addition, ODYSSEY OUTCOMES included 18,924 people, of who 5444 (28.8%) had diabetes and 37 of these had T1DM [97]. PCSK9 inhibitors appear to be safe, as the incidence of adverse effects was similar to placebo except injection-site reactions in the trials [53, 54]. Attaining low levels of LDL-C also

appears to be safe and further reduces the risk of ASCVD [101–103]. Furthermore, concomitant administration of insulin and PCSK9 inhibitors in people with T1DM does not negatively impact glycemia [104].

There is increasing evidence that elevated triglyceride-rich lipoproteins can significantly increase the risk of ASCVD [105, 106]. Currently available medications that can reduce triglycerides include statins, fibrates, niacin, and omega-3 fatty acids. Statin and fibrate combination therapy has not been shown to reduce the risk of ASCVD in the overall group of people enrolled in clinical endpoint trials, and therefore the combination is not recommended by some guidelines [44, 49, 50, 107, 108]. However, several further analyses have demonstrated that the combination is associated with reduced ASCVD risk in people with hypertriglyceridemia [109–113]. *Fenofibrate*, a PPRAα agonist, can also reduce the progression of retinopathy in people with T2DM and preexisting retinopathy, irrespective of baseline lipid levels [108, 114, 115]. The effect of fenofibrate on retinopathy in people with T1DM is being investigated in ongoing trials [116] specifically in T1DM (FAME-1 Eye) [117] and in trials by the University of Oxford (LENS trial) [118] and by the National Institutes of Health (NIH) [119], which include both T1DM and T2DM subjects. In addition, statin and niacin combination therapy is not recommended as it has not been shown to reduce the risk of ASCVD and is associated with increased adverse effects [44, 49, 50, 52]. So far, the evidence for medications that raise HDL-C, such as *cholesteryl ester transfer protein (CETP) inhibitors* and *niacin*, is substantially less robust than that for LDL-C-lowering therapies with regard to reducing the risk of ASCVD [120–124]. HDL-elevating therapies such as CETP inhibitors and infusions of reconstituted HDL (rHDL) are currently research tools. Furthermore, there is very little clinical trial data for the use of fibrates or niacin to reduce the risk of ASCVD in people with T1DM.

The role of *omega-3 fatty acid supplementation* using mixtures of eicosapentaenoic acid and docosahexaenoic acid in reducing the risk of ASCVD is controversial [106, 125]. In some large primary prevention trials (e.g., ASCEND and VITAL) where people with diabetes (including T1DM) were included, 1 g daily of omega-3 fatty acid supplementation did not reduce ASCVD events [126, 127]. In addition, the STRENGTH trial included people with diabetes and demonstrated that omega-3 carboxylic acid formulation of eicosapentaenoic acid or docosahexaenoic acid at 4 g daily (high dose) did not reduce ASCVD events in high-risk people treated with statin therapy compared with corn oil [128].

However, *icosapent ethyl*, a highly purified ethyl ester of eicosapentaenoic acid, was demonstrated to significantly reduce ASCVD events when prescribed at 2 g twice daily in the REDUCE-IT trial [129]. The trial included 8179 people with either established ASCVD or diabetes and at least one other ASCVD risk factor, with fasting triglycerides of 1.5–5.6 mmol/L (135–499 mg/dL) and LDL-C of 1.1–2.6 mmol/L (41–100 mg/dL) on statin therapy [129]. Of note, the number of people with T1DM in the trial was only 57 [129]. The benefits of icosapent ethyl in reducing the risk of ASCVD appeared similar across baseline triglyceride levels,

therefore suggesting that mechanisms beyond triglyceride lowering may be contributing factors [129, 130]. The treatment has been endorsed by guidelines, but further studies are needed in people with T1DM [44, 52, 131].

Barriers to Optimal Clinical Care

Despite the importance of lipid care, dyslipidemia is often under-recognized and undertreated in people with T1DM, particularly in those who have not developed vascular complications [132, 133]. A number of studies have demonstrated that many children, adolescents, and adults with T1DM do not attain lipid and lipoprotein goals and that statin therapy is underutilized despite the high prevalence of ASCVD risk factors [6, 132–138]. Failure to achieve treatment goals for ASCVD risk factors, including for lipid and lipoproteins, is associated with an increased risk of mortality and ASCVD in people with T1DM [19, 139]. This highlights the need to identify barriers to lipid care, which are likely to be multifactorial and include both clinician and patient factors.

One possible reason for the undertreatment of lipids in people with T1DM may be concerns of drug side effects, particularly of statins, which are frequent topics in the media. However, statins are generally well tolerated and adverse effects were rare in randomized controlled trials [140]. Other barriers may include clinician uncertainty around guideline recommendations in people with T1DM, therapeutic inertia, medication cost, time constraints of a T1DM consultation, and preference to prioritize glycemic control or implement lifestyle modifications before considering lipid-lowering medication. In addition, clinicians may be reluctant to prescribe statin therapy in adolescent people or in younger females with T1DM in their childbearing years. Statins have been shown to be safe for adolescents with T1DM in the short term but are contraindicated in pregnancy and should be avoided if pregnancy is being planned [141, 142]. Thus, more knowledge of barriers and enablers of lipid care in T1DM and increased education of both patients and clinicians are required to improve the clinical care and health outcomes related to lipids in people with T1DM.

Conclusions

In this chapter, the assessment and pharmacological management of dyslipidemia in people with T1DM are discussed. Early assessment of ASCVD risk and aggressive multifactorial risk factor control are essential for reducing the burden of ASCVD; however, risk stratification in T1DM remains challenging and guidelines recommend differing approaches to management. Additional risk stratification methods may aid in the decision-making process for deciding when and how aggressively to treat dyslipidemia, and T1DM-specific risk engines may have future utility in this

space. There is good evidence that intensive lowering of LDL-C can effectively lower ASCVD risk in people with T1DM. Achieving guideline-recommended lipid goals; under-use of drugs, usually statin therapy; and ensuring adherence to therapies remain major challenges. Further research in people with T1DM is needed to improve ASCVD risk stratification, evaluate the long-term safety and efficacy of statin use in the young, and assess the utility of non-statin lipid pharmacotherapies in this cohort. In the meantime, guideline recommendations should serve to inform clinical judgement and be tailored to the individual.

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Chapter 29
Adjunct Drug Treatment to Reduce Vascular Disease in People with Diabetes

Alicia J. Jenkins

Introduction

There is a pandemic of diabetes mellitus, with an estimated 537 million people currently living with diabetes, with about 80% of them living in disadvantaged regions [1]. About 85–95% have type 2 diabetes, of whom about 45% may not be aware of their diagnosis and hence be at particularly high risk of diabetes complications [1]. The incidence of youth-onset type 2 diabetes is increasing, predominantly related to high rates of obesity and sedentary lifestyles in youth [1]. Youth-onset type 2 diabetes is associated with even higher rates of chronic complications than for type 1 diabetes, likely contributed to by the higher rates of (type 2 diabetes) associated risk factors of obesity, hypertension, and dyslipidemia, and often poor mental well-being and suboptimal engagement with the healthcare system [2–4]. The incidence and prevalence of type 1 diabetes is also increasing globally, with growing recognition that the onset of this currently incurable autoimmune condition, which often commences in childhood, can occur at any age [1, 5].

Both of these common types of diabetes are associated with the risk of macrovascular and microvascular complications, including those related to atherosclerosis, and the microvascular complications of retinopathy, nephropathy, and peripheral and autonomic neuropathy. Whilst these conditions usually develop over years, with

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long periods of asymptomatic tissue damage, people with type 2 diabetes may have chronic complications at diagnosis due to its late diagnosis, and prediabetes, which can exist for years before progressing to type 2 diabetes, is associated with accelerated atheroma, but usually not microvascular complications [6].

Compared to the background population, people with diabetes are more than twofold likely to develop cardiovascular disease (CVD), with those with type 1 diabetes onset aged 10 years or less having up to 30-fold higher risk of CVD [7]. People with diabetes are also 15–20 times more likely to have a nontraumatic lower limb amputation [8]. Diabetes is also the leading cause of working-age adult-onset vision loss globally [9] and of kidney disease, including end-stage renal disease (ESRD) [10], which is also associated with high rates of CVD and mortality.

Multiple Risk Factors for Vascular Complications

The hallmark of diabetes mellitus for diagnosis, monitoring, and titration of glucose control treatment is hyperglycemia. Hyperglycemia is a major risk factor for the development and progression of microvascular complications, and improved glycemic control is usually associated with lower risk of chronic diabetes complications. Associations between glycemia and macrovascular disease exist, but are less strong than for microvascular complications, with other factors, such as dyslipidemia and hypertension, increasing in relative strength [6]. As well as glucose-related factors, there are many non-glucose modifiable traditional risk factors to be assessed and managed in people with diabetes [11]. These are summarized in Table 29.1 and are usually included in clinical practice guidelines, although there are often variations between national guidelines and changes over time as the evidence base grows. In addition, there are multiple novel and emerging risk factors, examples of which are in Table 29.2, that are also implicated in the pathogenesis of chronic diabetes complications and hence may also represent therapeutic targets [6]. There is interplay between glucose control and many other traditional and novel risk factors, which is discussed in another book chapter herein by this author (Dr. Jenkins). For example, hyperglycemia can induce dyslipidemia and increase inflammation, oxidative stress, mitochondrial damage, and vascular endothelial dysfunction.

The presence of multiple risk factors, even at a low level of severity, can place people with diabetes at moderate to high risk of developing or progressing macrovascular and microvascular complications. Macrovascular complications include cardiovascular disease, including coronary artery disease, cerebrovascular disease, peripheral vascular disease, and heart failure, and microvascular complications are diabetic retinopathy, nephropathy, and peripheral and autonomic neuropathy [1, 11].

It is not uncommon for people with diabetes, and also without diabetes, to have multiple vascular risk factors. In an Australian Health Survey of adults, over 94% had three or more concurrent vascular risk factors, 41% had four concurrent risk factors, and 28% had five or six concurrent risk factors [12]. It is not uncommon for

Table 29.1 Clinically available risk factors for the chronic complications of diabetes

Potentially modifiable risk factors
Glucose related
Hyperglycemia, Hypoglycemia, High glucose or HbA1c variability
Lipid related
Elevated LDL cholesterol, Low HDL cholesterol, High triglycerides, High non-HDL cholesterol, High ApoB, Low ApoA1, High lipoprotein(a), High lipid variability
Obesity
BMI, Waist hip circumference, Waist circumference
Insulin resistance
Metabolic syndrome
Hyperuricemia
Hypertension
Pulse pressure ≥60 mmHg
Smoking
Lifestyle-related risk factors
Poor nutrition, Low physical activity, High sitting time, Poor sleep quality
Clotting and fibrinolysis
For example fibrinogen
High platelet count/platelet dysfunction
Microvascular complications
A risk factor for macrovascular disease and other microvascular complications, Renal function: albuminuria, eGFR, creatinine clearance, Retinopathy: ETdRS score, macular volume, macular oedema
Existent macrovascular disease
Coronary artery calcification CAC, CT-angiography burden, Carotid IMT, ECG changes, e.g., left ventricular hypertrophy, MI, silent MI, Echocardiography, e.g., systolic or diastolic dysfunction, wall dyskinesis, Heart failure, Systolic and diastolic dysfunction
Unmodifiable risk factors
Genetics
Positive family history
Increasing age
Longer diabetes duration
Age of diabetes onset (though may be delayed by type 2 diabetes prevention programs or by experimental immunomodulatory therapies for type 1 diabetes)

patients or for their clinicians to disregard risk factors when they are low level or fluctuating. For example, blood pressure levels can vary widely in an individual even during a day, and episodic single measures in a clinic may miss elevated blood pressure levels. Furthermore, unless sought by a 24-h blood pressure monitor, one may miss overnight blood pressure non-dipping, an early sign of elevated blood pressure, which is associated with increased risk of cardiometabolic disease, including CVD and diabetes [13]. Similarly, even if risk factors are identified and non-drug interventions are inadequate, risk factors are not always treated pharmacologically, nor are

Table 29.2 Some novel and emerging risk factors for diabetes complication not usually assessed in clinical practice and included in most diabetes care guidelines

Glucose and insulin resistance related
1,5-anhydroglucitol, Glycated albumin, HOMA-IR, HOMA-beta, C-peptide levels, Insulin levels, Adiponectin, Resistin, Estimate glucose disposal rate or other measures of insulin sensitivity/resistance
Lipoproteins
Qualitative changes in lipoproteins, e.g., glycation, oxidation, immune complexes, Changes in subclasses, e.g., more small dense LDL, Lipoprotein-related enzymes, e.g., PON, LCAT, CETP, Lipoprotein function, e.g., antioxidant and anti-inflammatory effects of HDL, Lipidomics signatures, Lp(a) phenotype or genotype, HDL dysfunction
Inflammation
Systemic: ESR, white cell count, CRP, TNF-alpha, interleukins, NFκBeta Vascular inflammation: sVCAM-1, sICAM-1, sE-selectin
Oxidative stress
Isoprostanes, Myeloperoxidase, Oxidized LDL and oxidized LDL/LDL, Advanced glycation end products, e.g., blood levels, skin AGEs, ocular AGEs, Mitochondrial DNA copy number
Growth factors
Vascular endothelium growth factor, Pigment epithelium-derived factor, Matrix metalloproteinases (MMPs)
Thrombosis related
High PAI-1 levels and activity, Low tPA levels and activity, High fibrinogen, Platelet dysfunction, Elevated platelet levels, Elevated coated platelets
Adiposity related
% Body fat, including visceral fat, subcutaneous fat, Adipokines, e.g., adiponectin, leptin
Atherosclerosis burden
Coronary artery calcification, CT-coronary angiography, Carotid IMT, Aortic IMT, Endothelial dysfunction, Coronary artery (slow) flow, Ankle brachial index
Microvascular complications
Retinopathy: ETDRS, macular volume, retinal vessel caliber, and geometry, Renal disease: albuminuria, GFR, cystatin C, KIM-1, NGAL, Neuropathy: peripheral, e.g., nerve conduction studies; autonomic
Genetics and epigenetics
Polygenic risk score, Individual genetic markers, Telomere length, microRNAs and microRNA signatures, DNA methylation, Histone modification
Omics' signatures
Proteomics signatures, Lipidomics signatures, Metabolomics signatures, Phenomics signatures
Cell signaling
PKC activity, AMPK activity, Wnt pathway, PPARα activity, LDL receptor activity, PCKS9 activity

drugs always titrated or added so as to achieve recommended risk factor targets [6, 12]. Both patient and clinician factors are likely implicated [6].

As there are multiple risk factors for diabetes complications, there are usually evidence-based recommended targets for glycemia, blood pressure, and lipids and

Table 29.3 Risk factor targets for diabetes patients from major bodies

	RACGP/diabetes Australia[a]	ADA[b]	ESC/EASD[c]	IDF[d]
HbA1c	≤7% (53 mmol/mol)	<7% (53 mmol/mol)	<7.0% (53 mmol/mol)	<7% (53 mmol/mol)
SBP	≤140 mmHg	<140 mmHg[e]	<130 mmHg	≤130–140 mmHg
DBP	≤90 mmHg	<90 mmHg[e]	<80 mmHg	≤80 mmHg
LDL-C	<2.0 mmol/L <1.8 mmol/L if established CVD	–[f]	Moderate CV risk: <2.6 mmol/L High CV risk: <1.8 mmol/L and ≥50% reduction Very high CV risk: <1.4 mmol/L and ≥50% reduction	<2.6 mmol/L Established CVD or high CV risk: <1.8 mmol/L
BMI	<25 kg/m^2	<25 kg/m^2[g]	–	–
WHR	–	–	–	–
Waist Circumference	♂ <94 cm ♀ <80 cm	–	–	–

[a] RACGP Management of type 2 diabetes: A handbook for general practice 2020
[b] American Diabetes Association Standards of Diabetes Care 2021
[c] 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD)
[d] Source: Adapted from Jenkins et al., in P. Toth and C. Cannon (Eds), Comprehensive Cardiovascular Medicine in Primary Care, 2nd ed., Humana Press 2018
[e] Target lower by 10 mmHg may be appropriate in individuals at higher CV risk (existing CVD or 10-year ASCVD risk ≥15%)
[f] Prescription of lipid-lowering medications dependent on patient's age and CV risk
[g] <23 kg/m^2 for Asian American individuals

recommendations for nonsmoking, optimal weight, physical activity, and nutrition. There are usually variations between national and international diabetes associations (Table 29.3), and the recommendations and treatment targets may also differ by age, ethnicity, and presence or absence of chronic complications. Furthermore, the accessibility of drugs to treat risk factors or systems to monitor them (e.g., home glucose monitoring by finger-prick blood glucose levels or interstitial fluid continuous glucose monitoring (CGM), home blood pressure monitoring, and laboratory-based lipid profiles) may differ between or within regions based on regulatory or economic aspects [14–16].

Treating Multiple Risk Factors Is Beneficial

Treatment of multiple risk factors for the chronic complications of diabetes is effective. An example is the Steno-2 trial in which 160 adults with type 2 diabetes and increased albuminuria were randomized to intensive therapy (including renin-angiotensin-aldosterone system (RAAS) and statin drugs) or standard therapy for a mean of 7.8 years and then observed for a further 5.5 years, with the study primary endpoint being all-cause mortality [17]. The intensive treated group had 0.5% (6 mmol/mol) lower HbA1c, 15 mmHg lower systolic blood pressure, and 1.5 mmol/l (58 mg/dL) lower total cholesterol, with 24 vs. 40 deaths in the intensive vs. standard therapy group (HR 0.54, 95% CI 0.32–0.89, $p = 0.02$), with the intensive treatment group also having lower rates of CVD death (HR 0.43; 0.19–0.94, $p = 0.04$) and CVD (HR 0.45; 0.23–0.86, $p = 0.02$), and only 1 vs. 6 people developed ESRD, $p = 0.04$ [17].

The personal and socioeconomic gains of treatments that avoid premature death or debilitating chronic complications are substantial.

Mnemonics to Guide Diabetes Care

There are several variations of a mnemonic to assist busy clinicians and trainees in diabetes care. Our initial mnemonic was **GLOBES**, being **G**lucose, **L**ipids and lipid drugs, **O**besity, **B**lood pressure and blood pressure drugs, and **E**motion and **S**moking [18]. The globes referred to diabetes being a global problem, that the eye is a globe that is impacted by each of those elements (glucose, lipids, obesity, blood pressure, emotions, and smoking), and that a global or holistic approach to the person with diabetes should be taken. **GLOBE^{2}S^2** was the next version: reflecting **G**lucose, **L**ipids and lipid drugs, **O**besity, **B**lood pressure and blood pressure drugs, **E**motion and **E**ducation and **S**moking and **S**creening [18]. A subsequent version was **GLOBES STRIVED**, representing **GLOBES** as previously, and **STRIVED** reflecting **S**creening, **TR**eating to target, **I**nflammation (or infection), **V**accinations, **E**ducation, and **D**evices (STRIVED) [19]. I now suggest **GLOBES CAD STRIVE**. The **CAD** represents **C**lotting, **A**dvocacy, and **D**evices. **STRIVE** now represents **S**creening, **TR**eating to target, **I**nflammation (or infection), **V**accinations, and **E**ducation. This is summarized in Table 29.4.

Each element of GLOBES CAD STRIVE is now briefly overviewed. There are also additional treatments to be considered in people with chronic complications, and others have developed mnemonics to remind clinicians of treatment strategies for coronary artery disease and for heart failure, which are relevant to people with and without diabetes. These will be described later in this chapter. More details of the various drug classes with example doses, side effects, and contraindications are

Table 29.4 Versions of a mnemonic for diabetes care

GLOBES
Glucose, lipids and lipid drugs, obesity, blood pressure and blood pressure drugs, emotions, smoking
GLOBE^{2}S^2
Glucose, lipids and lipid drugs, obesity, blood pressure and blood pressure drugs, emotions, education, smoking, screening
GLOBES STRIVED
Glucose, lipids and lipid drugs, obesity, blood pressure and blood pressure drugs, emotions, smoking (GLOBES)
Screening, treating to target, inflammation, vaccinations, education, devices (STRIVED)
GLOBES CAD STRIVE
Glucose, lipids and lipid drugs, obesity, blood pressure and blood pressure drugs, emotions, smoking (GLOBES)
Clotting, advocacy, devices (CAD)
Screening, treating to target, inflammation, vaccinations, education, devices (STRIVE)

provided in a book chapter my colleagues and I wrote regarding the management of diabetes and its complications in primary care [6].

GLOBES

Glucose, **L**ipids, **O**besity, **B**lood Pressure, **E**motions, **S**moking

Glucose

Diagnosis and monitoring measures: Glucose levels are central to the diagnosis and monitoring of prediabetes and diabetes mellitus, irrespective of the diabetes type (e.g., type 1 or type 2 diabetes, gestational diabetes, cystic fibrosis-related diabetes, post-pancreatitis, or post-pancreatectomy diabetes or iatrogenic diabetes). Diabetes is usually diagnosed by fasting or random venous blood glucose levels, not capillary blood tests, which are not accurate enough for diagnosis, or by an oral glucose tolerance test (oGTT) and/or HbA1c levels. The monitoring of glycemic control is usually by serial HbA1c levels (usually every 3–4 months, and sometimes, for insulin-treated subjects, by self-monitoring of capillary blood glucose levels or interstitial fluid glucose levels by continuous glucose monitoring (CGM), with the latter discussed in the Devices section) [11, 20].

Glucose metrics: There are several aspects of glucose control relevant to diabetes care. *Individual blood or interstitial fluid glucose levels* can guide the person with diabetes, for example, regarding their safety to drive a car or to exercise, and to help select an insulin dose to cover food to be consumed or to correct a high glucose level. Widely used by clinicians is the mean level of glucose control, usually assessed by *HbA1c* levels (reflecting mean blood glucose over the past 3 months). HbA1c levels are often used in guidelines as a treatment target, to assess chronic complication risk and to guide decisions about pharmacologic glucose control drug use [11, 20]. With *continuous glucose monitoring* (CGM) or the variant of flash glucose monitoring (FGM), as well as the concurrent interstitial fluid glucose levels, readings (every 5–15 min for 3–14 days, depending on which model is used) are graphed. Alarms can alert the wearer and sometime a carer for low, high, or rapidly changing glucose levels [21–24]. A standardized one-page ambulatory glucose profile (AGP) report for CGM profiles has been developed [25], and CGM-related targets for people with type 1 or type 2 diabetes, including in pregnancy, have been recommended by an international consensus group [26]. The AGP provides the mean glucose levels, an estimated HbA1c value and glucose variability (usually CV%) and time in target (70–180 mg/dL) range, and above and below it, as well as a small image of each day's glucose trace. *Glucose variability* may be calculated from the CV or SD of serial glucose levels from interstitial fluid glucose or blood glucose monitoring or from serial HbA1c measures, or potentially other short-term measures of mean glycemia such as fructosamine, though these are less often used in clinical practice or research.

In people with diabetes, all aspects of glucose control, including hyperglycemia, hypoglycemia, and higher glucose variability have been linked with increased risk of microvascular and macrovascular complications and with mortality [27–31]. Importantly, meta-analyses have confirmed substantially reduced risk of microvascular complications (diabetic retinopathy, kidney disease, and neuropathy) with better glycemic control, usually as reflected by HbA1c levels, and also some cardiovascular disease benefit, albeit less than for microvascular complications [30, 31].

Hypoglycemia is associated with increased risk of CVD and mortality [32–38]. There are several mechanisms for hypoglycemia-induced adverse vascular events. Hypoglycemia prolongs the cardiac QT interval, which with a hypoglycemia-related catecholamine surge and potential hypokalemia due to a relative excess of insulin may induce a cardiac arrhythmia and even sudden death, sometimes referred to as the "dead-in-bed" syndrome, which was first recognized in young people with type 1 diabetes [36–38]. Hypoglycemia can also induce endothelial dysfunction (with vasoconstriction), increased inflammation, oxidative stress, and a pro-thrombotic tendency, which can last for several days after hypoglycemia [32–35]. Another indirect association between hypoglycemia and cardiovascular events may be that frailty and reduced ability for self-care may increase the risk of cardiovascular events, death, and hypoglycemia [32].

Glycemia and chronic complications: Landmark trials in type 1 diabetes, the Diabetes Control and Complications Trial and its observational follow-up, the Epidemiology of Diabetes Intervention and Complications (DCCT/EDIC) Study [39–41], and in type 2 diabetes, the United Kingdom Prevention of Diabetes Study

(UKPDS), showed positive relationships between glycemia and neurovascular complications [42, 43]. Importantly, these trials also showed major benefit of reduced complications with better glucose control.

Briefly, in the type 1 diabetes DCCT trial, intensive vs. standard diabetes management for a mean of 6.9 years, achieving mean HbA1c levels of 7.1% vs. 8.9% (54 vs. 74 mmol/mol), respectively, significantly reduced the development of all forms of microvascular complications by 27–76% and (due to very low number of macrovascular events in this young cohort) of macrovascular events by a nonsignificant 41%. During the observational follow-up, during which all subjects were encouraged to follow intensive therapy, and the mean HbA1c was similar in both groups, about 8.0% (64 mmol/mol) [39–41], there were many years of significantly reduced microvascular and macrovascular complications in the group in the DCCT intensive treatment group (related to metabolic memory) (discussed below) [44].

In the UKPDS (n = 3867), adults with newly diagnosed type 2 diabetes were randomized to intensive vs. standard glucose control, achieving mean HbA1c levels of 7.0 vs. 7.9% (53 vs. 63 mmol/mol) for a mean of 10 years. For every 1% reduction in HbA1c (in % units), there was a 21% reduction in any diabetes-related endpoint, 21% reduction in diabetes-related death, 14% reduction in all-cause mortality, 43% reduction in peripheral vascular disease, 37% reduction in microvascular complications (all $p < 0.0001$), and 12% reduction in stroke ($p = 0.035$) [42, 43]. The "intensive treatment" goal in these earlier trials is now the standard, common treatment target in clinical practice for most people with diabetes.

Metabolic memory: The DCCT/EDIC and UKPDS studies also showed the existence of metabolic memory or the legacy effect for glucose [43–45]. These terms refer to the body's ability to continue to respond to (by complication status) good or poor glucose control for years after the glucose control has worsened or improved. Advanced glycation end products (AGEs) and/or epigenetics are implicated [43, 45]. Vascular metabolic memory may also exist for other risk factors and their treatment, including for lipids [46].

Glucose Targets

The generally recommended HbA1c target for most nonpregnant adults with diabetes in guidelines is 7% (53 mmol/mol) or less. The American College of Physicians suggested a general HbA1c target of 7–8% (53–63 mmol/mol) [47]. Whilst a HbA1c <7% (<53 mmol/mol) is recommended for most people with diabetes, subset analyses of major trials suggest that more intensive glucose control may have cardiovascular benefits in adults with short-duration type 2 diabetes, and hence, a more stringent target (e.g., HbA1c ≤6 or 6.5% (42 or 48 mmol/mol)) should be considered. A meta-analysis based on the presence of microvascular complications also suggests that a lower diagnostic HbA1c level and targets may be appropriate [48]. However, a recent meta-analysis, including recent trials, suggests that a HbA1c between 7% and 7.7% (53 and 61 mmol/mol) reduces both micro- and macrovascular complications in people with type 2 diabetes, irrespective of known diabetes duration [49]. Most guidelines recommend a personalized approach based on such

factors as age, life expectancy, comorbidities, frailty, medications, risk of hypoglycemia and hypoglycemia awareness status, and social circumstances, such as if they live alone or in a care home [50].

Treating Glucose Levels

Nondrug Measures

Many recommended lifestyle measures that impact glucose levels also favorably impact appetite, weight, mood, blood pressure, lipids, cardiorespiratory fitness, and mental well-being. Commonly recommended activities include regular aerobic and resistance exercise, limiting sitting time, healthy nutrition, and nonsmoking [11]. The effects of many of these lifestyle measures, specifically on lipids, are discussed in the book chapter herein by Dr. Peter Clifton. Even after starting pharmacologic agents, these lifestyle measures should still be continued. Patients may benefit from the support of allied healthcare professionals such as a dietician, exercise physiologist, psychologist, and smoking cessation programs.

Glucose Control Drugs

When lifestyle alone is insufficient to achieve the desired glycemic control (usually HbA1c) targets, glucose control drugs are added, usually oral medications (in type 2 diabetes), one at a time and waiting several months after commencement to recheck the effect on measures of glucose control before increasing their dose or adding a second-line drug. Given that off-target effects may exist, which may detract from the benefits of improved glycemic control, regulatory bodies, such as the US Food and Drug Administration (FDA), require that the cardiovascular event safety of any new glucose control drug be demonstrated.

The various classes of glucose drugs, their main mechanism(s) of action, and some common side effects and contraindications are summarized in Table 29.5. Metformin, a low-cost, relatively safe, and well-tolerated drug, is usually the first-line oral agent and is also used often in prediabetes to retard progression to type 2 diabetes [11]. As more classes of glucose control drugs emerge, and their availability and costs vary, and there are sometimes ethnicity-based differences in responsiveness, there are variations in the order in which glucose control drugs are added. Often oral agents, first one and then two, three, or four classes, are used before adding injectable drugs such as GLP-1 agonists and insulin. Factors to consider when choosing glucose control drugs include cardiovascular and kidney disease status.

Glucose-lowering drugs reduce complication risk by improving glycemia, by improving lipid levels (usually by about 5–10%) and lipoprotein quality (such as by reducing nonenzymatic glycation), and by other pleiotropic effects. Commonly used modern glucose control drugs such as SGLT2 inhibitors and GLP-1 receptor agonists, relative to placebo, can reduce the risk of cardiovascular events and

Table 29.5 Clinically available glucose-lowering drugs

Drug class	Examples	Mechanism(s)	Route of administration	Potential side effects	HbA1c reduction
Biguanides	Metformin	Improves peripheral glucose uptake, slows stomach emptying, reduces hepatic gluconeogenesis	Oral	Gut upset Metallic taste Lactic acidosis (rare) Vitamin B12 reduction	1%
Alpha-glucosidase inhibitor	Acarbose Miglitol Voglibose	Inhibit alpha-glucosidase enzyme in small intestine	Oral	Gut upset	0.6%
Bile acid-binding resin		Slow glucose absorption	Oral	Gut upset	
Sulfonylureas	Glibenclamide Gliclazide Glimepiride	Stimulate insulin secretion	Oral	Hypoglycemia, gut upset, rash Weight gain	1.25%
Thiazolidinediones	Rosiglitazone Pioglitazone	PPAR-gamma agonist, reduces hepatic gluconeogenesis, peripheral insulin sensitization	Oral	Edema, heart failure, macular edema, fractures	0.8%
Incretin—DPP4 inhibitor	Linagliptin Sitagliptin Saxagliptin	Inhibit DPP4 activity to slow breakdown of incretin hormones: increases insulin secretion, inhibits glucagon release, slows gastric emptying	Oral	Gut upset Nasopharyngitis	0.75%
Incretin—GLP-1 agonist	Liraglutide Exenatide	Activates GLP-1 to increase insulin secretion, suppresses glucagon, slows gastric emptying	Injection	Gut upset, increased heart rate, gallstones	1%
SGLT-2 inhibitors	Dapagliflozin Empagliflozin	Inhibits renal glucose reabsorption leading to glycosuria	Oral	Genitourinary infection Euglycemic diabetic ketoacidosis, dehydration, hypotension	0.7%

(continued)

Table 29.5 (continued)

Drug class	Examples	Mechanism(s)	Route of administration	Potential side effects	HbA1c reduction
Insulins	Ultrarapid, e.g., Fiasp Rapid e.g. Novorapid, Humalog Short-acting, e.g., Actrapid Intermediate: Protaphane, Isophane Premix of rapid and intermediate-acting insulins, e.g., NovoMix Long acting: Levemir, glargine Ultra-long acting: Degludec	Promotes cellular glucose uptake Inhibits lipolysis Inhibits endogenous glucose production	Injection	Hypoglycemia Weight gain	0.9–1.1%

cardiovascular mortality [51, 52]. With the natural progression of type 2 diabetes as diabetes duration increases, many people with type 2 diabetes will require multiple drugs from different classes of glucose-lowering drugs. Apart from insulin, where background and prandial insulin coverage may be required, no more than one member of each drug class should be prescribed.

Individual glucose control drugs are discussed in a previous book chapter by the author [6], but given the rapid evolution of new glucose control drugs and evidence, reference to major diabetes body guidelines is recommended. Excellent examples are from the Australian Diabetes Society [53] and the American Association of Clinical Endocrinology [54].

Glucose Control in Type 1 Diabetes and Complications

Insulin pumps: For people with type 1 diabetes, exogenous insulin is essential for life, and there are various options for its delivery, with options being by multiple daily injections or by an insulin pump. Interestingly, for the same HbA1c level, insulin delivery by an insulin pump is associated with about 45% reduction in the risk of cardiovascular mortality, death, and need for hospitalization: Hazard ratios

(pumps vs. injections) were as follows: coronary heart disease (CHD) death (HR 0.55, 95% CI 0.36–0.83), fatal CHD or stroke (HR 0.58 (0.40–0.85)), and all-cause mortality (HR 0.73 (0.58–0.92)) [55]. Other studies in type 1 diabetes showed similar benefits of pump use for microvascular complications. A 24-study (n = 9302 subjects) meta-analysis for diabetic retinopathy showed a relative risk (RR) for diabetic retinopathy of 0.45 (95% CI 0.24–0.83), independent of HbA1c levels [56]. In a prospective study of n = 989 12–20-year-olds with at least 5 years of type 1 diabetes and equal mean HbA1c of 8.7% (72 mmol/mol), the RR of diabetic retinopathy with pump therapy was 0.66 (95% CI 0.45–0.95) and for peripheral neuropathy RR 0.63 (95% CI 0.45–0.96), both $p < 0.03$ [57]. Potential mechanisms may be a 20–30% lower daily insulin dose when insulin is delivered by a pump rather than multiple daily insulin injections and less glucose variability, which, as discussed earlier, has been associated with lower complication risk, perhaps related to less inflammation and oxidative stress.

Adjunct glucose control drugs in type 1 diabetes: There is also interest in and some evidence of benefit of the use of adjunct glucose control drugs usually used in type 2 diabetes, in people with type 1 diabetes, particularly for those with features of type 2 diabetes, or so-called double diabetes. Such drugs include oral agents such as metformin and SGLT2 inhibitors, and injectable agents such as GLP-1 agonists and amylin-like pramlintide. There are several excellent reviews [58–60].

Metformin: In the REMOVAL trial of adjunct metformin for high cardiovascular disease risk adults with type 1 diabetes, adjunct metformin for a mean of 3 years had small but statistically significant benefits for LDL-C levels, glycemia, weight, and insulin dose reduction, and retarded the rate of progression of mean far wall carotid intima-media thickness (cIMT), the primary endpoint, though this did not reach statistical significance in the whole cohort [61], but did so in nonsmokers [62]. Metformin did significantly retard the progression of maximum far wall cIMT, which includes plaque (a pre-stated trial tertiary endpoint), in all subjects, and also significantly retarded renal disease progression (eGFR loss, not albuminuria) in all subjects [61, 63]. Benefit did not differ by body mass index (BMI) status. Major side effects of metformin are related to gut upset, which potentially may have been less with extended-release metformin preparations, and reduction in vitamin B12 levels [61].

SGLT2 inhibitors: In clinical trials, SGLT2 inhibitors, initially developed for use in people with type 2 diabetes, showed that in adults with type 1 diabetes, SGLT2 inhibitors significantly improve glycemia, including HbA1c and CGM time in range, and some CVD risk factors, such as weight and lipids. Side effects were increased risk of genitourinary infections and low but increased rates of diabetic ketoacidosis relative to the control arm [64, 65]. Even with hybrid closed loop (HCL) or with glucose sensor augmented pumps (SAP), adjunct empagliflozin significantly increased time in glucose target range, by up to 17.5% (HCL plus empagliflozin vs. SAP plus placebo) [66]. To date (May 2022), some regions (Europe and Japan) have approved SGLT2 inhibitor adjunct therapy for some people with type 1 diabetes, but the US FDA has not. As well as improving glycemia in type 2 diabetes, SGLT2 inhibitors also significantly reduce the risk of kidney and cardiovascular

events, but such long-term trial evidence in people with type 1 diabetes is not yet available.

GLP-1 agonists: Short-acting injectable GLP-1 agonists also show benefit as adjunct therapy in type 1 diabetes [67]. Liraglutide, an incretin-based injectable drug used for type 2 diabetes and for weight loss in obesity, even in the absence of diabetes, has also been trialed in adults with type 1 diabetes for glucose control and for other risk factor outcomes [68]. In five trials ($n = 2445$), liraglutide significantly improved HbA1c levels (in % units) by up to 0.24% and induced weight loss up to 4.9 kg and decreased total daily insulin needs, mainly due to bolus dose requirements. There was no significant change in hypoglycemia, nor increase in diabetic ketoacidosis. The main side effects were gastrointestinal upset and increased heart rate [67, 68].

Pramlintide: The pancreas also secretes amylin, and an injectable synthetic human amylin analogue has been developed for prandial glucose control in people with type 2 diabetes. In a randomized controlled trial in 651 adults with type 1 diabetes and mean (SD) HbA1c 8.9 (1.0)% (75 mmol/mol), mealtime placebo or varying doses of pramlintide were added to their insulin therapy for 1 year. Pramlintide (60 μg three or four times daily) significantly reduced HbA1c levels (in % units) by 0.29% ($p < 0.011$) and 0.34% ($p < 0.001$), respectively, vs. 0.04% reduction with placebo, and the proportion of subjects achieving HbA1c <7% (53 mmol/mol) trebled with pramlintide. There was no increase in concomitant insulin use, and there was a small but statistically significant weight loss (≈1 kg). Nausea was the most common adverse event [69].

Further trials, follow-up, and reports of off-label use of adjunct glucose control drugs are merited.

Lipids and Lipid Drugs

Particularly in Western society, the typical lipid profile and lifestyle favor atherosclerosis. To achieve recommended LDL-C targets and/or LDL-C reductions so as to minimize the risk of atherosclerosis and the clinically evident-related vascular complications, most people with diabetes will usually require lipid-lowering drugs. Lipids are also implicated in the pathogenesis of microvascular complications, and many lipid drugs show benefit associated with reductions in LDL-C or triglycerides. As well as the direct effects of these lipid drugs, there are also likely pleiotropic effects that may reduce vascular complications, such as favorable effects on inflammation, oxidative stress, growth factors, cell signaling, and molecular effects [70–74]. Lifestyle measures, as discussed in the book chapter herein by Dr. Peter Clifton, should be instituted and continued, ideally with support from other clinicians, allied healthcare professionals, and community and peer support.

Table 29.6 summarizes clinically available lipid drug classes, their main mechanism of action, and common side effects. Much more details and the evidence base for their use in primary and secondary prevention for macrovascular disease and for

Table 29.6 Clinically available lipid-lowering drugs

Drug class	Examples	Mechanism(s)	Route of administration	Common side effects	% Lipid change
Mainly LDL lowering					
Statins	Atorvastatin Rosuvastatin Pravastatin Simvastatin	HMG CoA reductase inhibitor, upregulates LDL-receptor (LDL-R), thereby increasing LDL removal from blood	Oral (daily)	Myalgia Abnormal liver function tests	↓25–50% LDL-C ↓10–20% TG
NPC1L1 inhibitor	Ezetimibe	Inhibits brush border enzyme, reducing intestinal cholesterol absorption	Oral (daily)	Gut upset Rhinitis	↓15–25% LDL-C
Resins	Cholestyramine Colesevelam	Bind cholesterol-rich bile acids and remove them via gut	Oral (multiple times daily)	Gut upset Interfere absorption of some tablets	↓15–25% LDL-C
PCSK9 inhibitors	Evolocumab Alirocumab	Monoclonal antibody that inhibits PCSK9-related reduction in LDL-R	Injection 2–4 weekly	Myalgia Flu-like symptoms Injection-site reaction Can raise triglycerides	↓60% LDL-C ↑HDL-C 5–10%
ACL inhibitor	Bempedoic acid	Inhibits hepatic ATP citrate lyase, in the hepatic cholesterol synthesis pathway	Oral (daily)	Limb pain Anemia	↓ 20–40% LDL-C
Mainly triglyceride lowering					
Fibrates	Fenofibrate Fenofibric acid Bezafibrate Gemfibrozil	PPARα agonist, activates lipoprotein lipase, increases HDL synthesis, decreases hepatic production ApoC	Oral (daily or for gemfibrozil twice daily)	Myalgia Gut upset Rhinitis	↓40–80% TG ↓5–15% LDL-C ↑HDL-C 10–30%
Fish oils/ EPA		Reduce hepatic VLDL synthesis, upregulate lipoprotein lipase	Oral 1–3 times daily	Gut upset Bruising, bleeding	No change LDL-C
Nicotinic acid/ niacin		Inhibits lipolysis in adipose tissue Decreases the rate of HDL breakdown	Oral	Gut upset Flushing Worsens glycemia Abnormal liver function	↓15–30% LDL-C ↓25–45% TG ↑HDL 20–35%

(continued)

Table 29.6 (continued)

Drug class	Examples	Mechanism(s)	Route of administration	Common side effects	% Lipid change
Mainly LDL and Lp(a) lowering					
PCSK9 inhibitor	Evolocumab Alirocumab	Monoclonal antibody that inhibits PCSK9-related reduction in LDL-R	Injection 2–4 weekly	Myalgia Flu-like symptoms Injection-site reaction	↓20–30%

microvascular complications in diabetes are discussed in multiple other book chapters (Chapters 15–22) herein. As with glucose- and blood pressure-lowering drugs, it is not uncommon for people with diabetes to need more than one lipid-lowering drug, but no more than one drug per class should be used. The first-line lipid-lowering drug in diabetes for LDL lowering is usually a "statin." If not tolerated or inadequate LDL-C reduction, a drug reducing intestinal cholesterol absorption could be added, such as ezetimibe. If still insufficient lipid lowering or drug tolerance issues, then a PCSK9 inhibitor could be added. The first-line drug for moderate or severe hypertriglyceridemia is usually a fibrate. For more details about the evidence base, mechanisms of action, and side effects of these various drug classes, there are excellent chapters in this book by Dr. Martin et al. (statins), Dr. Banach et al. (statin intolerance), Dr. E Brinton et al. (fibrates), Dr. S Philip (fish oils), Dr. H Bays (cholesterol absorption inhibitors and resins), and Dr. P Toth (PCSK9 inhibitors).

Statin intolerance, most commonly presenting as myalgia with or without elevations in CK levels, occurs in less than 5% of statin trial participants but is reported at severalfold higher rates in clinical practice. Diabetes is a risk factor for statin intolerance. The overdiagnosis or misdiagnosis of statin intolerance leads to people missing out on the health benefits of statin therapy, and also the consequences of the missed diagnosis of what is causing the symptoms or abnormal laboratory tests that were mistakenly attributed to statin intolerance [75]. Several comprehensive guidelines to statin intolerance have been published and also a short practical guide for its diagnosis and management [75–79]. Alternate drugs suggested include low-dose alternating-day statins, ezetimibe, PCSK9 inhibitors, and bempedoic acid (the activating enzyme for which is not present in muscle, hence muscle side effects are uncommon [79]).

Obesity

Being overweight or obese is a risk factor for prediabetes, for type 2 diabetes, and for chronic complications of type 2 and type 1 diabetes. Being overweight or obese is now also common in people with type 1 diabetes, with this combination of type 1 diabetes with excess adiposity or insulin resistance being termed "double diabetes." Double diabetes is associated with increased risk of chronic complications, mortality, worse glycemic control, higher insulin doses, and worse risk factors such as dyslipidemia and hypertension [80–83]. Consideration of the prevention of adiposity and means for weight loss in overweight or obese people with type 2 diabetes [84, 85] and for people with type 1 diabetes are topical [86–89].

An initial weight loss of 5–10% of body weight for overweight or obese patients with diabetes is recommended [11]. Diets such as very-low-calorie diets (VLCDs) and pharmacological therapies (e.g., orlistat) may be considered [84, 86, 87]. To reduce potential weight gain and to facilitate weight loss, glucose-lowering drugs that promote weight loss (e.g., metformin, SGLT2 inhibitors, GLP-1 analogues) or are weight neutral (e.g., DPP-4 inhibitors), discussed above, should be considered. Bariatric surgery, for those who are overweight and obese and particularly for those with morbid obesity and/or comorbidities, can lead to substantial weight loss, improved metabolic control, and may even reverse type 2 diabetes [85]. Whilst less commonly used to date, bariatric surgery in people with type 1 diabetes can improve BMI, HbA1c, insulin dose, and blood pressure and increase HDL-C levels [89].

Blood Pressure and Blood Pressure Drugs

The incidence and prevalence of hypertension are increased in people with diabetes, and if inadequately treated increase the risk of both microvascular and macrovascular complications. Hypertension, including in the general population, often first manifests as lack of the normal blood pressure reduction overnight, called non-dipping [13], and is best identified by 24-h blood pressure monitoring. Whilst supine or sitting and standing blood pressure should be assessed at each clinic visit, home blood pressure measurements are often more reliable than in-clinic measures. People with diabetes, particularly if they have prehypertension or hypertension, should be encouraged to purchase a blood pressure monitor for home use. This can prove helpful for remote or telehealth consultations, such as were widely used during the COVID-19 pandemic, when changes in lifestyle, such as nutrition and exercise, and stress could worsen blood pressure control [90–92]. Patients should bring their home blood pressure monitor to a clinic visit to check their technique and to calibrate it against a clinic device.

A 29-trial meta-analysis by the Blood Pressure (BP) Lowering Treatment Trialists Collaboration (BPLTTC) of individual subject data from over 160,000 type 2 diabetes patients showed that lowering systolic blood pressure by 5 mmHg for

4–5 years with most types of blood pressure drugs reduced coronary heart disease risk by 20%, stroke by 28%, and major CVD events by 22%, with additional reductions in heart failure [93]. The major blood pressure-lowering types of drugs (ACE inhibitors, angiotensin receptor blockers, diuretics, beta-blockers, and calcium channel blockers) protect against cardiovascular, cerebrovascular, and microvascular (e.g., renal) complications [93]. Hence, diagnosing hypertension and treating it to target are clinically worthwhile.

Individualizing BP Targets

Recommended blood pressure targets vary somewhat between guidelines (see Table 29.3 and recent guidelines and reviews [94–98]). Targets vary according to cardiovascular and renal risk. A blood pressure of <140/90 mmHg is reasonable in patients at lower CVD risk (ASCVD risk <15%), but a lower target of 130/80 mmHg is advised in those at higher CVD risk (ASCVD risk >15%) if achievable [94]. Targeting lower systolic blood pressures even in those with renal damage (e.g., <120 mmHg) has not been shown to reduce major cardiovascular events in people with diabetes, and it increases serious adverse events of hypotension and kidney dysfunction [95]. A combination of lifestyle (low salt, DASH diet, minimum alcohol, weight loss, exercise, nonsmoking) and pharmacological treatments is recommended to optimize blood pressure. Several reviews and guidelines have been published, such as by the American Diabetes Association and the UK and European agencies [94–97]. Usually, one blood pressure agent is commenced at a time, unless the initial systolic blood pressure is 20 mmHg or more above goal, when dual antihypertensive therapy is advised [94–98], but a combination of ACEis and ARBs should not be used due to adverse effects on kidney function [98]. Taking at least one blood pressure drug at bedtime, rather than on wakening, has been recommended as it improved ambulatory blood pressure and significantly reduced cardiovascular risk by 67% [99], but the 2022 American Diabetes Association guidelines suggest that this is insufficiently validated [94]. Nonetheless, ensuring good blood pressure control across the 24-h time period is desirable, such as by checking with a 24-h blood pressure monitor or home blood pressure checks at different times of the day.

Table 29.7 summarizes features of some blood pressure-lowering agents. Drug choices can be informed by guidelines [93–98] and are influenced by locally available drugs and combination tablets. Often multiple blood pressure-lowering drugs may be needed to meet recommended blood pressure targets and to avoid side effects associated with high individual drug dosages. Combination tablets can help with patient adherence and reduce costs. In general, drug doses can be titrated after 4 weeks of a regimen as this is the usual time to reach the maximal blood pressure-lowering effect. A common strategy in people with diabetes and hypertension is to start with an ACE or ARB drug, and if insufficient blood pressure control to add a diuretic, and if still insufficient blood pressure control, another drug class such as a calcium channel blocker or beta-blocker, particularly if coronary artery disease coexists. A mineralocorticoid antagonist may also be considered, provided that hyperkalemia is not a concern. Kidney function should be monitored so as to guide

Table 29.7 Clinically available blood pressure-lowering drugs

Drug class	Examples	Mechanism(s)	Usual route of administration	Common side effects	Comment
RAAS drugs					
ACE inhibitors	Ramipril Quinapril Enalapril	Blocks conversion of angiotensin I to II and bradykinin degradation and decreases aldosterone	Oral	Persistent dry cough Elevated K+, decline renal function, hypotension, rash, angioedema	Contraindicated in pregnancy, renal artery stenosis, angioedema
Angiotensin receptor blockers	Irbesartan Valsartan Telmisartan	Antagonizes interaction between angiotensin II and angiotensin receptors, lowers aldosterone levels	Oral	High K+, low blood pressure, decreased renal function, headache, gut upset, abnormal liver function	Contraindicated in pregnancy, and in angioedema
Calcium blocker	Amlodipine, verapamil Diltiazem	Inhibits calcium influx in vascular smooth muscle and heart, causing vasodilation, reduced myocardial contractility, and sinoatrial and atrioventricular node depression Decreases aldosterone	Oral	Dizziness, flushing, constipation, edema, palpitations, hypotension	Contraindicated in sick sinus syndrome, Wolfe-Parkinson-White syndrome, second- or third-degree heart block. Not used with non-dihydropyridine blockers with beta-blockers
Beta-blockers	Metoprolol, atenolol Labetalol	Decrease heart rate and cardiac output, peripheral vasodilation, central effects	Oral	Hypotension, fatigue, Raynaud's, increase glucose levels, bronchospasm	Contraindicated in airways disease, sick sinus syndrome, with cocaine and non-dihydropyridine calcium channel blockers (can cause atrioventricular block)

(continued)

Table 29.5 (continued)

Drug class	Examples	Mechanism(s)	Usual route of administration	Common side effects	Comment
Diuretics Thiazide type (a) Loop diuretic type (b) Potassium-sparing diuretic (c)	(a) Chlorothiazide, indapamide (b) Frusemide (c) Amiloride	Decrease sodium reabsorption at various sites in renal tubules, increasing urinary sodium and water loss, reducing plasma and extracellular fluid volume	Oral, some, e.g., frusemide also IV	Dehydration, hypotension, increased uric acid levels, gout, low magnesium, hyponatremia, muscle cramps, some thiazides worsen glycemia and lipids	Contraindicated in late renal impairment, e.g., eGFR <50 mL/min
Aldosterone antagonists	Spironolactone Eplerenone	Acts in renal tubules to block aldosterone effects, causing renal sodium and water loss and potassium (K+) retention	Oral	Hyperkalemia Spironolactone (nonspecific) so also binds to sex hormone receptors, leading to menstrual irregularities in women and gynecomastia and erectile dysfunction in men	Contraindicated Addison's disease, renal impairment
Renin inhibitor	Aliskiren	Binds to renin and blocks its binding to angiotensinogen		Hyperkalemia, especially if also on ACE inhibitor hypotension, dizziness, gut upset, increases uric acid, rarely angioedema	Contraindicated in late-stage renal disease, e.g., CrCl <60 mL/min
Alpha-blockers	Prazosin Phentolamine Phenoxybenzamine	Blocks alpha-adrenergic receptors, inducing blood vessel dilation	Oral	Postural hypotension, dizziness, tachycardia, nasal stuffiness, peripheral edema, erectile dysfunction, tremor	Usually short-term use only. Contraindicated in breastfeeding. Avoid with renal impairment, coronary artery disease
Central adrenergic agonists	Methyldopa	Decreases adrenergic outflow from the central nervous system, decreases peripheral resistance and blood pressure Can be used for hypertensive crisis Reduces plasma renin but negligible effect on kidney function	Oral or IV	Postural hypotension, bradycardia, dizziness, gut upset, drowsiness, headache, fatigue	Largely replaced by modern drugs

drug choice and doses and effects on electrolytes and renal function. Consideration of regularly updated guidelines such as those by the national bodies, such as the American Diabetes Association, is prudent [94, 96, 97].

Resistant hypertension is more common in people with diabetes. This is defined as not meeting blood pressure targets (usually ≤140/90 mmHg) whilst adherent to lifestyle measures and adequate doses of three classes of antihypertensive drugs, including a diuretic. As well as excluding underlying conditions such as secondary hypertension (e.g., renal artery stenosis) and "white coat" hypertension, addition of mineralocorticoid receptor antagonist therapy should be considered. This class of drug also has cardioprotective and renoprotective effects but, particularly if used with a RAAS drug, can increase the risk of hyperkalemia [94].

Emotions

Mental health issues including anxiety and depression are common in the general population and are often more common in people living with a chronic illness, including type 1 and type 2 diabetes [11, 100–107]. In addition, there is a specific entity of diabetes distress, which refers to the negative emotions arising from living with diabetes and the high burden of daily self-management. Diabetes distress has many similar features of anxiety and depression and can be screened for using short survey tools (e.g., PAID and DDS-2) [101, 102, 104–107]. An estimated 25% of people with diabetes will experience depression, and 40–45% will experience diabetes distress [101, 103]. Suboptimal mental health is associated with higher HbA1c levels and poorer self-care such as nutrition, exercise, prescribed medication use, and more missed healthcare professional visits [101]. Awareness of the condition, regular discussion with patients, counselling such as by a diabetes educator or counsellor, family and peer support, and sometimes referral to a psychologist or psychiatrist can be helpful. There are several recent reviews and practical guides regarding diabetes distress diagnosis and management [104–107]. Anxiety or depression or another coexistent mental illness will sometimes require medications. For example, antipsychotic medications are associated with increased risk of diabetes due to weight gain and adverse effects on insulin secretion and insulin sensitivity [108].

Smoking

Smoking is a major risk factor for cardiovascular disease in the general population and in people with diabetes. It is also a risk factor for type 2 diabetes and for the microvascular complications of both type 1 and type 2 diabetes [11]. Smoking's adverse effects include dyslipidemia, qualitative changes in lipoproteins, worse glycemia, endothelial dysfunction, increased blood pressure, inflammation, and oxidative stress. Smoking cessation reduces mortality risk by one-third after only a few years [109]. People with diabetes are more likely to stop smoking if they receive the

appropriate counselling and support [110], which may include pharmacological therapy (e.g., nicotine patches). The combination of counselling and pharmacologic therapy is more effective than either therapy alone [111]. E-cigarette or "vaping" is not recommended as an aid to smoking cessation, and a meta-analysis shows lower rates of smoking cessation in e-cigarette users than nonusers [112]. Vaping itself can increase the risk of myocardial infarction, even in the general population [113]. A systematic review suggests that vaping can worsen glycemia, triglycerides, blood pressure, and abdominal obesity [114]. Further studies of the long-term effects of vaping on the risk of type 2 diabetes and double diabetes in type 1 diabetes and of complications and mortality in people with type 1 and type 2 diabetes are merited. More importantly, effective strategies to prevent the uptake of smoking and vaping and second-hand smoke inhalation are desirable.

CAD

Clotting, **A**dvocacy, **D**evices

Clotting

Diabetes is associated with pro-clotting changes in platelets and with impaired fibrinolysis, which favor thrombosis. The routine use of antiplatelet agents for people with type 1 or type 2 diabetes as a primary prevention is controversial due to bleeding risk, but is recommended for secondary prevention of cardiovascular disease, unless contraindicated [11, 94]. The 2022 American Diabetes Association guidelines recommend aspiring (75–162 mg/day) in people with diabetes and atherosclerotic cardiovascular disease. If allergic to aspirin, clopidogrel (75 mg/day) can be used [94]. The ADA suggests that aspirin could be considered as primary prevention in diabetes for both sexes aged >50 years and <70 years and ≥1 other major risk factor who are not at increased risk of bleeding (e.g., older age, anemia, kidney disease). For older patients, the risk-benefit ratio does not seem favorable. Similarly, aspirin is not recommended for those <50 years and at low risk of atherosclerotic CVD due to the bleeding risks, nor is it recommended for those <21 years due to the risk of Reye's syndrome [94].

After an acute coronary syndrome event or revascularization, dual-antiplatelet therapy (with low-dose aspirin and a P2Y12 inhibitor) should be considered for at least 1 year. Longer term dual-antiplatelet therapy may be given to diabetic patients with a prior coronary intervention, high risk of further ischemia, and low bleeding risk. Aspirin combined with low-dose rivaroxaban may benefit people with diabetes, stable coronary or peripheral vascular disease, and low bleeding risk. A cardiologist could help guide decisions [6, 94].

The Antithrombotic Trialists' Collaboration individual patient-level meta-analysis included six trials of aspirin for primary prevention in the general population, with >95,000 participants and almost 4000 subjects with diabetes [115]. Aspirin reduced the risk of serious vascular events by 12% (relative risk 0.88 [95% CI 0.82–0.94]), with the greatest reduction for nonfatal MI, with no significant benefit for coronary heart disease death or stroke [115]. In the ASCEND (A Study of Cardiovascular Events iN Diabetes) trial (n = 15,480 diabetes patients with no evident cardiovascular disease), aspirin 100 mg daily vs. placebo over 7.4 years was associated with a 12% reduction in vascular events, but major (predominantly gut) bleeding was significantly increased from 3.2 to 4.1% in the aspirin group [116]. Two other large trials with diabetes subgroups tested aspirin for primary prevention of vascular events (ARRIVE and ASPREE) and found no major benefit on vascular events, but the side effect of bleeding was significantly increased [117, 118].

Further research is merited for both primary and secondary prevention.

Advocacy

An estimated 80% of people with diabetes globally live in disadvantaged regions, where their access to insulin and to other drugs and aspects of diabetes care is limited [1]. Even in advantaged regions, many people cannot afford the access to all healthcare professionals, drugs, and devices that may optimize their health outcomes [14, 16, 119, 120]. Whilst there is always a moral obligation, there is a legal obligation to provide such medical care [15]. International human rights law places obligations on governments to ensure the accessibility and affordability of insulin (a World Health Organization (WHO) essential medicine) and other drugs and to aspects of diabetes care, including information. However, drugs being listed on the WHO lists do not necessarily equate to continuous availability, availability in all urban, rural, and remote parts of the country, and their affordability. Monitoring systems such as glucose testing devices are not included in such essential medicines list. A human rights approach facilitating the improvement of diabetes services and equitable access to diabetes care provides a strong framework for advocacy and policy. My colleagues and I recently published a paper and extensive online supplementary material, a white paper, to provide guidance [15]. There needs to be sustained effort by people with diabetes, their families and community, healthcare professionals, industry, governments, and nongovernment organizations (NGOs) to improve access to diabetes care, including affordable medications and related devices, in all regions. This includes during natural and man-made disasters, situations in which people with diabetes particularly children, those with type 1 diabetes, elderly or frail, pregnant women with diabetes, and people with chronic complications are especially vulnerable [15].

Devices

An increasing array of devices are available to assist in the care of people with diabetes. Some are patient centric and others are clinician centric. Some people with diabetes may choose to use food, weight, and physical activity trackers or smart phone apps. Body weight, body composition scales, and a blood pressure cuff for at-home use may be helpful. During the COVID-19 pandemic, when many diabetes consultations were by phone or telehealth, the availability of such monitoring systems and uploadable glucose monitoring devices and insulin pumps was helpful. Clinician-prescribed 24-h blood pressure monitoring can be used to diagnose hypertension and monitor therapy. Loss of nocturnal blood pressure dipping is often the first sign of hypertension.

Glucose and ketone monitoring systems have advanced rapidly in recent years. Self-monitoring of finger-prick blood glucose and of urine or blood ketones have been possible for decades. With SGLT2 inhibitors and their potential for euglycemic diabetic ketoacidosis, more ketone monitoring is being performed by people with type 2 and type 1 diabetes using SGLT2 inhibitors.

Recently available types of interstitial fluid glucose monitors include real-time monitoring (where the glucose data are available to the user) or masked mode (for clinician or research use with delayed data access by the prescriber or wearer). Depending on the system used, glucose readings are made every 5–15 min for 6–14 days. Data can also be downloaded and a standardized one-page report is provided, including the mean and CV of glucose levels, an estimated HbA1c, and the time in recommended glucose target range, and above and below that target range. An international consensus group has recommended targets for people with type 1 diabetes, type 2 diabetes, and diabetes in pregnancy. A medically implantable system that provides interstitial fluid readings for up to 90 or 180 days has been developed, and others with even longer life spans are in development.

Other devices that can assist diabetes care include SMS messages and reminder services and telehealth, as was used extensively during the COVID pandemic.

STRIVE

Screening, **TR**eating to target, **I**nflammation, **V**accinations, **E**ducation

Screening

All people with type 1 or type 2 diabetes should undergo regular screening for complications and vascular risk factors, usually on an annual basis [11, 94], though less frequent than annual screening for diabetic retinopathy may suffice based on

individual risk [121]. People with youth-onset type 2 diabetes are at particularly high risk of chronic complications (even higher than those with comparable duration of type 1 diabetes) [2–4], likely related to risk factors such as obesity, hypertension, and dyslipidemia.

In general, weight/BMI and blood pressure should be checked at each visit and HbA1c and glucose monitoring should be checked at each 3–4 monthly visit, with foot (neurovascular, skin, and nail) status, lipids, and kidney function checked at least annually, and more often if interventions are being made. If risk factors, such as dyslipidemia or hypertension, are identified, they should be treated and their levels reassessed, usually after 1–2 months of therapy, with titration of therapy as required [11].

As yet, there is no recommendation for routine screening for asymptomatic coronary artery disease in adults with diabetes, provided that there is aggressive risk factor management. Consultation with a cardiologist should be considered for those considering commencing an exercise program or if there are atypical symptoms or evidence of silent ischemia on an EKG [94]. Coronary artery calcification scoring and CT coronary angiograms can identify subclinical disease, but as yet there are no studies to provide guidance.

Adults with diabetes should also undergo age, sex, family history, and environmental exposure appropriate screening for cancers as they are at the same or increased risk of most cancers than their nondiabetic peers [94]. This may relate to the diabetes milieu or comorbidities such as obesity or smoking. Whilst prostate cancer incidence is lower in men with vs. without diabetes, perhaps related to lower testosterone levels in diabetes, especially with poor glucose control and obesity, it is still common enough to merit regular screening in men with diabetes.

Some anticancer immunotherapies or chemotherapy regimens, such as checkpoint inhibitors, or corticosteroids that are often included in chemotherapy regimens, can also induce diabetes or worsen glucose control [122]. Clinicians should monitor for this and counsel patients accordingly.

TReating to Target

Most people with diabetes do not meet all recommended treatment targets such as for HbA1c, lipids, blood pressure, kidney function, BMI, and nonsmoking. In our survey of 282 Indigenous Australians with type 2 diabetes, a group at high risk of diabetes complications, who were attending Indigenous-led primary care practices, the median number of nine risk factors at target was three, with achievement rates of individual factors ranging from 20% for obesity to 64% for nonsmoking [123, 124]. These results were similar to surveys in other Indigenous health services in Australia and to results in non-Indigenous Australians [124].

Multiple reasons for risk factors not meeting recommended targets are likely contributory. This includes the often demanding and complex multidrug and lifestyle-based treatment regimens, clinical inertia by the treating diabetes care team [125], and suboptimal adherence to recommendations by the person with

diabetes. Clinical inertia may be mitigated by educational or learning interventions targeting cognitive barriers to medication management, and empowered patients. Clinician diligence, a regular review of targets met, and audits of patient records, which some electronic medical records may facilitate, may be helpful. Electronic decision support tools can also assist with identifying subjects not at target, and suggesting therapeutic steps may be helpful [6, 125]. Adequate time spent with the person with diabetes and adaptation of consultations to their level of health literacy are key. Culturally appropriate means of health care and education should be provided.

Patient nonadherence to diabetes care plans may arise due to challenges of maintaining a healthy lifestyle, complexity of their drug regimen, perceived or real side effects or risks, inability to afford all prescribed medications, and mental health issues such as diabetes distress or depression. Adequate health insurance is essential to ensure access to healthcare professionals and to treatments. We are aware that it is not uncommon for some patients to reduce their tablet doses or to alternate taking for example their blood pressure vs. their statin drugs so as to reduce their medication costs. This would contribute to higher variability in risk factors and poorer control. In a nonjudgmental way, clinicians should inquire about the frequency of missed doses rather than just up-titrating prescribed doses. Sample packs can be a short-term stop-gap measure, using lower cost or combination drugs where possible, social worker input, application to any drug company or other aid programs, and advocacy.

Inflammation/Infections

People with diabetes are at increased risk of infection and poor wound healing, particularly if they have suboptimal glucose control. Common infections include urinary tract infections, which may be silent, particularly in the elderly and during pregnancy; fungal infections of the skin; and genitourinary tract and periodontal disease [126]. Periodontal disease has been associated with increased risk of cardiovascular disease, diabetic microvascular complications, and death [127, 128]. Poor wound healing of diabetic foot ulcers, which may be promoted by ischemia, neuropathy, infection, and injury, can contribute to infection and need for amputation. Appropriate care, including debridement and many additional, usually topical drugs, can promote wound healing and delay or prevent amputations [129]. Of lipid drugs, the FIELD trial demonstrated an almost halving of the rate of lower limb amputations in people with type 2 diabetes, driven by below-ankle “microvascular amputation” protection [130]. Our related basic science paper suggested a PPAR-α-independent mechanism related to TXNIP [131].

As recently observed in the COVID pandemic, people with diabetes are also at increased risk of infection and of adverse outcomes such as requiring hospitalization, ventilation, death, and long COVID [90–92, 132, 133]. Worse glucose control preinfection is associated with high risk of infection and a poorer prognosis from

COVID infection [134]. People with diabetes should be considered for any appropriate antiviral therapy and supportive care.

Vaccinations

Due to their increased risk of infection and often poorer outcomes, people with diabetes should have all age-appropriate vaccinations. This usually includes vaccination such as against influenza (annually), pneumococcal pneumonia, tetanus, COVID, and shingles, and others as appropriate to age, sex, past history, occupation, environment, and any travel-related exposure risks [11].

Education

The diabetes knowledge base is rapidly increasing; hence, it is important for both clinicians and people with diabetes to remain up to date. Education should be regarded as an ongoing process, and the manner and amount of information provided to a person with diabetes be personalized to their current state of knowledge, desire for information, literacy, and health literacy, and should be delivered in a culturally appropriate manner and format. Asking them to "teach back" or explain what they understand of the key points is a good means of checking patient understanding and actively involving them in the discussion.

In an American Association of Diabetes Educators' systematic review, diabetes self-management education was associated with a statistically and clinically significant 0.74% HbA1c (in % units) reduction [135]. People with diabetes should be educated at diagnosis, and repeat education should be provided over their lifetime. Diabetes is a complex condition, and personalized treatment goals and available medications, tests, and devices change over time. Ideally, patients should be referred for structured diabetes education and to a dietician and other allied healthcare professionals, such as a podiatrist and exercise physiologist and psychologist if available. Various care team members should avoid providing conflicting advice as this is confusing and worrying for patients and may promote inaction. Clinicians may assist patient education by referral to reliable websites, such as those provided by national diabetes associations, such as the American Diabetes Association (www.diabetes.org) and Diabetes Australia (www.diabetesaustralia.com.au). Accurate information may be misunderstood by the person with diabetes, and some social media sites or lay public members may, well-meaningly or otherwise, provide diabetes patients with unreliable information, including the use of unproven therapies. Clinicians should be open to discussing such topics.

Education is also key to support people with diabetes to undertake and continue the many required lifestyle changes, medications, and devices that can optimize their health outcomes. The personal and financial costs, actual and potential side effects, and drug interactions can be challenging.

Other Mnemonics for Cardiovascular Disease

Coronary Artery Disease

As an estimated 60% of deaths in people with diabetes are due to cardiovascular disease, it is critical that clinicians implement proven primary and secondary prevention therapies. Cardiologist Professor Anthony Keech has developed a series of mnemonics for atherosclerotic cardiovascular disease secondary prevention: **Fairly fast SA^{2}A^{2}B Convertible** (fish oil, fibrate, statin, dual-antiplatelet therapy, ACEi, aldosterone antagonist, beta-blocker, clopidogrel) is an evidence-based mnemonic for the secondary prevention of cardiovascular disease in the general population, which is also relevant to people with diabetes. The rationale for each element in this mnemonic is detailed in the related publication [136]. These elements should be discussed with the treating cardiologist and also with the patient and their family as to the reason for each of the medications. Medication tolerance and ongoing adherence should be regularly reviewed.

Heart Failure

We and others have reviewed the pathophysiology and diagnosis of heart failure in people with diabetes [137–142]. Heart failure in people with diabetes may be due to ischemia, hypertension, or diabetic cardiomyopathy, or a combination thereof. Other conditions such as valvular disease, inherited cardiomyopathy, and viral myocarditis or drug-induced cardiomyopathy may also occur. Generally, heart failure is more common and has a poorer prognosis in people with diabetes than in nondiabetic subjects. Heart failure can be divided into that with reduced or preserved ejection fraction (HFrEF or HFpEF), with the latter accounting for about 50% of heart failure. A long subclinical phase is recognized, but as yet no routine cardiac imaging for its diagnosis, such as echocardiography or cardiac MRI, is recommended. Treatment of HFpEF is particularly difficult with as yet there being no specific treatments, nor has any therapy or combination thereof been shown to reduce mortality.

Prof. Anthony Keech and Dr. Jordan Fulcher and colleagues have developed and published a mnemonic for the treatment of heart failure. BANDAID2 (beta-blocker, ACEi/ARB/angiotensin receptor-neprilysin inhibitor (ARNI), nitrate-hydralazine, diuretics, aldosterone antagonist, ivabradine, devices, and digoxin) is an evidence-based mnemonic for the treatment of heart failure with reduced ejection fraction (HFrEF) [122]. Further detail is provided in the original paper [143]. In addition, as per more recent trials, SGLT2 inhibitors (or GLP-1 analogues if the former is not tolerated or contraindicated) should be considered in type 2 diabetes patients with heart failure. In class II or III HFrEF patients without prior angioedema, current guidelines recommend switching ACEi/ARB therapy to an ARNI (e.g., Entresto), as in a large RCT [144], ARNI compared to enalapril reduced the composite outcome of cardiovascular mortality or HF by 20% [145].

Table 29.8 Summary of evidence of some drug treatments on diabetes complications

Risk factor	Main example drugs	Reduces CVD events		Reduces CVD mortality	
		Primary prevention	Secondary prevention	Primary prevention	Secondary prevention
Glucose	Biguanides, sulfonylureas, DPP-4 inhibitors[b], thiazolidinediones[b], insulin	Contrasting evidence	Contrasting evidence	Contrasting evidence	Contrasting evidence
	Empagliflozin (SGLT-2 inhibitor)	Insufficient evidence	Yes	Insufficient evidence	Yes
	Canagliflozin (SGLT-2 inhibitor)	Insufficient evidence	Yes	Insufficient evidence	No
	Liraglutide (injectable GLP-1 agonist)	Insufficient evidence	Yes	Insufficient evidence	Yes
Lipids/ lipid drugs	Statins	Yes	Yes	Yes	Yes
	Ezetimibe	Yes	Yes	No	No
	Fenofibrate	Yes, with dyslipidemia	Yes, with dyslipidemia	No	No
	PCKS9 inhibitors	Insufficient evidence	Yes	Insufficient evidence	No
Obesity	Orlistat	Insufficient evidence	Insufficient evidence	Insufficient evidence	Insufficient evidence
	Bariatric surgery (morbidly obese)	Yes	Yes, less than 1° prevention	Yes	Yes, less than in 1° prevention
Blood pressure	ACEI/ARBs, diuretics, calcium channel blockers, beta-blockers, spironolactone	Yes	Yes	Yes	Yes
Smoking	Nicotine replacement, varenicline, bupropion	Yes	Yes	Yes	Yes
Obesity	Orlistat	Insufficient evidence	Insufficient evidence	Insufficient evidence	Insufficient evidence
	Bariatric surgery (morbidly obese)	Yes	Yes, less than in 1° prevention	Yes	Yes, less than in 1° prevention
Clotting	Aspirin, clopidogrel (in patients who cannot tolerate aspirin)	Yes[a, b]	Yes[a, b]	? No	Yes
Devices	Insulin pumps vs. injections in T1D			Yes	

"No" suggests negative results in ≥1 large RCT

T1D type 1 diabetes

1° = primary

[a] Consider in high-vascular-risk patients (10y ASCVD risk >10%) after consideration of bleeding risks

[b] Can cause adverse outcomes in certain settings

Table 29.9 Summary of evidence of some risk factor treatments on diabetes microvascular complications

Risk factors	Main example drugs	Reduces retinopathy		Reduces nephropathy		Reduces neuropathy	
		Primary prevention	Secondary prevention	Primary prevention	Secondary prevention	Primary prevention	Secondary prevention
Glucose	Biguanides, sulfonylureas, DPP-4 inhibitors, GLP-1 agonist, thiazolidinediones, insulin	Yes, in recent onset T2D [43, 45]	Yes, in recent-onset T2D diabetes [43–45]	Yes [43]	Yes [43]	Yes [44, 146]	Yes [44, 146]
Lipids	Statins	? Contrasting evidence [129]	No	Reduces albuminuria but not eGFR/BUN[a]	Reduces albuminuria but not eGFR/BUN[a]	? Yes	? Insufficient evidence
	Fenofibrate	No	Yes	Protective for eGFR and albuminuria[a]	Protective for eGFR and albuminuria[a]	Yes	Yes
Blood pressure	ACEI/ARBs	Yes [127]	Contrasting evidence	Yes, but ↑ risk of CVD death[a]	Yes, ↑ benefit	No	No
	Diuretics, calcium channel blockers, beta-blockers	Yes [127]	Contrasting evidence	Yes, but ↑ risk of CVD death[b]	Yes	No	No
Insulin pumps Smoking		Yes Yes	? ?	? Yes	? ?	Yes	? ?

"No" suggests negative results in ≥1 large RCT

a Currently no renal indication for statins or fenofibrates

? unknown

[b] Currently no indication for antihypertensive agents in the primary prevention of diabetic nephropathy

Summary

There are multiple risk factors for the microvascular and macrovascular complications of diabetes and hence multiple treatment targets that can be addressed using drugs for glucose, lipids, blood pressure, and clotting. Tables 29.8 and 29.9 provide a simple summary of the current state of benefit for diabetes complications.

Conclusions

There are many types of diabetes and diabetes complications, with many related risk factors, and thankfully, an increasing range of effective therapies that can ameliorate the chronic complications of diabetes, and even diabetes itself, particularly type 2 diabetes. There are multiple factors to address including glucose, lipids, blood pressure, emotions, smoking (GLOBES), clotting, advocacy, and devices (CAD), screening, treating to target, inflammation/infection, vaccinations, and education. A multidisciplinary team holistic approach to patient-centered care is ideal, which can be challenging given the large number of people with diabetes and increasing complexity of care.

Even more therapies and devices are likely to arise in future, enhancing the complexity of care regimens for both the patient and their treating clinicians. Attention to optimal models of care will be key. Shared roles within the team and electronic decision support tools may assist in the optimization and delivery of the care strategy for each person with diabetes. The effort will be worth it to reduce the personally and socioeconomically costly microvascular and macrovascular complications of diabetes. Advocacy at individual, local, national, and global levels, from the grassroots up and from the top down, is also important to ensure more equitable access to quality diabetes care for all people with or at risk of diabetes.

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Chapter 30
Emerging Lipoprotein-Related Therapeutics for Patients with Diabetes

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Introduction

Dyslipidemia, predominantly elevated low-density lipoprotein (LDL) cholesterol, lowered high-density lipoprotein (HDL) cholesterol, and elevated triglycerides, is a major risk factor for the development of atherosclerosis, which is in turn the principal pathophysiological process, underlying the mortality arising from cardiovascular disease (CVD) [1–4]. Recent data has indicated that lifetime exposure to cholesterol may be a risk factor for CVD [5]. The atherothrombotic events leading to tissue ischemia and heart attacks, strokes, and lower limb amputations are a major cause of morbidity and mortality in people with and without diabetes [6, 7]. The impact of atherothrombotic disease is such that it is a major cause of mortality in those without diabetes and it is the major cause of premature mortality in people with diabetes [8]. Diabetes affects the lipid profile and strongly accelerates the process of atherogenesis, but the actual mechanisms have remained elusive and the contribution of diabetic dyslipidemia is also strong but of uncertain etiology [6, 9]. The major pathophysiological and therapeutic implication is that diabetes is itself an independent risk factor for atherothrombotic CVD, so when combined with other risk factors, the impact is amplified [7, 10]. Considering dyslipidemia as an independent risk factor, then the person with diabetes is very vulnerable to the combined effect of these risk factors, let alone should they have other risk factors such as hypertension or other lifestyle risk factors such as cigarette smoking [11, 12].

The aim of this chapter is to briefly update the important issues on the latest concepts around the development of atherosclerosis, to consider developments in our understanding of the role of lipids and lipoproteins in the etiology of atherosclerosis and the role of medical treatments in preventing or reducing the process of atherosclerosis. The chapter will then be used to expand on the "emerging" agents for the treatment of lipid and lipoprotein disorders—there is a large amount of information available on existing agents already in widespread clinical use, so this chapter will look forward and focus on those agents which are just outside widespread clinical usage, which appear to have a high probability of joining the therapeutic armamentarium for the treatment of lipid and lipoprotein disorders in people with diabetes.

Recent Developments in the Understanding of the Etiology of Atherosclerosis and Its Thrombotic Consequences

The understanding of the biochemical and cellular process underlying atherosclerosis has not changed greatly in the last two decades since the introduction of the concept of inflammation—the role of the immune system in perpetuating unresolving inflammation in the wall of large blood vessels [13–16]. It has in part evolved that some see hyperlipidemia and inflammation as two distinct elements of atherosclerosis, but the most convincing descriptions see these as parallel processes with

benefits arising from treating both elements. The clinical consequences of atherosclerosis, which can be morbidity and even premature mortality, arise from the atherothrombotic events of blood clot formation within a vessel and downstream tissue ischemia [17–19]. The critical pathological parameter has been considered to be the formation and breakdown of atherosclerotic plaques—lipid-laden complex entities with thin fibrous caps, which are speculated to randomly rupture causing acute focal vessel occlusion [20, 21]. In very recent times, some have moved the focus to the erosion theory whereby the atherothrombotic event occurs on an area of vessel wall denuded of endothelium; however, there is not a great deal of evidence in support of this hypothesis and the clinical prominence of vulnerable plaques and plaque rupture remains as the focus of a huge amount of basic and clinical research work [22, 23].

Lipids, Lipoproteins, and the Development of Atherosclerosis

In the area of the contribution of lipids and lipoproteins to atherogenesis, elevated levels of LDL and its associated ApoB-containing lipoproteins retain its status as the major lipid-related causative factor in the development of atherosclerosis [24]. Similarly, the hypothesis that the LDL is retained in the vessel wall due to binding and retention by modified proteoglycans with hyper-elongated glycosaminoglycan chains has been supported by ongoing in vitro and in vivo evidence [25–27]. Therapies which target elevated levels of LDL, and especially some such as statins, which have additional beneficial pleiotropic anti-inflammatory actions, thus hold a primary place in the therapeutic armamentarium to mitigate the lipid contribution to atherothrombotic complications leading to CVD [28–31].

There has been ongoing intense research and conceptualizations of the relationship of lipids to atherosclerosis, and some movements in this area have occurred in recent times. The most prominent areas have been the role of HDL and triglyceride-rich lipoproteins (TGRL). Over the years, triglycerides (TGs) have played a subordinate role to LDL as a focus of interest as a causative factor for atherosclerosis [32–35]. However, genetic studies have emerged which support a causative role for TGRL such as chylomicrons, VLDL, and their remnants in atherosclerosis. Lipoprotein lipase (LPL) is a widely expressed water-soluble enzyme, which uses ApoC-II as a cofactor to hydrolyze TGRL to free fatty acids (FFAs) and monoacylglycerols. ApoCIII, Angptl3, and Angtl4 inhibit the activity of LPL to hydrolyze TGs in TGRLs, leading to an accumulation of TGRL particles [34, 35]. Clearly, modulating the activity of LPL can alter the distribution of TGRLs and alter the atherogenic profile of blood. Agents modulating these parameters have emerged as potential therapeutic agents and are discussed in this chapter.

Medical Management of Dyslipidemia for Reducing Atherosclerosis

In the last several decades, HMGCoA reductase inhibitors (statins) have established themselves as one of the most efficacious and widely used drugs for the treatment of hyperlipidemia and the primary and secondary prevention of atherosclerosis and consequent CVD events [36]. Literally, millions of patients have taken statins, and this therapeutic category has contributed to the reduction in cardiovascular events and deaths. Indeed, some claims which overstate the side effects of statins leading to reduced prescribing or taking of statins may have led to unnecessary morbidities and mortalities [37]. However, although highly efficacious, in multiple trials, the effectiveness of statins remains around a 30% reduction in cardiovascular events [38]. Hence, the need has existed to discover additional therapies, which can improve the response of the therapy of dyslipidemia. There have been two major approaches—adding to the lipid-lowering impact of statins or alternative biochemical mechanisms which complement the action of statins, for example, anti-inflammatory agents or other LDL-lowering agents.

In this chapter, to maintain context, we will only consider agents which target favorable changes in lipid profiles such as PCSK9 inhibitors and the new class of ATP citrate lyase (ACLY) inhibitors such as bempedoic acid. Furthermore, in the context of statin therapy, it has emerged that there are patients who cannot tolerate statins due to adverse effects, commonly muscle-related side effects, and those who do not wish to take statins due to their analysis of information and misinformation in the public domain. These latter groups will require alternative therapies to statins such as other LDL-lowering drugs and/or agents blocking interactions between lipoproteins and extracellular matrix (discussed in another chapter in this book).

Specific Approaches to the Modification of Lipid Profiles to Reduce Cardiovascular Disease

Ongoing research into lipoprotein and lipid metabolism, and especially its relationship to the development and progression of atherosclerosis, has been remarkably successful in identifying new therapeutic targets, which can be exploited to normalize dyslipidemias. In this context, work establishing the validity of PCSK9 as a target has opened the field to newer methods of targeting this protein, to other LDL-lowering agents such as ACLY inhibitors, products related to omega-3 fatty acids, apolipoprotein C3, angiopoietin-related protein 3 (Angptl3), PUFAs, specifically icosapent ethyl and the PPAR system, notably PPAR-α agents, have provided pathways leading to new agents to address the dyslipidemia and related complications of patients with diabetes. In some cases, in parallel with more general work, the rapid development of new biological agents, notably antibodies and

mRNA-modulating agents, has also been successfully applied to the area of lipoprotein abnormalities and CVD. This chapter explores some of these agents, which are opening new frontiers for the treatment of dyslipidemia and reducing cardiovascular and microvascular disease risk. For each target and agent(s), we have set out to define the basic biochemical mechanism of its involvement in lipid and lipoprotein metabolism and identify pleiotropic actions both on and off target, actions in cells and animals, preliminary data in clinical trials, and speculations on its potential clinical utility including potential adverse drug effects.

PCSK9 Inhibitors and CRISPR Drugs

Ever since the discovery by Nikolai Anichkov in St. Petersburg (1913) that cholesterol feeding to rabbits would induce atherosclerosis [39], therapy targeting cholesterol has been the main therapeutic strategy to prevent the development and progression of atherosclerosis. Prior to Anichkov's discovery, Ignatowski had described a relationship between cholesterol-rich food and experimental atherosclerosis [40] and Windaus showed that atheromatous plaques contained 6 times more free cholesterol and 20 times more esterified cholesterol compared to the normal arterial wall [41]. Forty-five years later, the first association between plasma cholesterol and atherosclerotic heart disease was published, summarizing the results of the "Coronary Heart Disease in the Framingham Study," indicating the high risk of myocardial infarction in subjects with high plasma cholesterol levels [42]. Diet was the only way of controlling plasma cholesterol until 1987 when the cholesterol-lowering "statin" agent lovastatin became available [43, 44]. Lovastatin inhibits HMGCoA reductase, which is required for cholesterol synthesis; its design was based on weaker structurally related inhibitors isolated from *Penicillium citrinum* 10 years earlier [45].

Today, a large variety of cholesterol-lowering agents targeting its biosynthesis are available for clinical use and can be combined with agents from other lipid drug classes. Many clinical trials have confirmed the importance of lowering plasma cholesterol levels to reduce the frequency of ischemic heart disease, myocardial infarction, and stroke [46–48], including studies in high-risk patients, such as those with type 2 diabetes [49] or rheumatoid arthritis [50, 51]. Combination therapy is frequently used in high-risk patients with type 2 diabetes, where rates of cardiovascular events can remain elevated even with statin therapy. Addition of ezetimibe, a once-daily oral agent that targets the Niemann-Pick C1-like protein to reduce cholesterol absorption from the intestine, has been combined with statins in both type 2 diabetes [52] and patients with acute coronary syndromes [53]. Similarly, fibrates such as gemfibrozil or fenofibrate, which lower blood triglyceride levels and cause mild reductions in LDL cholesterol levels, have been used with statins in type 2 diabetes, but this combination appears somewhat limited in efficacy for additional cardiovascular event reduction [54]. Both middle-aged and older people can benefit from cholesterol lowering. Therapeutic targets vary depending on whether a patient

is high or very high risk. In high-risk patients, the recommended therapeutic LDL-C goal is <100 mg/dL (2.6 mmol/L), whilst in very high-risk patients, <70 mg/dL (1.8 mmol/L) is a therapeutic option [55]. Despite the proven benefits of cholesterol lowering with statins, nonadherence is a growing concern, driven in part by the lack of understanding of the significance of the treatment in preventing coronary heart disease and ischemic strokes as well as skepticism on treatment efficacy and complexity of medication regimens [56]. Adherence to recommended therapy is highest following myocardial infarction and lowest in those with hyperlipidemia who do not have coronary heart disease or diabetes [57]. Statin intolerance also contributes to nonadherence, increasing the dependence on other less efficacious therapies such as ezetimibe [58].

In this section, we focus on newer cholesterol-lowering therapies to overcome some of the complications and limitations of lowering cholesterol with statins. We also focus on new developments in anti-inflammatory therapies, which can be combined with statin therapy to more effectively reduce plaque inflammation and progression than with cholesterol-lowering agents (predominantly statins) alone [59].

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

PCSK9 Biology

Seminal studies by Marianne Abifadel and associates report that two gain-of-function mutations in the PCSK9 gene encoding proprotein convertase subtilisin/kexin type 9 were associated with autosomal dominant hypercholesterolemia and uncovered a key player in cholesterol homeostasis [60]. This was followed by reports of low LDL cholesterol levels in African Americans, but not European Americans, resulting from frequent nonsense mutations in PCSK9 [61] in the former; together, the two studies indicated that common sequence variations in PCSK9 have large effects on plasma cholesterol levels in selected populations and opened a new chapter in cholesterol therapeutics. Subsequent studies indicated that nonsense mutations in PCSK9 were associated with not only reductions in LDL cholesterol but also substantial reductions in the risk of coronary heart disease [62].

PCSK9 is a 74 kDa zymogen comprised of four domains: the N-terminal pro-domain, the signal peptide, the catalytic domain, and the C-domain. The pro-domain acts to modulate the effects of PCSK9 by releasing the catalytic domain. Its main activity is related to its binding to specific target proteins and escorting the resulting complexes towards intracellular degradation compartments. The first protein target to be identified was the LDL receptor (LDLR) on the surface of hepatocytes (Fig. 30.1). Under normal circumstances, LDL binds to the LDLR on the hepatocyte surface and the LDL/LDLR complex is internalized and delivered to endosomes. In the low-pH environment of the endosome, the LDLR changes its conformation

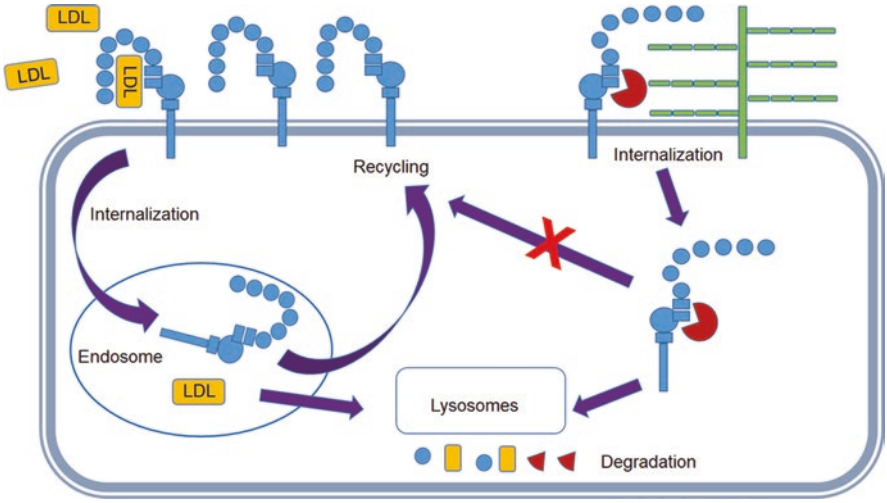

Fig. 30.1 Proposed mechanism by which PCSK9 targets LDLR for degradation. At neutral pH, LDLR on the hepatocyte cell surface adopts an open extended form and binds LDL predominantly via the ligand-binding domain (circles). This is followed by internalization into endosomes, whereupon LDL is targeted to lysosomes for degradation and LDLR is recycled to the cell membrane. LDLR can also bind with PCSK9, which is bound on the cell surface to membrane proteoglycans. Upon binding, the LDLR-PCSK9 complex is internationalized into endosomes before being targeted to lysosomes for destruction. This effectively reduces membrane LDLRs and their capacity to remove LDL, resulting in hyperlipidemia

enabling the release of bound LDL. The LDLR then recycles to the hepatocyte cell surface, whilst LDL is delivered to lysosomes; LDLRs can undergo multiple rounds of internalizations and recycling. Direct binding of PCSK9 to LDLR at the hepatocyte cell surface targets the LDLR for degradation. Although PCSK9 catalytic activity is not required for this function [63], a short segment of amino acids within the catalytic domain (amino acids 367 to 380) is required for binding to epidermal growth factor-like repeat A (EGF-A), the first of the three EGF-like repeats in the EGF precursor domain of LDLR. Also, the β-propeller domain and at least three copies of the ligand-binding repeats of the LDLR are required for PCSK9-mediated degradation of the receptor [64, 65]. Liver heparin sulfate proteoglycans on the hepatocyte cell surface are PCSK9 receptors and essential for PCSK9-induced LDLR degradation. The heparin sulfate-binding site is located in the PCSK9 prodomain and formed by surface-exposed basic residues interacting with trisulfate heparin sulfate disaccharide repeats; monoclonal antibodies directed against the heparin sulfate-binding site are potent inhibitors of PCSK9 [66]. Endocytosis of PCSK9/LDLR complexes occurs via both clathrin- and caveolae-dependent mechanisms, with caveolae-mediated endocytosis being dependent on PCSK9 interacting with cyclase-associated protein 1 [67]. PCSK9 effects are not restricted to LDLR, rather PCSK9 can also induce degradation of other members of the LDLR family, particularly two of the most structurally related receptors, VLDLR and ApoER2,

which have 59% and 46% identity, respectively, to LDLR; this occurs independently of any effects on LDLR [68]. Low-density lipoprotein receptor-related protein 1 is similarly affected [69]. PCSK9 can also induce the degradation of CD36, a scavenger receptor and fatty acid transporter involved in triglyceride accumulation and immunity [70], as well as non-acetylated intermediates of the nascent membrane protein BACE1 (β-site amyloid precursor protein-cleaving enzyme 1), which is involved in the generation of amyloid β-peptide implicated in Alzheimer's disease [71].

The cargo receptor SURF4 physically associates with intracellular PCSK9 and promotes its cellular secretion [72]. In plasma, PCSK9 can be found in two main forms: an intact heterodimer which is considered as the more active form exhibiting stronger binding to and degradation of LDLR and a furin-cleaved heterodimer which binds more weakly to LDLR and is considered the less active form [73, 74]. In contrast, intracellular PCSK9 is only found in its proprotein form or as an intact heterodimer ready for secretion [75], suggesting that furin cleavage is mediated largely extracellularly. Direct regulation of PCSK9 in plasma can also occur via interactions with LDL. PCSK9 also associates with lipoprotein a (Lp[a]) but does not bind with other apoB-containing lipoproteins such as VLDL. Intact PCSK9 heterodimer associates with LDL, and the furin-cleaved PCSK9 exists as the unbound form; LDL appears to protect PCSK9 from furin cleavage, and it is this form that binds more strongly to LDLR [76].

PCSK9 is predominately expressed in liver and intestine, with lower levels in kidney. Transcription of the PCSK9 gene, up or down, is determined by the abundance and activities of specific nuclear factors acting in cooperation or in competition with cis-regulatory elements. PCSK9 is regulated by cholesterol via the sterol regulatory element-binding protein (SREBP). The promoter region of the PCSK9 gene contains an SP1 site and steroid regulatory element (SRE) that makes transcription of PCSK9 dependent on steroids. In mice, expression is downregulated by dietary cholesterol and upregulated by overexpression of SREBP1a and SREBP2. Statins, which inhibit the rate-limiting enzyme in cholesterol biosynthesis, HMGCoA reductase A, increase PCSK9 mRNA via SREBP2, which can be potentiated by hepatocyte nuclear factor-1α (HNF1α) binding to an element located 28 nucleotides upstream of SRE [77]; upregulation of HNF1α expression by statins contributes to PCSK9 production [78]. A histone nuclear factor P (HINFP) located between the HNF1 and SRE is also important for both basal and sterol-regulated PCSK9 transcription; knockdown of HINFP greatly reduces acetylated histone H4 on the PCSK9 promoter and lowers PCSK9 protein. HINFP appears to be a coactivator in SREBP-mediated PCSK9 gene transactivation [79]. HNF1α and SREBP2 appear to be at the crossroads of transcriptional pathways that regulate the expression of PCSK9 as mTOR, which downregulates HNF1α results in reduced PCSK9 transcription and sirtuin 6, which has inhibitory effects on SREBP2, having similar effects [80, 81].

Despite the recent intense focus on PCSK9 in atherosclerotic cardiovascular diseases, PCSK9 biology and its impact extend beyond the cardiovascular system. Recently, it has been shown that PCSK9 can disrupt the recycling of MHC class I

molecules to the cell surface by physically associating with them and promoting their relocation to and degradation in lysosomes, in a manner similar to its negative regulation of the LDLR [82]. MHC I is expressed on the cell surface of all nucleated cells where its function is to display peptide fragments of proteins generated by cells to cytotoxic T cells; patients with high tumor PCSK9 mRNA expression have a worse overall survival than those with low mRNA expression. Inhibition of PCSK9 has been shown to potentiate immune checkpoint therapy for cancer [82]. Cardiovascular events in patients receiving checkpoint inhibitors are significantly higher and mediated potentially by accelerated progression of atherosclerosis [83, 84].

Because PCSK9 is produced by many cell types in addition to hepatocytes, it may also contribute to atherosclerosis by mechanisms independent of the effects on hepatocytes. In ApoE-deficient mice, overexpression of PCSK9 increases atherosclerosis without affecting plasma lipids [85]. The effects of ApoE are exerted via ApoER2, a member of the LDLR family, which is expressed by platelets, endothelial cells, monocytes, and macrophages [86], and decreased by PCSK9. A reduction in ApoER2 increases both inflammation and foam cell formation [68]. PCSK9 is expressed in carotid atherosclerotic plaques together with other PCSKs including PCSK5, PCSK6, and PCSK7 whose significance is still unknown [87]. In plaques, secretion of PCSK9 by vascular smooth muscle cells can significantly affect the functions of adjacent cells such as macrophages, affecting their uptake of VLDL and LDL [88] as well as ABCA1-mediated cholesterol efflux [89]. PCSK9 also induces pro-inflammatory responses in macrophages, increasing their expression of cytokines such as IL-1β, IL-6, and TNF-α and chemokines CXCL2 and MCP1 that promote atherosclerosis [90]. PCSK9 in dendritic cells is important for atherosclerosis, and its inhibition reduces the differentiation and proliferation of T cells [91]. PCSK9 regulates endothelial cell apoptotic responses, and its deletion with siRNA inhibits oxidized LDL (oxLDL)-induced apoptosis [92]. Recent studies indicate that PCSK9 affects immune cell functions that may impact atherosclerosis. Deficiency in PCSK9 decreases plasma IL-17, atherosclerotic plaques, and IL-17-producing T cells [93]. In CD8+ T cells, LDLR interacts with the T-cell receptor (TCR) complex and regulates TCR recycling and signaling, thus facilitating the cytotoxic functions of CD8+ T cells; PCSK9 prevents recycling of LDLR and TCR to the plasma membrane, thereby reducing the effector function of cytotoxic CD8+ T cells. Inhibiting PCSK9 potentiates their cytotoxic effects by promoting LDLR-mediated TCR recycling [94, 95]. In rheumatoid arthritis, low levels of PCSK9 are associated with remission when treated with anti-TNF-alpha therapies, which may also impact their high susceptibility for developing atherosclerosis, but mechanisms are unclear [96]; methotrexate which is used in the treatment of rheumatoid arthritis and psoriasis decreases PCSK9 levels [97], but such reductions apparently do not significantly impact atherosclerosis [98]. Mendelian randomization studies indicate that patients carrying loss-of-function PCSK9 genetic variants display not only lower LDL cholesterol but also an increased risk of developing type 2 diabetes [99].

Current and Future Directions in PCSK9 Therapy

The discovery of PCSK9 as a regulator of plasma cholesterol has resulted in a new era in lipid-lowering therapy. As well as the existent PCSK9 inhibitors, multiple other therapeutic agents/strategies have been developed or are in development to lower PCSK9. These range from antibodies and novel siRNA strategies to small molecules and vaccinations aimed at generating long-term production of antibodies. We will now provide an overview.

Monoclonal Antibodies

At least six monoclonal antibodies are being investigated for reducing LDL cholesterol levels, with evolocumab and alirocumab already approved by the FDA as adjunct therapies to diet and maximally tolerated statin therapy in high-CVD-risk patients. Evolocumab is a fully human monoclonal IgG2 antibody selected for its ability to bind both wild-type and gain-of-function mutant PCSK9. Although the mechanism through which evolocumab targets PCSK9 has not been fully elucidated, examination of the crystal structure of PCSK9 in complex with a Fab fragment from a similar human monoclonal antibody indicates that it binds to the catalytic domain of PCSK9 with the antibody epitope on PCSK9 being adjacent to the region of PCSK9 required for LDLR interaction [100]. This interaction sterically hinders the interaction of PCSK9 with the LDLR, effectively blocking the ability of PCSK9 to initiate degradation of the LDLR. Multiple clinical trials have shown that evolocumab and alirocumab, which are given by subcutaneous injection every 2–4 weeks, very effectively lower LDL cholesterol in combination with statins and also greatly improve clinical outcomes.

In the ODYSSEY LONG TERM trial, Robinson et al. [101] examined 2341 patients at high risk for cardiovascular events who had LDL cholesterol levels of 1.8 mmol per liter (70 mg/dL) or more whilst receiving statins. Participants were randomized to receiving either alirocumab or placebo for 78 weeks. Alirocumab lowered cholesterol by 62% by 24 weeks, and this was maintained during the study period. In the post hoc analysis, the rate of major adverse events—death from coronary heart disease, nonfatal myocardial infarction, fatal and nonfatal strokes, or unstable angina requiring hospitalization—was halved in those receiving alirocumab. Similarly, in a multicenter, randomized, double-blind placebo-controlled 2.8-year study involving 18,924 patients who had experienced an acute coronary syndrome 1–12 months earlier and were on high-intensity statin therapy, the addition of alirocumab also reduced the risk of recurrent ischemic effects compared with placebo [102]. Evolocumab also exhibits essentially similar effects. In a randomized, double-blind, placebo-controlled trial involving 27,564 patients with atherosclerotic cardiovascular disease treated with statins, the addition of evolocumab for 2.2 years significantly reduced plasma cholesterol and cardiovascular death,

myocardial infarction, stroke, and need for coronary revascularization and hospitalization for unstable angina [103]. Mendelian randomization studies indicate that PCSK9 variants are associated with not only lower LDL cholesterol but also higher fasting glucose, body weight, waist-to-hip ratios, and increased risk of type 2 diabetes [104]. In mice, PCSK9 controls LDLR expression in the pancreas limiting cholesterol overload in beta cells, an effect which appears independent of circulating PCSK9 and probably related to locally produced PCSK9 [105, 106]. Recent clinical trials with evolocumab indicate that these observations may not have any influence on PCSK9 therapy as PCSK9 inhibition significantly reduces cardiovascular risk in patients with/without diabetes and during the 2.2-year follow-up did not appear to increase the risk of new-onset diabetes or worsen glycemia [107]. Similar findings have been reported in participants with the metabolic syndrome receiving evolocumab together with statins [108].

More recent developments in PCSK9 antibody therapies have focused on extending the half-life and duration of action of antibodies. Such antibodies bind with high affinity to PCSK9 in plasma at pH 7.4 and dissociate at the endosomal pH of 5.5–6.0 so as to escape from targeted mediated endosomal degradation [109], an effect dependent on the atypical neonatal Fc receptor. This receptor functions as a recycling receptor that is responsible for maintaining IgG [110]. Bococizumab is one such anti-PCSK9 antibody; it appears to have no therapeutic benefit with respect to major adverse cardiovascular events in low-risk patients, but is of significant benefit to high-risk patients. Its further development has been discontinued [111].

PCSK9 Vaccines

Passive immunotherapy using monoclonal antibodies is now well established but costs up to US$10,000 p.a., greatly limiting their availability even for high-risk patients. Anti-PCSK9 vaccines are an important emerging therapy, and successful vaccination resulting in the generation of anti-PCSK9 antibodies in vivo would greatly reduce treatment cost, making anti-PCSK9 therapy much more widely available. It would also greatly reduce treatment frequency, with current PCSK9 antibodies being administered once or twice monthly.

The fundamental feature of a PCSK9 vaccine is the capacity to trigger the generation of host anti-PCSK9 antibodies, which effectively prevent the interaction of PCSK9 with LDLR. Momtazi-Borojeni et al. [112] used a combination of AFFITOME technology [113] and a nanoliposome platform technology to design a novel anti-PCSK9 vaccine formulation called liposomal immunogenic fused PCSK9-tetanus peptide plus alum adjuvant (L-IFPTA). The main challenge in designing such a vaccine against a self-antigen, in this case PCSK9, is to break down B-cell tolerance whilst avoiding activation of destructive autoreactive T-cell responses; it is important to exclude peptide antigens that are able to induce specific T-cell responses, but B cells also need help from CD4+ T helper (Th) cells for efficient activation and differentiation into antibody-producing plasma cells [114]. This

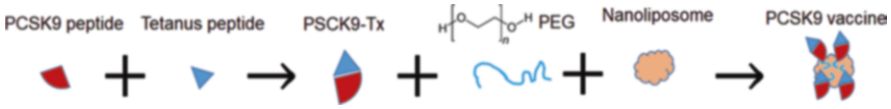

Fig. 30.2 PCSK9 vaccines have been created by incorporating antigens, specifically a PCSK 9 peptide fragment coupled to a peptide fragment of tetanus toxin, making the immunizing antigen different from the native PCSK9 sequence, and which is now identified by the immune system as foreign, thus overcoming self-tolerance. The antigenic peptide is then incorporated into nanoliposome particles for immunization

was achieved by conjugating a B-cell epitope, which mimics the N-terminal sequence of the PCSK9 that binds to LDLR to a foreign Th epitope. Thus, this vaccine contains two different epitopes, one belonging to PCSK9 and the other to tetanus toxin proteins; CD4+ T-cell responses are enhanced by coupling tetanus peptide, a foreign Th epitope, to the PCSK9 fragment. The surface-displayed peptide nanoliposomes generated (L-IFPTA) (Fig. 30.2) elicit far higher and more durable titers of anti-PCSK9 antibodies compared to peptide alone and effectively inhibit PCSK9-LDLR interactions; plasma anti-PCSK9 antibodies decayed slowly with the plasma half-life of about 5 months [112]. Vaccination did not affect the numbers of pro-inflammatory CD4 + IFN-γ Th1 cells or cytotoxic CD8 + IFN-γ + T cells. Vaccination of hypercholesterolemic mice reduced plasma cholesterol, LDL cholesterol, and VLDL cholesterol levels without affecting triglyceride levels; these effects were associated with reductions in the size and severity of atherosclerotic lesions [115]. This L-IFPTA vaccine also effectively immunized healthy nonhuman primates [116]. Other somewhat similar vaccine strategies have also been developed demonstrating therapeutic efficacy, but all are still at the preclinical stage [117–119]. Although significantly more work is required and the L-IFPTA strategy is the most advanced where safety and effectiveness appear impressive, progression to phase I trial in the near future will be required to advance this promising strategy.

PCSK9-Related Small Interfering RNA (siRNA)

Unlike monoclonal antibodies which target extracellular PCSK9, including plasma PCSK9, siRNAs have been designed to prevent intracellular translation of PCSK9 mRNA to protein [120]. Specifically, these molecules silence translation of their complementary target mRNA in a sequence-specific manner by forming effector RNA-inducing silencing complexes [121]. The design of therapeutic siRNA necessitates that the siRNAs only exhibit gene-silencing activity without immunological effects such as induction of pro-inflammatory cytokines and type 1 interferons [122, 123]. Early siRNA molecules designed to silence PCSK9 mRNA in vivo were administered using lipidoid nanoparticles composed of a novel cationic component $98N_{12}$-5, cholesterol, and poly-(ethylene glycol)-lipid; this approach, although highly efficacious, does not provide tissue specificity [124]. Using this approach, a

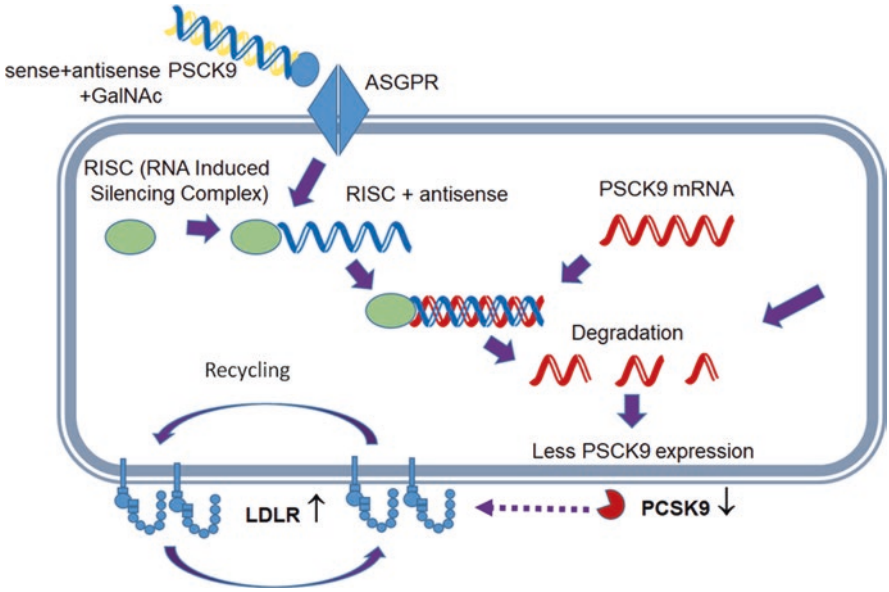

Fig. 30.3 PCSK9 siRNA-mediated inhibition of PCSK9 protein expression in hepatocytes. *N*-acetylgalactosamine carbohydrate (GalNAc)-conjugated PCSK9 small interfering RNAs (siRNAs) are taken up by hepatocytes via the asialoglycoprotein receptor (ASGPR). Once in the cell, they mediate PCSK9 mRNA degradation via the RNA-induced silencing complex (RISC), reducing PCSK9 mRNA translation to protein, resulting in reduced PCSK9 secretion. This, in turn, results in elevated cell surface levels of LDLR via a reduction in its lysosomal degradation

single dose of siRNA targeting PCSK9 induces a rapid, durable, and reversible lowering of plasma PCSK9, apolipoprotein B, and LDL cholesterol levels without affecting plasma HDL cholesterol levels or triglycerides, with effects lasting for 3 weeks [125].

A number of novel strategies are now available for drug delivery to the liver [126, 127]. Nair and associates explored the use of carbohydrates to deliver siRNA targeting liver transthyretin [128] and demonstrated that siRNA targeting the rodent transthyretin gene conjugated to *N*-acetylgalactosamine greatly suppressed expression in the liver with uptake not exceeding the uptake capacity of liver asialoglycoprotein receptors (ASGPR) (Fig. 30.3). During chronic therapy, there was no evidence of tachyphylaxis or sensitization of the ASGPR with high therapeutic efficacy being maintained for at least 40 weeks, indicating that targeting this receptor may be an effective therapeutic strategy for specifically delivering siRNA molecules to the liver. ASGPR is highly expressed by hepatocytes [127] and minimally by other cell types [129].

Inclisiran, a long-acting RNAi targeting PCSK9, has recently been introduced for human therapy and causes substantial reductions in LDL cholesterol; effects are sustained for periods up to 6 months after a single subcutaneous injection. N-acetylgalactosamine is conjugated to inclisiran in much the same manner as

siRNA targeting liver transthyretin [128]. To achieve long-term reductions in PCSK9 and LDL cholesterol, the siRNA is further modified by a combination of phosphorothioate, 2′-*O*-methyl nucleotide, and 2′-fluoro-nucleotide modifications that increase its resistance to attack by various nucleotide-modifying enzymes [128]. Preclinical studies in nonhuman primates indicated high efficacy in reducing plasma PCSK9, by more than 80% with a very slow return to baseline over 90–120 days after administration. In humans, inclisiran also reduced plasma PCSK9 levels by around 75%, which was maintained for 6 months with no serious adverse events [130]. The ORION I trial was the first phase II study on inclisiran, a multicenter, randomized, placebo-controlled trial conducted on 501 patients at high risk of CVD [131]. Participants were required to have been on maximal tolerated doses of statin and/or ezetimibe for 30 days and were randomized to either inclisiran or placebo. The greatest reduction in LDL cholesterol was observed in subjects receiving two doses of 300 mg, where the mean reductions in plasma PCSK9 and LDL cholesterol were 69.1% and 52.6%, respectively, 180 days after initiating therapy. Other effects on lipids included a 25–46% reduction in total cholesterol along with ApoB (23–41%) and VLDL cholesterol (12–21%) as well as Lp(a) (~19%). Similar to results obtained with PCSK9 antibodies, inclisiran did not affect C-reactive protein concentrations [132]. Whilst large reductions in LDL cholesterol are strongly associated with reductions in cardiovascular events and one would expect inclisiran to also greatly impact major adverse cardiovascular events (MACE; cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke), such clinical trials with inclisiran are, at the time of writing, still in progress. The ORION 5 study is a 5-year double-blind placebo-controlled randomized, multicenter study investigating its effects on MACE outcomes in nearly 15,000 patients with preexisting CVD disease aged 55 years or older [133, 134].

In Vivo CRISPR Base Editing to Reduce PCSK9

Early reports that frequent nonsense mutations in PCSK9 are associated with low LDL cholesterol levels raise the possibility of therapeutically inducing such mutations to control hyperlipidemia [61]. Both adeno-associated virus (AAV) and liposome technologies have been developed to deliver clustered regularly interspaced short palindromic repeat-CRISPR-associated protein 9 (CRISPR-Cas9) into postmitotic cells which target PCSK9 [135, 136]. Gene editing methods include CRISP-Cas9 and Cas12 nucleases [137, 138] as well as CRISPR cytosine and adenosine base editors [139, 140]. Recently, efficient in vivo delivery of a CRISPR adenine base editor encapsulated in lipid nanoparticles was successfully delivered to cynomolgus monkeys, introducing a single-nucleotide PCSK9 loss-of-function mutation. Its introduction greatly reduced plasma PCSK9 and LDL cholesterol levels, which were sustained for at least 8 months. Lipid “base editor” carrier molecules included polyethylene glycol (PEG), which were cleared from the circulation within 2 weeks, and early moderate rises in liver enzymes—aspartate aminotransferase and

alanine aminotransferase—resolved after 2 weeks. CRISPR cytosine or adenosine base editors have less off-target effects than CRISPR-Cas9 double-stranded DNA breaks and appear to be ideal for inducing therapeutically important PCSK9 mutations, with the CRISPR adenine base editor discernable editing only occurring at the PCSK9 target site [136]. Translation of these findings to humans may be easier compared to using the self-cleavage AAV-CRISPR system [135], as it potentially minimizes immunotoxicity [141]. However, in translating the CRISPR-Cas9 systems to clinical trials [135, 136], it will be important to take into account the immune status of individuals. In a recent study probing human serum for the presence of anti-Cas9 antibodies, 78% and 58% of subjects possessed antibodies against anti-SaCas9 and anti-SpCas9. In addition, anti-Sa Cas9 T cells and anti-Sp T cells were present in 78% and 67% of donors [142]. Targeting the "base editors" specifically to the liver by modifying the lipid nanoparticles so that they also have on their nanoparticle surface asialoglycoprotein receptors (ASGPR) would increase specificity for liver [128, 129].

Small-Molecule Inhibitors of PCSK9

Small-molecule inhibitors potentially offer the most convenient mode of therapy, which can be much less expensive than those using highly technical approaches together with more invasive modes of administration. However, achieving high specificity can be challenging. One approach to develop small-molecule inhibitors is to prevent LDLR-PCSK9 interactions, similar to what has been achieved with antibodies. PCSK9 acts by binding to the EGF(A) domain of LDLR on the cell surface via its catalytic domain. Zhang and associates identified a 13-amino acid linear peptide (Pep2-8) from phage-displayed peptide libraries that bound to PCSK9 with modest affinity but not to other proprotein convertases [143]. Pep2-8 effectively inhibited LDLR binding to PCSK9 by engaging the same β-sheet hydrogen bonds as EGF(A) [143]. Schroeder and associates reported complementary studies through the synthesis of truncated EGF(A) peptides that restored LDLR recycling in the presence of PCSK9 [144]. More recently, a cryptic peptide-binding site on PCSK9 was described that enabled more targeted design of antagonists [145]. This vacated N-terminal groove of PCSK9, which is adjacent to the EGF(A)-binding site, is accessible to small peptides, which can prevent interactions between PCSK9 and the LDLR. Evison and associates have demonstrated that a small molecule nilotinib, a tyrosine kinase inhibitor used to treat chronic myelogenous leukemia, interacts with the groove of PCSK9, leading to the enzyme's active site [146]. They developed a related compound possessing little tyrosine kinase inhibitory activity that remained highly effective in disrupting PCSK9-LDLR interactions; it effectively reduced total plasma cholesterol levels in mice.

Other approaches to hinder the interaction between LDLR and PCSK9 have focused on Adnectin BMS-962476 developed by Bristol-Myers Squibb/Adnexus. Adnectin is a synthetic protein based on the tenth type III domain of human

fibronectin. Its variable loops can be designed to efficiently introduce a surface that binds therapeutically relevant targets with high affinity and specificity [147]. BMS-962476 is composed of a PCSK9-targeting polypeptide conjugated with polyethylene glycol to enhance its pharmacokinetic profile [148]. The crystal structure of human PCSK9-Adnectin complex indicates multiple sites of interactions that include the N-terminus of Adnectin and Adnectin BC loop residue tyrosine 29 (Y29). In mice overexpressing PCSK9, BMS-962476 reduced total plasma cholesterol levels by up to 35%, as well as free plasma PCSK9 levels. In a first in human study, BMS-962476 was well tolerated and dose-dependently reduced plasma LDL cholesterol and PCSK9 by 48% and 91%, respectively [149]. Other less developed approaches to inhibit PCSK9-LDLR interactions or PCSK9 transcription are also in progress [150, 151].

PCSK9 Therapy and Type 2 Diabetes

Diabetes is associated with a two- to fourfold increased risk of atherosclerotic CVD. Statins are associated with reductions in both blood LDL cholesterol levels and the risk of atherosclerotic CVD as well as modest hyperglycemia, increased body weight, and a modest increased risk of type 2 diabetes, which does not offset their substantial benefits. Recently, Mendelian randomization studies by Schmidt and associates have focused on whether PCSK9 is also associated with increased risk of type 2 diabetes [104]. They indicate that four independent PCSK9 variants (rs11536680, rs11591147, rs2479409, and rs11206510) were associated with lower LDL cholesterol as well as higher fasting glucose concentrations, body weight, waist-to-hip ratio, and increased risk of type 2 diabetes; other similar studies support these conclusions [152, 153]. Despite this association, such studies cannot establish whether the cause lies with LDL cholesterol lowering by any mechanism or by mechanisms directed by gene products. However, they have raised the question as to whether PCSK9-lowering strategies might induce type 2 diabetes. Studies in genetically modified mice have provided some answers [105]. In mice deficient in PCSK9, glucose clearance was found to be significantly impaired but insulin sensitivity was unaffected. Detailed analysis of pancreas morphology indicated increased accumulation of cholesteryl esters, paralleled by increased intracellular insulin levels and decreased plasma insulin. In mice in which PCSK9 as well as LDLR were deleted, this phenotype was reverted, indicating that LDLR and PCSK9 were largely responsible for the phenotype. Also this phenotype was not apparent in AlbCre+/PCSK9$^{loxP/loxP}$ mice where PCSK9 was only deleted from hepatocytes, indicating that circulating, liver-derived PCSK9, the principal target of PCSK9 monoclonal antibodies, does not impact *beta*-cell function and insulin secretion. Similarly, given that therapeutic siRNA specifically targets liver PCSK9, this therapeutic strategy would also be expected not to affect plasma glucose or insulin levels.

It also indicates that evolving therapeutic strategies such as in vivo CRISPR-based editing strategies may need to be more liver specific to avoid such complexities. Current clinical trial evidence indicates that therapy with anti-PCSK9 antibodies does not affect the incidence of diabetes [154, 155].

Combined Lipid Lowering and Anti-inflammatory Strategies

Recent studies indicate that the immune system, both immune cells and cytokines, is also extremely important in the development and progression of atherosclerosis. Macrophages, CD4+ and CD8+ T cells, as well as γδ-T cells are already present in early arterial lesions, including fatty streaks, and their effects become more pronounced and diverse as lesions progress to atheroma and the more complex atherosclerotic plaques responsible for both heart attacks and strokes. Studies in atherosclerotic mice have identified many potential anti-inflammatory therapeutic targets within immune cells and families of cytokines/chemokines that can prevent the progression of atherosclerosis even in the presence of severe hyperlipidemia [156]. Combining statins with canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β in patients, has been shown to significantly lower the rates of recurrent major adverse cardiovascular events by mechanisms that are independent of LDL cholesterol lowering [157]. The study focused on those with high levels of inflammation, based on C-reactive protein (CRP) being 2 or more mg per liter. Primary endpoints included nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death, whilst secondary endpoints included hospitalization for unstable angina that required revascularization and incidence of new-onset type 2 diabetes amongst subjects with prediabetes; reduction with 150 mg canakinumab was approximately 20% compared with placebo; the magnitude of the effects is not surprising given that the actions of only a single pro-inflammatory cytokine were inhibited; interleukin-1β is produced by macrophage inflammasomes [158]. The study definitively proved that inflammation in humans is an important driver of atherosclerotic plaque progression, and treatments are likely to be more efficacious if inflammation is more broadly targeted. More recently, colchicine was reported to have similar effects with roughly similar efficacies [159]. The mechanisms by which colchicine exerts its beneficial effects are unknown, but it may also involve preventing macrophage inflammasome activation, in particular NLRP3 inflammasomes. The beneficial effects of colchicine in treating gout appear due at least in part to preventing NLRP3 inflammasome activation by uric acid crystals [160]; other pathways may also be important, but they also involve myeloid cell activation [161].

Recent studies of T cells in human atherosclerotic plaques indicate an imbalance between pro- and anti-inflammatory cells within vulnerable rupture-prone atherosclerotic plaques [162]; in particular, the number of CD4+ regulatory T cells (Tregs)

is 3.5-fold lower in unstable/vulnerable human atherosclerotic plaques than in stable plaques. These cells play an indispensable role in suppressing excessive immune responses including inflammatory responses deleterious to the host [163, 164]. They express particularly high levels of CD25 (i.e., interleukin (IL)-2α receptors) and can be selectively expanded using anti-IL2 antibodies that favor the activation of IL-2Rα^{hi} Tregs [165]. Anti-IL-2 antibodies complexed to interleukin-2 (IL-2) that favor such activation and Treg expansion have been shown in atherosclerotic mice to prevent not only the development of atherosclerosis but also progression of established atherosclerosis, by reducing plaque pro-inflammatory cell numbers as well as pro-inflammatory cytokine levels [166]. Regulatory T cells are now considered an important new therapeutic target for atherosclerosis [167], and two strategies are being developed to translate such findings to the clinical setting. Combined with statin therapy, effective broad-spectrum anti-inflammatory therapies are expected to have a greater impact on atherosclerosis than lipid-targeted therapy alone.

Interleukin-2 Muteins

IL-2 muteins are IL-2 molecules with an altered amino acid sequence and are often fused with IgG to prolong their half-life. Elucidation of the quaternary complex of interleukin-2 (IL-2) with its α, β, and γ_c receptors has greatly facilitated the engineering of IL-2 muteins with variable affinity to either IL-2Rα, IL-2Rβ, or IL-2Rγ [168] (Fig. 30.4). Like IL-2, IL-2 muteins suffer from limited bioavailability in vivo due to rapid degradation, which is prevented by fusion with a monoclonal antibody or a crystallizable Fc fragment of an antibody. Novel human IgG1 and IgG4 Fc engineered to completely abolish immune effector functions have been used for this

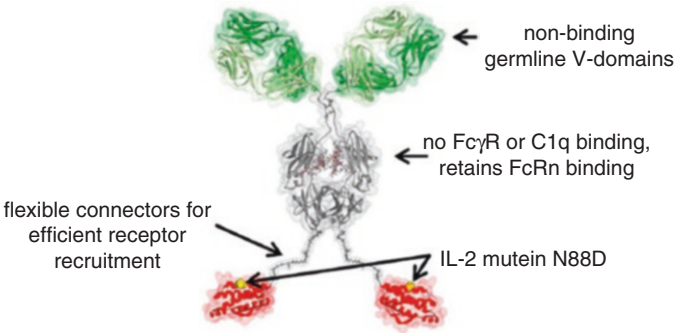

Fig. 30.4 Structure of an IgG-IL-2 protein mutein. IgG devoid of Fcγ receptor or C1q binding is coupled to the IL-2 mutein N88D through flexible connectors to prolong half-life and duration of action. Specificity for activating Tregs is obtained through the N88D point mutation indicated by the yellow within the IL-2 mutein

purpose; hIgG1-P329 LALA (LALA-L234A paired with L235A) and hIgG4-P329G SPLE (SPLE-S228P paired with L235E) completely abolished FcγR and C1q interactions [169]. Recently, a human IL-2 mutein harboring an N88D substitution which reduced its affinity for IL-2Rβγ was fused to a non-targeted effector-function-silent human IgG1. This fused IL-2 mutein is long-lived and preferentially amplifies Tregs in macaques [170]. Others have shown that the fusion of IL-2 with IL-2Rα, joined by a non-cleavable linker, has significantly greater in vivo efficacy than IL-2 at Treg expansion, both with respect to plasma half-life and selectively for Tregs [171]. Amgen has also produced an Fc-IL-2 mutein (AMD-592) designed with a greater half-life than IL-2 and increased affinity for IL-2Rα. It preferentially expands Tregs and lowers the production of pro-inflammatory cytokines. AMD-592 (efavaleukin alfa) is in phase II clinical trials [172, 173]. Pandion Therapeutics has developed a highly selective IL-2 mutein by introducing mutations in IL-2 that significantly decreased CD122 (IL-2Rβ) binding affinity in addition to other mutations that increased CD25 binding affinity. Its administration to mice and monkeys selectively expanded Tregs without significant effects on other immune cells and without increasing pro-inflammatory cytokine production. It is also in clinical trials [174]. Other IL-2 muteins are in development [175, 176]. One potential limitation with IL-2 muteins is the possibility of off-target side effects due to expansion of large numbers of polyclonal Tregs [176]. Clinical trials will be required to resolve this issue. However, a short-term phase I/II study using low-dose IL-2 which significantly elevated Tregs indicates that therapy with IL-2 muteins is likely to be both safe and well tolerated [177]. It is also highly likely that it may be useful in people with type 2 diabetes, not only for preventing accelerated atherosclerosis progression, but also for improving insulin sensitivity and providing protection from diabetes complications. Tregs are significantly reduced in type 2 diabetic patients with chronic complications [178]. Expansion of Tregs can significantly improve insulin sensitivity, as well as ameliorate diabetic nephropathy, and suppress progression of atherosclerosis [179], but this will need to be confirmed in future clinical trials.

CAR-Tregs

CAR-Tregs open the therapeutic window to prevent the progression of life-threatening atherosclerosis using cell therapy. Tregs (CD4+ Foxp3+ T cells) are a small subset of immune T cells constituting approximately 5% of CD4+ T cells in blood that are dedicated to curbing excessive inflammation and pro-inflammatory immune cell over-activation. They very effectively prevent atherosclerosis progression in mice [166]. Chimeric antigen receptor (CAR) technology, which was initially developed to generate CAR-T cells for treating blood cancers such as B-cell lymphomas, is now being extended to treat conditions associated with autoimmunity and severe inflammation through the development of CAR-Tregs. Like CAR-T cells, these cells have the potential to expand in vivo and can survive and remain active for at least 4 years after adoptive transfer into humans [180]. A

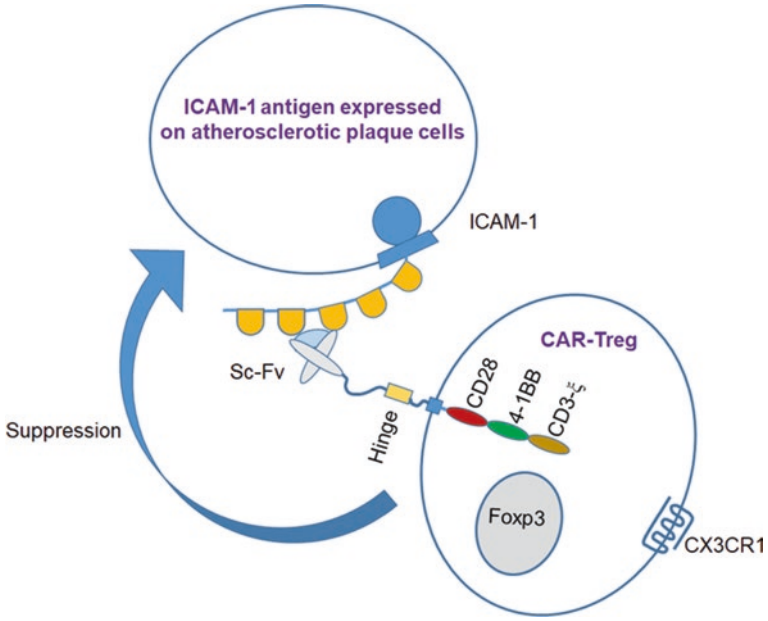

Fig. 30.5 Schematic representation of a CAR-Treg designed to interact with ICAM-1 antigen presented on the cell surface of synthetic phenotype vascular smooth muscle cells, macrophages, dendritic cells, and CD8+ T cells, which are highly prevalent within atherosclerotic plaques. The chimeric antigen receptor expressed on the Treg cell surface is composed of scFV specific for ICAM-1 fused with intracellular domains (e.g., CD28, 4-1BB) and an intracellular activation domain CD3ζ. The chemokine receptor CX3CR1 is also expressed on the Treg cell surface to increase homing to atherosclerotic plaques; CX3CL1, the chemokine for CX3CR1, is highly expressed in human plaques

second-generation chimeric antigen receptor on Tregs has been developed [181, 182] that targets (CD19+) B cells for example, involved in inflammation, including atherosclerosis [183, 184]; it consists of a signal peptide sequence, a FMC63 scFv (anti-hCD19 antibody) sequence, a CD28 sequence through the extracellular, transmembrane, and cytoplasmic domains linked to CD3ζ [181, 185] (Fig. 30.5); other variants have also been produced [186]. Generation of CAR-Tregs is not trivial and involves multiple steps, including isolation, frequently by leukapheresis, purification, and in vitro expansion, followed by CAR gene delivery using lentiviral vectors [187]. Given the low abundance of Tregs (<3%) within the peripheral blood monocyte population, achieving high purity for therapeutic use can be challenging. Despite such challenges, a HLA-2-A2-specific CAR-Treg (TX200) has been developed [188, 189] and has progressed to stage 1 clinical trials for the prevention of immune-mediated rejection following HLA-A2-mismatched kidney transplantation for end-stage renal disease [189]. Other studies are also close to clinical translation. In relation to atherosclerosis, those at extremely high risk of cardiovascular death

due to atherosclerosis would be high-priority candidates for such therapies given their expected high cost; CAR-T-cell therapy in Australia costs approximately AUS$598,000 per patient, and one might expect CAR-Treg therapy to be similar. One would envisage the development of a "luxury model" CAR-Treg for treating extremely high-risk atherosclerotic patients, which would include not only a CAR but also chemokine receptors to assist in homing to atherosclerotic plaques [190]; recently, it has been shown that CX3CR1-transduced T regulatory cells are more effective in homing to atherosclerotic plaques and in suppressing progression of atherosclerotic plaques [191].

As to CAR specificity, targeting ICAM-1 is one possibility. ICAM-1 is a member of the immunoglobulin superfamily and is either rarely expressed or not expressed under normal conditions. However, it is widely expressed by a variety of cells when stimulated by inflammatory factors including cytokines. It can be expressed by synthetic-type vascular smooth muscle cells [192], phagocytic macrophages [193], dendritic cells [194], and CD8+ T cells [195], major immune cell types present in atherosclerotic plaques. ICAM-1 is highly expressed in human atherosclerotic plaques [196], and sequences for a single-chain variable fragment (scFV) specific for intercellular adhesion molecule 1 (ICAM-1), which effectively interacts with ICAM-1, have been developed [197]. One can expect this type of cell therapy to advance significantly over the coming years and hopefully result in off-the-shelf Treg therapy for atherosclerosis combined with current lipid-lowering strategies. Production of off-the-shelf Tregs targeting the same antigen in atherosclerotic plaques would markedly lower costs enabling a greater proportion of high-risk patients to be treated. Given the high specificity of CAR-Tregs, the therapy should also be highly effective in preventing accelerated atherosclerosis and its complications in very-high-risk diabetic patients.

Conclusions Regarding Inhibitors of PCSK9 and of Inflammation

There has been enormous progress in atherosclerosis therapies since plasma LDL cholesterol was first linked to myocardial infarction and stroke in the "Coronary Heart Disease in the Framingham Study" [42]. This progress has continued since the development of statins. Targeting PCSK9 has opened a new exciting therapeutic chapter in atherosclerosis, and many therapies targeting PCSK9 are still in development. Anti-inflammatory therapies have also been shown to impact the development/progression of established atherosclerosis, particularly exploiting the ability of Tregs to prevent excessive inflammation and pro-inflammatory immune cell overactivity. These therapies are still evolving but very likely will also greatly impact atherosclerosis-related death, myocardial infarction, stroke, and unstable angina in many patients including those with inflammatory, autoimmune disorders and patients with type 2 diabetes. Many of the new approaches have been possible

due to newly developed, diverse, and powerful technologies, which were unavailable during development of statins and demonstrate the power of bringing these technologies from the laboratory to the clinic, by providing access to novel anti-atherosclerotic therapies.

ATP Citrate Lyase (ACLY) Inhibitors

Another relatively new class of predominantly LDL-lowering drugs is that of ATP citrate lyase (ACLY) inhibitors, with the first in this class in clinical use, with the FDA and the EU approval, being bempedoic acid [198]. As yet, there are no published diabetes-specific trials with either cardiovascular or microvascular complications of diabetes as primary endpoints. Such data from trials, meta-analyses of diabetes subgroups in general population trials, and audits from large clinical or insurance databases will likely arise in future.

Mechanism of Action of Bempedoic Acid

Bempedoic acid is a synthetic prodrug that is activated by the enzyme very-long-chain acyl CoA synthase 1 (ACSVL1) to the active form ETC-1002-CoA, which inhibits the enzyme adenosine triphosphate-citrate lyase (ACLY), which is upstream of HMGCoA reductase, which is targeted by statins. ACL inhibition inhibits cellular cholesterol synthesis, leading to upregulation of LDL receptors (LDLR) and increased clearance of LDL. ACSVL1 is predominantly found in the liver, and is not in skeletal muscle, reducing the risk of muscle side effects [199] as noted in clinical trials [200–205].

Bempedoic acid also activates AMPK in various cell types, which may potentially improve (lessen) insulin resistance and reduce the risk of hyperglycemia and new-onset diabetes seen with statins [202].

Pharmacokinetics of Bempedoic Acid

Bempedoic acid is a 180 mg tablet taken once a day, with or without food. Blood levels peak at 3.5 h after administration, and the mean drug half-life is 21 days. Renal impairment reduces its clearance, and people with an eGFR<30 mL/min/1.73 m^2 or on dialysis have been excluded from trials to date [202].

Table 30.1 Summary of four phase III clinical trials of bempedoic acid

Trial/year	Subjects	Intervention	Baseline LDL-C mg/dL mean (SD)	% change LDL-C
CLEAR Tranquility 2018	269 adults with statin intolerance on no or low-dose statin + LDL-C ≥100 mg/dL	1 W screen 4 W run-in on 10 mg ezetimibe Rando. 2:1 to BPA or placebo for 12 W	BPA 123 (27) Placebo 130 (31)	BPA −23.5 Placebo +5 Placebo adjusted −28.5 $P < 0.001$
CLEAR Serenity 2019	345 adults for primary or secondary CVD prevention; intolerant ≥2 statins	5 W screen Rando. 2:1 BPA or placebo for 24 W	BPA 159 (40) Placebo 157 (39)	BPA −23.6 Placebo −1.3 Placebo adjusted −21.4 $P < 0.001$
CLEAR Harmony 2019	2230 adults with CVD+/or heterozygous FH on max. statin ≥4 W + LDL-C ≥70 mg/dL	Rando. 2:1 BPA or placebo for 52 W	BPA 102 (30) Placebo 104 (29)	BPA −12.6 Placebo +1 $P < 0.001$
CLEAR Wisdom 2019	779 adults with CVD+/or heterozygous FH on max. statin ≥4 W + LDL-C ≥100 mg/dL at visit 1 and ≥70 mg/dL 1 W pre-rando	1 W screen Placebo run-in 4 W Rando. 2:1 BPA or placebo for 52 W, with primary outcome LDL-C at 12 W	BPA 122 (38) Placebo 119 (38)	BPA −15.1 Placebo +2.4 Placebo adjusted −17.4 $P < 0.001$

BPA bempedoic acid; *FH* familial hypercholesterolemia; *LDL-C* low-density lipoprotein cholesterol; *Rando* randomization; *W* week

Clinical Trials of Bempedoic Acid

Four phase III clinical trials in North America and Europe have evaluated the safety and efficacy of bempedoic acid to date, the results of which led to the FDA approval in February 2020. The Cholesterol Lowering via Bempedoic Acid, an ACL-inhibiting Regimen (CLEAR) suite of trials included CLEAR Tranquility [203], CLEAR Serenity [205], CLEAR Harmony [206], and CLEAR Wisdom [207]. These trials in North America and sometimes also in Europe were multicenter randomized double-blind placebo-controlled trials in adults, with each trial testing a once-daily 180 mg dose of bempedoic acid alone or with a statin background. These four trials are summarized in Table 30.1.

Overall, from these CLEAR trials, bempedoic acid (180 mg/dL) reduced LDL cholesterol levels about 18% when taken with a statin and by 24% as monotherapy, with higher reduction when combined with ezetimibe (10 mg).

In the four CLEAR trials [203, 205–207] summarized above, there were no significant differences in serious adverse effects, nor in discontinuation rates between

treatment arms, except for the CLEAR Wisdom trial in which more in the active vs. placebo arm discontinued due to adverse events (10.9% vs. 8.6%).

The results of studies long enough to more reliably inform regarding cardiovascular event effects are pending. A large secondary prevention study in 12,000 statin-intolerant patients with over 4-year follow-up, CLEAR OUTCOMES, is ongoing, due to report in 2022 [208, 209].

Whilst we await these trial outcomes, other evidence supports likely benefit. A Mendelian randomization analysis has predicted a reduction in CVD risk per unit decrease in the LDL cholesterol level in carriers of loss-of-function mutation in ACLY, which is similar to that in carriers of loss of function for HMGCR [210]. In a recent meta-analysis of cardiovascular outcomes from four bempedoic acid studies including 3483 participants, there was a 17%, non-statistically significant reduction in MACE, but a 50% reduction of noncoronary revascularizations [211].

Bempedoic Acid Trials in Type 2 Diabetes

To date, two phase II trials of bempedoic acid have been conducted in adults with type 2 diabetes [201]. In the ETC-1002-005 trial, participants discontinued all glucose and lipid control drugs and were randomly assigned to bempedoic acid 80 mg daily for 2 weeks and then 120 mg for 2 weeks or to 4 weeks of placebo. Bempedoic acid lowered LDL cholesterol levels by 43% vs. 4% in the placebo group, $P < 0.0001$, and also lowered CRP levels (by 41% vs. 11% in the placebo), $P = 0.0011$. There was no worsening of glycemia [212]. In the 1002FDC-058 trial of 180 mg bempedoic acid and 10 mg ezetimibe vs. placebo in type 2 diabetes the (placebo adjusted), LDL cholesterol reduction was 40% and that of CRP was 25%, both $P < 0.0001$, with no deterioration in glycemia [213].

Pleiotropic Effects of Bempedoic Acid

Little is reported yet regarding pleiotropic effects of bempedoic acid to date, but as with statins, bempedoic acid significantly lowers CRP levels (as measured by high-sensitivity CRP assays) [201, 212–214].

Side Effects of Bempedoic Acid

Overall, based on individual phase II and phase III trials, an open-label extension study of the CLEAR WISDOM trial [215] and several meta-analyses and systematic reviews, including by national lipid bodies, support that bempedoic acid is relatively well tolerated and generally safe [212, 213, 215–217]. Post-marketing

monitoring should continue and will likely inform in relation to tolerability and safety in the broader general community.

Common side effects in human trials include modest rises in renal function tests of blood urea nitrogen and serum creatinine.

Levels of uric acid are also increased, which may be mediated, at least partly, by bempedoic acid inhibiting the renal organic anion transporter 2 inhibitor. Not surprisingly, there is a higher incidence of gout with bempedoic acid (1.6/100 person-years) vs. 0.5/100 person-years for those allocated to placebo [201, 205, 206].

An uncommon (0.5%) but serious side effect of bempedoic acid is that of tendon rupture or inflammation, which was not reported in any of the placebo group subjects. The mechanism is not fully elucidated. Those at higher risk are thought to be people with kidney dysfunction, aged >60 years, or taking certain other medications (e.g., fluoroquinolones) [201].

Other side effects include lower hemoglobin and abnormal liver function tests, nasopharyngitis urinary tract infections, and arthralgia. Myalgia was still reported in trials, but has been related to statin background therapy; hence, the use of high-dose statins in combination with bempedoic acid is cautioned. Myalgia is less common with bempedoic acid than with statin therapy and hence may be suitable for people who cannot tolerate any statin or high-dose statin therapy. Unlike statins, bempedoic acid has not been associated with higher glucose levels of new-onset diabetes [200, 201].

Summary and Conclusions Regarding ACLY Inhibitors

A new class of lipid drug, ACLY inhibitors, is now available for clinical use in some countries for LDL lowering. This synthetic drug acts proximal to the site of HMGCoA reductase and upregulates LDL receptors, lowering LDL cholesterol levels, though to a lesser extent with monotherapy than for more potent statins. Activation of the prodrug is in liver, and not muscle, so myalgia is less common than with statin therapy, so is likely to benefit many statin-intolerant patients who developed myalgia. Unlike statins, which can increase glucose levels and rates of new-onset diabetes, this is not evident with bempedoic acid. The first drug of this class to be approved by the US and the EU regulatory bodies (in 2020) is bempedoic acid, which has shown LDL-lowering efficacy somewhat less than for high-intensity statins, and it can be combined with a statin, ezetimibe, or both. The results of a major CVD event endpoint trial are expected soon. Phase II trials have been conducted in adults with type 2 diabetes and show LDL cholesterol and CRP benefit, with no adverse effects on glycemia. Knowledge of the effects of bempedoic acid and any other ACLY inhibitors on cardiovascular and microvascular outcomes in both type 1 and type 2 diabetes is required.

The triglyceride-lowering long-chain fatty acids and their esters will now be discussed.

Long-Chain Fatty Acids and Their Esters: Impact on Dyslipidemia and CVD

Introduction

The activity of marine oils and their constituents has been one of the long-standing issues in the treatment of dyslipidemia and CVD prevention [218–222]. The initial observation is very well known—scientists discovered that native Greenland populations had lower rates of CVD compared to several Western populations and this population also had a higher intake of dietary fatty fish and fish products [218, 223]. The index biochemicals in fish oil products are unsaturated long-chain fatty acids (and their esters), which are precursors for eicosanoids and are nominally anti-inflammatory [224]. The initial observation has been the foundation of many studies with a variety of natural and synthetic products [219, 225, 226]. The evolution of knowledge has been mixed and controversial. Products investigated have varied from fish products and natural products to synthetic analogues and mimetic compounds, which are part of the natural products. The products are polyunsaturated fatty acids known as PUFAs. The ω-6 PUFAs and ω-3 PUFAs are generally considered to have beneficial health effects, but they have opposing effects on metabolic functions that might result in related pathological processes if the balance in the diet is altered [227]. In general, metabolites of ω-6 PUFAs are pro-inflammatory, whereas metabolites of ω-3 PUFAs have anti-inflammatory, repairing, and protective effects. The ratio of the ω-6/ω-3 PUFAs in the diet may determine the level of pro-inflammatory or anti-inflammatory balance [227, 228].

Hypertriglyceridemia promotes atherosclerosis and is an independent risk factor for CVD [229–231]. For triglyceride levels up to 500 mg/dL, triglycerides are carried in very-low-density lipoprotein (VLDL) particles, which provides an atherogenic milieu and increases the risk for CVD [219, 232]. At triglyceride levels above 500 mg/dL, triglycerides are located in chylomicrons and pose an elevated risk for the severe condition of acute pancreatitis, pathological inflammation of the pancreas. Various products have been licensed by the (United States) Food and Drug Administration (FDA) for use in humans for the treatment of dyslipidemia and hypertriglyceridemia and related conditions. The results have been equivocal at best [219]. However, the products are relatively safe and some studies have shown strong positive outcomes [233–235]. The most optimistic position is that certain agents might have benefits in some populations, so identifying such products and cohorts and noting the favorable safety profile mean that there may be a therapeutic use in areas where CVD has a broad and deep adverse impact on human health, and even a marginal product has the potential to benefit many people. In this section, we explore the products and target populations which might benefit from the ingestion of natural or synthetic products based on unsaturated long-chain fatty acids and their esters.

It should be pointed out that there is considerable controversy around the original finding that underpins this area of therapeutic medicine [219, 236]. The Eskimo/Inuit population consumed a diet high in fat which was against then current dietary guidelines. The issue is the level of coronary artery disease in this population and the contention that it was low (compared to Western populations) has been disputed [219]. Nevertheless, all subsequent trials of various fish oil and individual component products can be considered on the basis of the strength of the study design and the outcomes, and that is what is presented in this section.

For clarity, based on the results of recent large clinical trials, the therapeutic imperative in this area has evolved around two questions—the first question is if lowering elevated TGs (in this case with PUFAs) reduces cardiovascular events per se and the second is if PUFAs can reduce cardiovascular events due to or aside from their action to reduce TG levels. The concomitant roles of lipid-lowering and non-lipid-lowering effects of cardiovascular interventions are of considerable current interest [237]. The section should be considered in the context of these two questions.

Chemistry and Biosynthesis of PUFAs

The two main fatty acids that are essential in the diet are linoleic acid, which is otherwise referred to as omega-6 and α-linoleic acid, which is referred to as omega-3 fatty acid. The key biochemicals are long-chain fatty acids (and their esters) with multiple unsaturated (double) bonds. Products are named by the position number of the carbon atom having the first double bond [224].

Eicosapentaenoic acid (EPA, also icosapentaenoic acid) is an omega-3 fatty acid. In physiological literature, it is given the name 20:5($n-3$). It also has the trivial name timnodonic acid. In chemical structure, EPA is a carboxylic acid with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end (Fig. 30.6).

Docosahexaenoic acid (DHA) is a carboxylic acid with a 22-carbon chain and six (*hexa-*) *cis* bonds; the first double bond is located at the third carbon from the omega end.

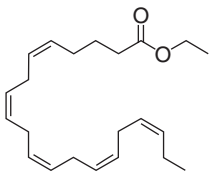

Fig. 30.6 Chemical structure of ethyl eicosapentaenoic acid ethyl ester (icosapent ethyl). The basic molecule is the ethyl ester of a 20-carbon carboxylic acid. Note the first double bond commencing at the third carbon atom from the aliphatic end of the molecule (giving the $n = 3$ or omega 3 designation). The 2-carbon chain is the ethyl group in an ester linkage to the carboxylic acid

Its trivial name is cervonic acid, its systematic name is *all-cis*-docosa-4,7,10,13,16,19-hexa-enoic acid, and its shorthand name is 22:6($n-3$) in the nomenclature of fatty acids.

The biochemicals, PUFAs, are catabolized by specific fatty acyl desaturase and elongate enzymes, which regulate the length of the carbon changes and the extent of desaturation. The biosynthesis of EPA in prokaryotes and eukaryotes involves polyketide synthase. The polyketide pathway includes multiple enzymes, namely 3-ketoacyl synthase, 2 ketoacyl-ACP-reductase, dehydrase, enoyl reductase, dehydratase/2-trans 3-cos isomerase, dehydratase/2-trans, and 2-cis isomerase. The biosynthesis of EPA varies in marine species, but most of the marine species' ability to convert C18 PUFA to LC-PUFA is dependent on the fatty acyl desaturase and elongase enzymes. The molecular basis of the enzymes will dictate where the double bond is formed on the resulting molecules. The EPA isoforms, $n-3$ and $n-6$ EPA, are not interconvertible in the human body and are important components of cell membranes [224].

The Paradigm Changing REDUCE-IT Trial of Icosapent Ethyl

The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) has been one of the more interesting clinical trials in cardiovascular medicine over the last decade [221, 232–234, 236, 238, 239]. Elevated plasma TGs are an established risk factor for cardiovascular events in patients with LDL-C levels well controlled with statins. PUFAs are known to reduce elevated TG levels, so the study was done to address the question if a PUFA, in this case icosapent ethyl, could reduce cardiovascular events in a high-risk population with elevated TGs [234].

REDUCE-IT utilized pure synthetic icosapent ethyl (Vascepa®, Amarin Pharmaceuticals, Dublin, Ireland). Icosapent ethyl is a synthetic analogue of EPA. The study was conducted in patients with elevated triglycerides and an elevated cardiovascular risk profile. REDUCE-IT was a prospective, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. The study screened 19,212 patients of whom 8179 were randomized [234]. There were subgroups representing primary and secondary CVD prevention. Groups had either established CVD or diabetes and additional risk factors. Subjects received either icosapent ethyl 2 g 2 × day (4 g IE per day) or placebo which was a mineral oil at 4 g/day [234]. It should be noted that the choice and actions of the placebo in such studies are neither simple nor neutral in terms of the study results.

The outcome after an average of 4.9 years was that the event rate in the primary prevention group (30% of total subjects) was 22.0% in the placebo group and 17.2% in the group that received IE. In the secondary prevention group (70% of total subjects), the event rate was 14.8% in the placebo group and a lower, 11.2%, in the IE-treated group. Thus, for the primary endpoint, IE treatment resulted in a 4% absolute reduction and a 25% relative risk reduction in the subjects given IE [234].

There had been previous CV studies in which patients in the USA showed lower response rates in such trials so there was an analysis of the cohort of subjects recruited in the USA [233]. This analysis showed that there was an appreciable effect of IE treatment in the US cohort, indeed greater than the response of the total cohort. Although this was not a statistically significant difference, the analysis indicated that there was no lesser effect in the US population included in the REDUCE-IT study, rendering the results of the REDUCE-IT trial relevant in considerations of future therapeutic strategies in the relevant broader US population [233].

The rationale or hypothesis of the REDUCE-IT study was that reducing elevated TGs would reduce the occurrence of ischemic events in the treated cohort. However, the results showed that there was no correlation between the lowering of TGs and the effect of IE. Based on established risk models, the observed median reduction of 14 mg per deciliter (equivalent to 0.36 mmol per liter) in non-HDL cholesterol level from baseline with icosapent ethyl would be expected to translate into a lower risk of cardiovascular events of only 6–8%—not the 25% observed in REDUCE-IT.

This is an example of the phenomena of whereby because a risk factor is elevated and shows a correlation with events, it does not automatically follow that reducing the risk factor reduces events. For example, and using an analogy, this is particularly apparent in studies of the hyperglycemia of diabetes. There are many pathological pathways and mechanisms causing the hyperglycemia, and different treatments may all reduce the hyperglycemia, but by different mechanisms, and therefore having variable effects on the cardiovascular event rates. The implication in the current REDUCE-IT study is that the favorable impact on reducing cardiovascular events arose from the pleiotropic actions of IE—that is, effects other than those arising from reducing plasma TG levels. Such effects may include anti-inflammatory, antithrombotic, and membrane stabilization antiarrhythmic effects [225]. These effects do not reduce the efficacy of IE but illustrate the complexity of linking the target of risk factors with cardiovascular outcomes.

REDUCE-IT had a positive outcome in terms of reducing events; somewhat confounding, the effects were not related to the lowering TG levels. As this trial stood out from numerous earlier unsuccessful trials of $n-3$ fatty acid products, further studies are necessary to fully appreciate the value and position of icosapent in the CVD therapeutic armamentarium.

The Positive Outcome JELIS Trial

JELIS (Japan EPA Lipid Intervention Study) was a trial of a pure EPA product given at 1.8 g/day [240]. The trial was conducted in Japan. Participants were randomized in an open-label manner to treatment with EPA (1800 mg/day; $n = 9326$) in addition to statin therapy or to statin therapy alone ($n = 9319$). Statin therapy was either pravastatin (10 mg/day) or simvastatin (5 mg/day). Posttreatment LDL cholesterol concentrations decreased 25%, from 4.7 mmol/L in both groups. There were 18,645

study participants (16% with diabetes), with a mean age of 61 years and mean follow-up of 4.6 years [240]. The significance of the trial being conducted in Japan is that the Japanese population consume a large number of fish in their normal diet.

Primary endpoints, which were similar to other related trials, were major adverse coronary events (MACE), defined as sudden cardiac death, unstable angina, myocardial infarction, or revascularization. The outcome was a positive beneficial effect of the intervention. The primary endpoint of MACE at the mean 4.6-year follow-up was significantly lower in the EPA-plus-statin group compared with the statin-alone group (2.8% vs. 3.5%, hazard ratio [HR] 0.81, $P = 0.011$), representing a 19% relative reduction in major coronary events ($P = 0.011$). These event rates are quite low, most likely reflecting the fact that the Japanese are already on a high dietary fish oil intake. It should be noted that this (1.8 g/day) is a relatively high dose of PUFAs, and the adverse event rate was higher in the test compared to placebo group. The study authors concluded that "EPA is a promising treatment for prevention of major coronary events, and especially nonfatal coronary events, in Japanese hypercholesterolemic patients," but the study results are similar to that of REDUCE-IT and might be more generalizable than implied in this statement [240].

Other Relevant PUFA Clinical Trials

The evidence is sound that elevated TGs are an independent risk factor for cardiovascular events in patients with well-controlled LDL-C levels on statin treatment. The TG in such subjects is carried in TG-rich lipoproteins including VLDL, chylomicrons, and remnant particles. Accordingly, PUFAs are known to reduce TG levels so trials have been undertaken to test if lowering TG levels reduces cardiovascular events. Furthermore, there have been other trials with agents other than PUFAs, such as niacin and PPAR-α ligands, the fibrates, to test this question.

STRENGTH was a trial of a mixture of EPA and DHA as the carboxylic acids in people with atherogenic dyslipidemia and high cardiovascular risk and on statins; most (70%) of the participants had diabetes [241]. The trial was a double-blind, randomized multicenter trial of the EPA/DHA mixture (4 g/day) with corn oil as the placebo. The trial randomized over 13,000 subjects. The primary endpoint occurred in 12.0% of the group treated with omega-3 fatty acids and 12.2% of those on placebo. As might be expected, there was a greater rate of adverse gastrointestinal events in the omega-3-treated group. The STRENGTH trial obtained a 19% reduction in plasma TG levels but no beneficial impact on cardiovascular events (the trial was cut short for futility of obtaining a positive outcome) [241]. The conclusion is that there is no benefit of lowering TGs using this EPA/DHE mixture nor is there any beneficial effect of the EPA/DHA mixture above and beyond its TG-lowering actions.

For reference, other clinical trials examining agents to reduce TGs include studies of niacin, such as the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial [242], and fibrates (Action to Control Cardiovascular Risk in Diabetes (ACCORD-Lipid)) in simvastatin-treated adults with diabetes [243]. The results of

these trials were similar to those described in detail for the STRENGTH trial in that there was no impact on cardiovascular events despite a lowering of TG levels.

Pleiotropic Actions Contributing to the Effects of PUFAs

The clinical trial data for the effect of EIP (on top of statin therapy) indicates that part of the MACE reducing outcomes are due to direct or pleiotropic actions impacting the pathophysiological mechanisms of CVD [244]. These apparently favorable biological actions are in addition to TG-lowering effects of PUFAs and are specific to PUFAs and not other TG-lowering interventions such as fenofibrate and niacin [232, 245]. In this context, PUFAs are amphiphilic molecules which can have a multitude of effects associated with intercalation into cellar membranes and more specific receptor-based actions due to interactions with G protein-coupled receptors and PPARs.

Pleiotropic actions can be identified in cellular studies or implied from animal or human studies, but their specific contribution to lowering cardiovascular risk is difficult to ascribe and quantitate. Furthermore, actions such as anti-inflammatory effects may result in benefits of PUFAs beyond the cardiovasculature and extend to beneficial effects on arthritis and inflammatory gut diseases. The major pleiotropic actions of PUFAs which may contribute to their favorable cardiovascular actions include regulation of hormone and metabolite concentrations, direct cellular effects mediated by fatty acid receptors, modulation of oxidative stress, and broad regulation of cellular behavior arising form the impact of PUFAs which intercalate into cellular membranes [245, 246].

EPA relative to DHA stabilizes the membrane structure and facilitates ROS scavenging, a key action as ROS are widely associated with the pathological mechanisms of atherosclerosis. PUFAs can also have effects on endothelial cells, where endothelial dysfunction is currently recognized as a central mechanism of atherosclerosis and hence CVD. EPA reduces arterial stiffness associated with reduced markers of oxidative stress and inflammation and is not dependent on changes in blood pressure or LDL levels.

Cholesterol domains in membranes facilitate the formation of extracellular cholesterol crystals, which are a hallmark of atherosclerosis. The intercalation of EPA into the alkyl chain core of the membrane lipid bilayer inhibits the cholesterol domain formation. Of note, hyperglycemia can stimulate cholesterol membrane domains, bringing in the relevance of this mechanism to people with diabetes.

PUFAs can act via PPAR transcription factors. PPARs are nuclear receptors with functions covering glucose and lipid metabolism, oxidative stress, and inflammation and other major systemic effects [247]. In cardiovascular physiology, PPAR-γ are associated with insulin resistance and PPAR-α with control of lipid metabolism, and these transcription factors are regulated by drugs including rosiglitazone and fenofibrate, respectively. PUFAs also act as ligands for PPARs. PUFAs can act like drugs by binding to PPARs, inducing conformational changes and triggering the transcription of specific genes including those encoding for various metabolic and cellular processes such as FA β-oxidation and adipogenesis and lipid homeostasis,

as well as regulation of processes related to aging comprising oxidative stress, inflammation, and neuroprotection.

Impact of Recent Clinical Trials on the Management of Hypertriglyceridemia

Residual risk for CVD events is present in patients treated with cholesterol-lowering medications, even to very low LDL-C levels. This indicates the presence and contribution of factors, including hypertriglyceridemia, to the risk of CVD in such patients. Recent clinical trials targeting hypertriglyceridemia with PUFAs have had a rapid and profound impact on recommendations for the treatment of patients with hypertriglyceridemia [219, 232, 235]. Hypertriglyceridemia is generally recognized as being moderate (500 mg/dL; 5.7 mM) or severe (>1000 mg/dL; 11.3 mM), the latter of which usually involves a genetic component of familial hypertriglyceridemia.

Icosapentaenoic acid is a chemically stable, highly purified FDA-approved prescription presentation of an EPA. The strength of evidence for the impact of IPE in the REDUCE-IT trial prompted several major organizations to update their guidelines and practice standards within a short period of the release of the trial results. As discussed earlier, IPE administration resulted in a 25% relative risk reduction in the CVD event rate in the primary composite (cardiovascular) endpoints and an absolute risk reduction of 4.8% in the primary composite endpoint of time to first event for pre-defined events.

Detailed descriptions for the use of IPE in patients with hypertriglyceridemia have been reported [232]. It should be noted, as discussed elsewhere, that the evidence for the efficacy of IPE in reducing CVD risk and events derives from an impact on hypertriglyceridemia and can also be ascribed to a raft of pleiotropic actions. There are no clinical guidelines for the use of IPE based on addressing pleiotropic actions. For patients with hypertriglyceridemia, the American Diabetes Association (ADA) recommends that in patients with atherosclerotic CVD or other cardiac risk factors and on a statin with controlled LDL-C but persistent hypertriglyceridemia (135–499 mg/dL; 1.52–5.63 mM), the addition of IPE should be considered so as to reduce CV risk. In the context of adding IPE to a statin, the guidelines further note that combining other relevant agents such as fibrates or niacin with statins has not been shown to provide additional CV benefit. In late 2019, the FDA further approved an indication for IPE as an adjunct to maximally tolerated statin therapy to reduce CV risk for multiple events in patients with elevated TG levels (>150 mg/dL) and established CVD or diabetes and with two or more additional risk factors for CV disease [219, 232].

The American Heart Association Scientific Statement (2020) recommends consideration of IPE use in patients with type 2 diabetes for further cardiovascular risk

reduction when TGs remain elevated despite maximally tolerated statin doses for the management of coronary artery disease.

Thus, despite the use of statins and other cholesterol-lowering medications, substantial residual CVD risk still exists and it represents a major healthcare and economic challenge. Interventions are required to reduce this risk, and taken in their entirety, trials of various PUFA products have specifically provided support for the role of the single purified product, IPE, for the addition of standard therapy to reduce this risk. These recommendations are specific for IPE and do not extend at present to other forms of EPAs, mixed EPA/DHA preparations, or other more basic and natural products. The use of IPE has been added to the global treatment guidelines for multiple peak medical organizations, and its use should be considered until, as always, more information arrives from clinical trials to further advise clinical practice [219, 232].

Summary and Conclusion Regarding Fatty Acids

This section was presented in terms of two questions—the role of lowering TGs in reducing cardiovascular events and the potential beneficial role of PUFAs aside from their action to lower plasma TGs. To the first question, clinical studies with EPA and DHA in various forms and products have had negative results insofar as lowering of TGs occurred but in the absence of reduced cardiovascular events. This data adds to similar negative data for fibrates and niacin and therefore the question if targeting elevated TGs is a viable strategy for reducing cardiovascular events. It is very difficult to prove a negative association, so the question remains open as to the validity of targeting elevated TGs, and further studies should continue with different therapeutic agents and different mechanisms of action. However, it does appear that certain EPA derivatives at high enough doses, and possibly with a threshold blood level, can reduce cardiovascular events in parallel to but not dependent upon a reduction in plasma TG levels; these would be due to so-called pleiotropic effects. This situation was supported by the REDUCE-IT and JELIS trials. In this context, further studies are justified on the mechanism of action of EPA derivatives, as well as clinical trials, noting that there may be a benefit for EPA derivatives aside from their TG-lowering activity and therefore in a broader clinical context than treating patients with elevated TGs.

Thus, in relation to the therapeutic status of fish oils and related products, recent quality evidence suggests that there is both less and more to the fish oil and cardiovascular disease issue. The low or lack of efficacy of marine products in lowering TGs and reducing cardiovascular events brings lots of concerns to this area. The robust efficacy of IE in the REDUCE-IT trial, albeit related to uncharacterized pleiotropic effects and not TG lowering, provides evidence for the valid use of IE and possibly other products for reducing cardiovascular events in specific high-risk populations, noting the value of efficacious agents in this population. CVD research is often not conclusive, and certainly in this area, further basic and clinical research

and clinical trials are required on the background of gaining additional information from monitoring of the outcomes of the ongoing use of IE therapy with the current and likely expanded clinical indications.

Emerging Molecular Therapies for Dyslipidemias

Molecular medicines are now more often being applied to dyslipidemia, commencing with PCSK9 inhibitors, discussed earlier in this chapter and in another chapter herein by Dr. Peter Toth. The next two sections discuss molecular based therapies targeting ApoCIII and then angiopoietin-related protein 3 (ANGPTL3).

ApoCIII-Targeting Therapies

ApoCIII is a key modulator of lipoprotein metabolism, such as via effects on lipoprotein lipase and hepatic lipase, and is one of the main active research areas of molecular based gene silencing. There are currently three main gene silencing therapies targeting ApoCIII (or ApoC3) that are in ongoing clinical trials. The treatments are (1) volanesorsen (IONIS-APO-CIIIRx), (2) AKCEA-APOCIII-LRx, and (3) ARO-APOC3.

Basic Biochemical Action/Mechanism of Action of ApoCIII Gene Silencing Therapies

Gene therapy targeting (antisense oligonucleotides (ASOs) and silencing RNA or short interfering RNA (siRNA)) by silencing ApoC3 shares a common biochemical action/mechanism of action. Both target ApoC3, an apolipoprotein encoded by the *APOC3* gene. ApoC3 is mainly secreted by the liver and, to a less extent, by the intestine [248]. It is predominantly associated with TG-rich lipoproteins (TRLs), e.g., chylomicrons and VLDL, and, to a lesser extent, with LDL and HDL particles. Accumulating preclinical and clinical evidences demonstrate that ApoC3 regulates TRL metabolism via (1) suppressing lipoprotein lipase (LPL) activity and (2) interrupting the interaction of ApoB and ApoE with their LDL receptors (LDLR), thereby increasing plasma levels of TRL via reducing lipolysis and hepatic uptake [249, 250].

ApoC3 Roles in Lipoprotein Metabolism

There are four main actions of ApoC3:

1. ApoC3 suppresses lipoprotein lipase (LPL) and hepatic lipase and disrupts the interaction of ApoB and ApoE apolipoproteins with their hepatic receptors. As a result, both the lipolysis and hepatic uptake of TRL are decreased.
2. ApoC3 stimulates the hepatic synthesis and secretion of VLDL [249].
3. ApoC3 exchanges between VLDL and HDL particles. When VLDL is hydroxylated by LPL, ApoC3 will transfer to HDL from VLDL, in an amount proportional to the extent of TG hydrolysis in VLDL. Subsequently, ApoC3 will redistribute to newly synthesized TG-enriched VLDL particles.
4. The ApoC3 distribution depends on the TG content of the triglyceride-rich lipoproteins (TRL). The majority of ApoC3 is in HDL when the TG level in TRL is low; on the other hand, it will transfer back to TRL particles when the TG content of TRL is higher.

ApoC3 Actions in Cells and Animal Models

Overview

A hallmark work based on 3734 participants of European or African ancestry in the USA revealed that loss-of-function mutations in *APOC3* were associated with lower TG levels and a decreased risk of coronary artery disease by 40% [251]. Almost at the same time, another large-scale clinical investigation demonstrated that mutation of *APOC3* was also associated with lifelong lower TG levels and reduced risk of ischemic heart disease by 36% and of ischemic vascular disease by 41% in the general population [252]. Consistent with this evidence, five people with the *APOC3R19X* null mutation had a reduction of plasma ApoCIII by 50% and displayed increased lipolysis of VLDL-TG and conversion of VLDL to LDL, without obvious effect on hepatic uptake of VLDL [253]. In contrast, the *APOC3* gain-of-function Gln38Lys mutation is associated with higher TG levels by 32% [254] and, when expressed in mice, promotes $VLDL_1$ production via lipogenesis de novo [255]. Thus, therapy decreasing ApoCIII may be of interest to reduce the risk of atherosclerosis.

Actions in Animals

A recent preclinical study revealed that loss of function by CRISPR/Cas9 technology protects against atherogenesis in hamsters [256], with a similar lipid profile found in *APOC3*-muted humans [257]. Decreased TG levels occurred without changes in hepatic VLDL secretion in atherogenic diet-fed hamsters, which was consistent with the phenotypes observed in ApoC3-deficient humans [257]. Moreover, ApoC3 was identified as an endogenous moderator, which drives NLRP3 inflammasome activation, which also provides another alternative molecular mechanism linking ApoC3 and atherosclerosis beyond its regulation of the lipid profile [258]. Although hypertriglyceridemia is common in people with the metabolic syndrome or with type 2 diabetes, whether it is an independent risk for beta-cell dysfunction and insulin secretion remains controversial. A study in ApoC3-transgenic mice identified normal insulin sensitivity and beta-cell health, supporting that hypertriglyceridemia per se might not be an independent risk factor for beta-cell dysfunction [259]. On the contrary, human APOC3-overexpressing mice were phenotyped by severe insulin resistance and hepatic steatosis on either a regular chow or a high-fat diet [260]. In sum, lipid-lowering therapy, which may not be a good avenue of beta-cell failure, is still a primary target for reducing cardiovascular risk in people with obesity and type 2 diabetes. In this regard, hypoglycemic therapy and hypolipidemia therapy are of equal importance for these patients.

Actions in Cells

In vitro, ApoCIII inhibits LPL and interrupts the interaction of ApoB and ApoE with their hepatic receptors, thus increasing circulating TRLs via decreasing lipolysis and hepatic uptake [261]. This is further enhanced by the capacity of ApoCIII to promote hepatic synthesis and secretion of VLDL [262]. On the other hand, a murine-specific ApoCIII-targeting ASO reduced the TG level via both low-density lipoprotein receptors (LDLR) and low-density lipoprotein receptor-related protein 1 (LRP1) pathway, independent of LPL, LDLR, or LRP1 separately [263].

Pleiotropic Actions of ApoC3

Beyond its direct regulation on lipid metabolism, ApoC3 appears to have pleiotropic biological effects:

1. ApoCIII enhances a pro-inflammatory response, e.g., by activating nuclear factor kappa beta (NFkB) signaling [264, 265], and vascular cell adhesion molecule-1 (VCAM1) expression [266], thereafter facilitating monocyte recruitment.

2. ApoCIII promotes oxidative stress and proliferation of VSMCs, which are also involved in early atherogenesis [264].

ApoC3 Therapies in Human Clinical Trials

Volanesorsen

Overview

Volanesorsen (IONIS-APO-CIIIRx) is a second-generation 2′-*O*-methoxyethyl (2′-MOE) chimeric ASO silencing the *APOC3* mRNA [267, 268]. Subcutaneous injection of volanesorsen with doses ranging from 50 to 400 mg significantly decreased TG levels in a dose-dependent manner in a healthy population [268]. A prospective, population-based study revealed that volanesorsen (weekly 300 mg injections) significantly reduced plasma levels of ApoC3 and TGs in patients with hypertriglyceridemia [267]. More encouragingly, volanesorsen treatment reduced plasma ApoC3 and TG levels in a 15-week trial in patients with diabetes. Of note, these changes were accompanied by better glucose disposal and insulin sensitivity [269], highlighting the therapeutical potential of volanesorsen in people with type 2 diabetes and hypertriglyceridemia.

Phase II Clinical Trial

In phase II, dose-ranging trials, weekly volanesorsen treatment ranging from 100 to 300 mg resulted in a dose-dependent decrease of TG levels of approximately 70% in 57 patients with untreated hypertriglyceridemia, or treated hypertriglyceridemia on standard fibrate therapy [270, 271]. Mover, volanesorsen treatment reduced ApoC3 on ApoB-100-, Lp(a)-, and ApoA-I-containing lipoproteins and plasma levels of ApoC2 and improved insulin sensitivity [267, 271], all of which helped to improve the metabolic phenotype in people with the metabolic syndrome.

Phase III Clinical Trial

There are two phase III clinical trials for volanesorsen in patients with severe hypertriglyceridemia: APPROACH (NCT02211209) and COMPASS (NCT02300233).

In the APPROACH trial, volanesorsen (300 mg weekly administered subcutaneously) vs. placebo was administered in 66 familial chylomicronemia syndrome (FCS) subjects for 52 weeks [272]. Volanesorsen treatment decreased TG levels by 77% compared with an 18% increase of TGs in the placebo-treated patients [272].

In the COMPASS trial, 113 participants with fasting TG ≥500 mg/dL were randomized to volanesorsen (300 mg weekly subcutaneously) vs. placebo for 26 weeks [273]. Volanesorsen treatment reduced TG levels by 72% vs. 1% in those in the placebo group [273]. Furthermore, volanesorsen treatment induced a striking TG reduction, greater than that attained with other agents, including fibrates or $n-3$ fatty acids [271].

Despite the encouraging efficacy and safety of volanesorsen against hypertriglyceridemia, further clinical trials exploring the effect of volanesorsen on cardiovascular risk are warranted.

AKCEA-APOCIII-LRx

AKCEA-APOCIII-LRx is another ASO agent targeting ApoC3 with GalNac modification, which is currently under investigation. This new-generation ASO integrates the GalNac conjugate, enabling delivery of drug to the liver.

In the phase I/IIa clinical trial of healthy volunteers with triglyceride levels ≥90 or ≥200 mg/dL, AKCEA-APOCIII-LRx led to a broad improvement of the lipid profile including significant TG reduction, and without significant thrombocytopenia [274] .

ARO-APOC3

ARO-APOC3 is a novel siRNA-based agent against ApoC3, currently in an early-stage clinical trial. Information regarding its lipid-lowering efficacy and safety is still lacking [275].

Other ApoC3-Based Therapies

A recent study demonstrated that both human APOC3 A43T (Ala43Thr) heterozygotes, a missense variant, and mice overexpressing human APOC3 A43T significantly decreased plasma ApoC3 and TG level. A monoclonal antibody targeting ApoC3 enhanced the clearance of ApoC3 and TRL catabolism [276]. This machinery holds potential as a novel therapeutic approach for hypertriglyceridemia; however, further preclinical and clinical investigations on efficacy and safety are warranted.

Adverse Effects of Some ApoC3-Targeting Therapies

Thrombocytopenia

A major side effect of volanesorsen is thrombocytopenia, as revealed by the APPROACH study, which could be severe, but was not associated with bleeding events [272]. Notably, the platelet reduction is reversible with interruption of dosing, and to some extent seems to be dose dependent. More frequent platelet monitoring and either interrupted dosing or reduced dosage, or both, might help prevent thrombocytopenia. Although the COMPASS trial did not observe thrombocytopenia [273, 277], volanesorsen was not approved by the FDA [277].

Injection-Site Reactions

Mild-to-moderate injection-site reactions occur in 12% of recipients [272].

No significant changes in renal or liver function were observed.

Summary and Conclusion Regarding ApoC-Targeting Molecular Therapies

After decades of significant progress in three phases of ApoC3 ASO (volanesorsen), volanesorsen was not approved by the Food and Drug Administration, mainly due to concern about the side effect of thrombocytopenia. However, it was approved by the European Medicines Agency (EMA) in 2019 for the treatment of familial chylomicronemia syndrome. Long-term safety and efficacy data for inhibition of ApoC3 is also essential. Long-term clinical trials, including in people with diabetes, are warranted.

Angiopoietin-Related Protein 3 (ANGPTL3): Antibodies, ASOs, and siRNAs

ANGPTL3 protein, encoded by the ANGPTL3 gene, is a member of the angiopoietin-like family and has key roles in angiogenesis and in the regulation of lipoprotein metabolism. ANGPTL3 is an inhibitor of lipases, including LPL and hepatic lipase, which increases circulating TG, LDL cholesterol, and HDL cholesterol levels. Thus, ANGPTL3 inhibition by various approaches, including antibodies, ASOs, and siRNAs, as discussed below, is an active area of research of relevance to understanding and managing lipoproteins in people with diabetes.

Basic Biochemical Action/Mechanism of Action

ANGPTL3 inhibitors reduce plasma ANGPTL3 levels and the resultant ANGPTL3–ANGPTL8 complex which downregulates lipoprotein lipase (LPL) [278]. Other lipases, including hepatic lipase, and possibly endothelial lipase, may be similarly affected [279]. As a consequence, the various forms of ANGPTL3 inhibitors upregulate lipase activities. ANGPTL3 production is limited to hepatocytes alone, whilst the accompanying ANGPTL8 is produced in adipose tissue as well as liver. ANGPTL8 production is enhanced in the fed state, and the resultant inhibition of LPL in brown adipose tissue, as well as skeletal and cardiac muscle, redirects lipolysis to white adipose tissue, where the ANGPTL3–ANGPTL8 complex counteracts ANGPTL3. This mechanism favors the use of TG for energy storage over energy use in the fed state, and vice versa during fasting [280].

This mechanism of action explains the ability of ANGPTL3 inhibitors to reduce plasma TG levels [281]. On the other hand, the mechanism behind the accompanying reduction in LDL-C is not as well established. This is particularly intriguing because ANGPTL3 inhibitors are capable of achieving clinically significant reductions of plasma LDL cholesterol levels in patients with homozygous familial hypercholesterolemia (HoFH) [282]. These patients lack LDLR-mediated endocytosis and are notoriously resistant to traditional therapies, such as statins, that stimulate this catabolic path. Less clear is the effect of ANGPTL3 status on HDL cholesterol levels. Observations associated with endogenous or pharmacological reduction in ANGPTL3 activity vary between reduction in HDL cholesterol levels and no effect. This is surprising because the inverse relationship between TG and HDL cholesterol levels dictates that ANGPTL3 inhibitors would be expected to increase HDL cholesterol levels. The postulated involvement of endothelial lipase [283] creates the possibility that any decrease in HDL cholesterol associated with ANGPTL3 inhibitor use may be associated with changes in HDL function which are not necessarily detrimental.

ANGPTL3 Actions on Lipid Metabolism in Cells and in Animal Models

Genetically modified mouse models indicate that under- or overexpression of ANGPTL3 can modulate the clearance of triglyceride-rich lipoproteins and hence plasma TG levels [284]. Animal and human studies established the pattern of tissue expression and the involvement of other cofactors, most notably AposC1, C2, C3, and A5. Human genetic studies revealed that heterozygosity for a loss-of-function variant of the gene which codes for ANGPTL3 results in a phenotype referred to as familial combined hypolipidemia [285]. This phenotype consists of reduced plasma levels of TGs (approximately −25%) and LDL (approximately −10%) and HDL cholesterol. The HDL cholesterol-lowering effect of ANGPTL3 inhibitors has been

attributed to mechanisms involving endothelial lipase, but upregulation of hepatic lipase may also be a contributing factor [286].

More importantly, turnover studies demonstrate that inhibition of ANGPTL3 reduces LDL cholesterol in a manner that is independent of LDLR activity. Whilst modest reduction in LDL synthesis is possible, the predominant effect appears to involve enhanced catabolism of intermediate-density remnants of TRL [287]. Clinicians are justifiably optimistic that this attribute will be of great therapeutic importance [288], but a beneficial effect on clinical outcomes is yet to be demonstrated. Studies of anti-ANGPTL3 antibody therapy in an appropriate atherosclerosis-prone mouse model demonstrated an encouraging reduction in coronary lesion size by approximately 40% [289]. Whilst laboratory studies help discern mechanisms due to difference in cells, animals, and humans, including in lipoprotein metabolism, human studies are essential.

ANGPTL3 Actions in Humans, Including Clinical Trials

The various forms of anti-ANGPTL3 therapy target ANGPTL3 at different points in its synthesis and secretion, so the relative impact may vary. Monoclonal antibodies against ANGPTL3 target most of the circulating protein. This may explain why the LDL cholesterol reductions associated with phase II and phase III trials are substantially greater (more than 50% decrease) than those seen in familial combined hypolipidemia. The lifelong hypolipidemia is associated with decreased risk of atherosclerotic CVD events (approximately 40% reduction [285]), which is consistent with reduced plaque volumes observed in animal studies of anti-ANGPTL3 antibody therapy [289].

Monoclonal antibody therapy is required on a frequent, usually weekly or monthly, basis, whereas gene silencing strategies such as antisense oligonucleotides (ASO) or small interfering RNAs (siRNA) directed against ANGPTL3 require less frequent dosing and are likely to be less costly over time. ANGPTL3 is a particularly suitable target for these therapies because ASOs and siRNAs can be specifically targeted for uptake by hepatic asialoglycoprotein receptors via conjugation with GalNAc residues [290].

Pleiotropic Actions and Adverse Drug Effects of ANGPTL3 Inhibitors

The highly effective targeting of gene silencing therapy towards the liver, the only tissue which synthesizes ANGPTL3, together with the exquisite specificity of target selection according to nucleotide sequence, makes off-target effects unlikely. Similarly, it also leaves little opportunity for pleiotropic effects, which might achieve serendipitous outcomes.

Injection-site reactions are the most consistent side effect in clinical trials to date, but they rarely lead to drug discontinuation.

The long half-life of the therapeutic effect of gene silencing techniques requires detailed consideration of all the physiological effects of ANGPTL3 to ensure that unwanted consequences do not occur. The lack of detrimental outcomes associated with ANGPTL3 loss-of-function variants is reassuring in this regard. On the other hand, ANGPTL3 levels decline in normal pregnancy [291]. The use of these agents in women of childbearing age could be challenging, whilst even more permanent approaches such as vaccination or CRISPR gene therapy are even less certain in this situation.

Summary and Conclusions Regarding ANGPTL3 Inhibition

The pattern of dyslipidemia often associated with type 2 diabetes features elevated TG and an excessive number of LDL particles. Furthermore, elevated levels of ANGPTL3 have been reported in patients with diabetes. Novel therapies which use the same strategies to target ApoC3 are also under development, as discussed in the previous section of this chapter, and they too target an inhibitor of LPL. The effects are likely to be similar, but not identical. Both have demonstrated remarkable ability to control cases of severe hypertriglyceridemia (>880 mg/dL, >10 mmol/L), which represents a high risk of acute pancreatitis. Anti-ApoC3 interventions achieve an equal or greater reduction in TG, which is associated with the expected reciprocal increase in HDL-C. On the other hand, the activation of an alternative catabolic pathway for LDL reduction appears to be a unique feature of anti-ANGPTL3 interventions. Both are likely to be suitable for use in patients with diabetes, and both are likely to reduce the risk of atherosclerotic CVD. There are theoretical reasons why they may differ in their ability to achieve this outcome, but it is highly likely that anti-ANGPTL3 interventions will have a unique role in the management of hoFH [292]. We await the outcomes of further research and clinical trials.

Other Relatively New and Emerging Lipid Drugs

Since the publication of the first edition of this book, other lipid drugs have emerged, are in development, or are being tested in different diabetes settings. As discussed in other chapters herein, PPARα agonists, such as fenofibrate, have shown microvascular complication protection, particularly for diabetic retinopathy in adults with type 2 diabetes. However, these positive results in the FIELD [293] and ACCORD Lipid trials [294] were pre-stated endpoints, but not primary endpoints. They have been supported by an Asian database audit [295] and a meta-analysis [296]. Currently, as recently reviewed, three clinical trials of fenofibrate for diabetic retinopathy in type 1 and type 2 diabetes are in progress [296, 297] It is envisaged that

more ACLY inhibitors will arise and more molecular based therapies will emerge, in addition to PCSK9 inhibitors and the ApoCIII-targeting treatments described herein. Lipoprotein(a) discussed in a dedicated chapter herein has a resurgence of interest regarding its role in cardiovascular disease and diabetes complications. Some molecular based therapies targeting lipoprotein(a) (Lp(a)) are in clinical trials [198]. As yet, there are no trials of Lp(a)-targeting molecular therapies, specifically in diabetes, nor reports of outcomes in diabetes subgroups.

Overall Summary and Conclusion

Quantitative and qualitative changes in lipoproteins are implicated in the cardiovascular and microvascular complications of diabetes, as discussed in other chapters herein. People with diabetes have already benefitted from existent lipid drugs and related treatment guidelines. With regard to statins, the Cholesterol Treatment Trialists Collaboration showed that people with diabetes gain as much cardioprotection from statins as do people without diabetes [298]. There are substantial knowledge gaps regarding the benefits or not of the new and emerging lipid drugs and various lipid drug combinations for the macrovascular and microvascular complications of type 1 and type 2 diabetes. Such data for the newer drugs will likely arise in the next few years. As for all lipid-modifying drugs in diabetes, whilst not as robust as primary endpoint randomized controlled trials, where long-term randomized controlled clinical trials are not available, the analysis of large insurance and clinical databases may provide information. The challenge, particularly considering that 80% of people with diabetes today live in disadvantaged regions, will be to equitably translate the use of effective lipid therapies into clinical practice.

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Part IV
Epidemiology of Diabetes and Diabetic Dyslipidemia

Chapter 31
Diabetes Epidemiology and Its Implications

Zachary Bloomgarden and Yehuda Handelsman

Introduction

It is by now commonplace knowledge that the number of persons with diabetes has been increasing in every region of the globe. More than 40 years ago, Kelly West suggested, "a preventive and a cure are already at hand for most diabetes. The cause is usually obesity; the preventive, and often the cure, is leanness" [1]. Subsequent epidemiologic research has, however, shown a number of additional factors. Increasing food intake, lack of physical activity, and environmental toxins contribute to the development of obesity, hence increasing population susceptibility. Diabetes is strongly associated with aging, so that an important driver of the increasing diabetes prevalence is simply the increasing number of older persons in the population. Furthermore, mortality among persons with diabetes has decreased, appearing to be at least in part due to reduction in cardiovascular disease [2], with reports to this effect from many areas, including the United Kingdom [3], Denmark [4], as well as the United States [5], adding to the number of prevalent cases of diabetes.

All the drivers of this epidemic of obesity, diabetes, and associated metabolic disease are similar from region to region. The implication is of a growing burden for healthcare systems throughout the world. By understanding the epidemiology of diabetes, we can endeavor to identify current trends and priorities for preventative and management approaches.

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Diabetes Definitions and Prevalence Estimates

The diagnosis of diabetes can be made based on fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L), plasma glucose ≥200 mg/dL (>11.0 mmol/L) 2 h after a 75 g oral glucose stimulation, or HbA1c ≥6.5% (47.5 mmol/mol). Many studies accept a person's history of diabetes, or of use of insulin or other hypoglycemic drugs, as an additional diagnostic criterion. The prevalence based on HbA1c may be inaccurate because of the direct association of HbA1c with increasing age [6], or because of variations in relationships between glycemia and HbA1c in different ethnic groups [7–9], or because of effects of kidney disease and anemia [10]. Overall, HbA1c-derived diabetes prevalence is similar to that based on FPG, although in individual population surveys, one or another approach may lead to as much as a 5–10% higher prevalence, while the prevalence based on either FPG or 2-h OGTT is 2–6% higher than that based on FPG alone [11]. This chapter addresses overall diabetes prevalence, primarily reflecting that of type 2 diabetes. The epidemiology of type 1 diabetes, which has also increased in recent years, has different associations and has been the topic of a number of recent reviews [12, 13].

In 2019, the International Diabetes Federation reported that the world population of persons aged 20–79 years with diabetes was 463 million persons, resulting in an age-standardized prevalence of 8.3% [14]; with the release of the IDF Diabetes Atlas 10th edition, the 2021 world population of persons with diabetes was reported as being 538 million persons, with prevalence of 10.5% of the adult population [15]. The International Diabetes Federation projects that there will be 644 million persons with diabetes in 2030, and 783 million persons with diabetes in 2045, for an age-standardized prevalence of 12.2%; the largest number is and will be in the Western Pacific region and then Southeast Asia, and the Middle East and North Africa have overtaken Europe in the prevalence of diabetes (Fig. 31.1) [10]. As high

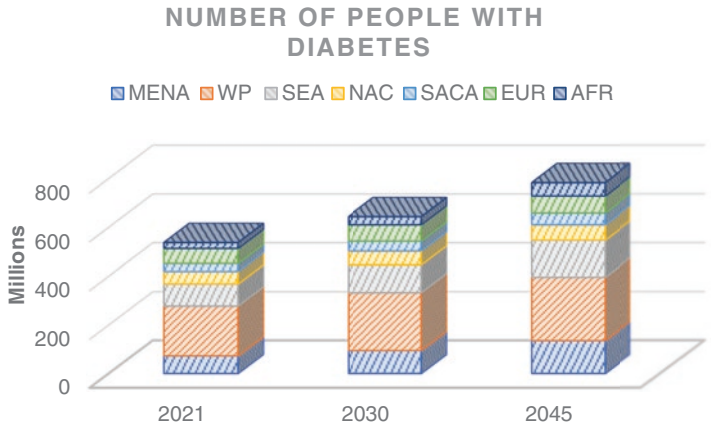

Fig. 31.1 Number of persons with diabetes, 2021. *AFR* Africa; *EUR* Europe; *MENA* Middle East and North Africa; *NAC* North America and Caribbean; *SACA* South and Central America; *SEA* Southeast Asia; *WP* Western Pacific. Redrawn from data in the International Diabetes Federation, IDF Diabetes Atlas 10th edition, downloaded December 12, 2021, from https://diabetesatlas.org/resources/ [15]

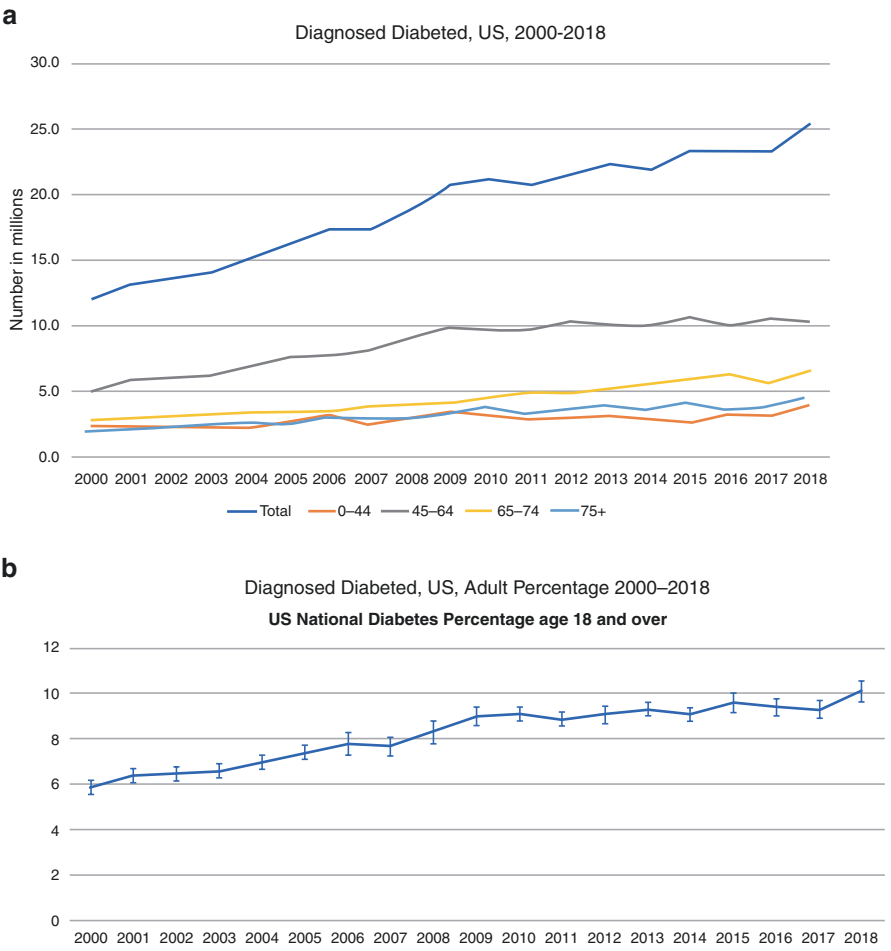

Fig. 31.2 Number of persons with diagnosed diabetes in the United States, 2000–2018. (**a**) Number of millions of persons, total and by age group, downloaded 21 August 2021 from www.cdc.gov/diabetes/data. (**b**) Percentage of Adult Population, downloaded 5 September 2021 from gis.cdc.gov/grasp/diabetes/DiabetesAtlas.html#

as these levels of prevalence appear, even greater lifetime diabetes risks are implied. Among 20-year-old urban men and women in India, for example, lifetime risks are 56% and 65%, respectively; specifically in overweight and in obese persons at age 20, lifetime risks are 71% and 87%, respectively [16].

Data from the Centers for Disease Control in the United States indicate that diagnosed diabetes prevalence increased progressively from 6% in 2000 to 10% in 2018 (Fig. 31.2a); considering the growth in the US population, the number of persons with diabetes increased from 12 to 25 million, with the increase at age 45–65 growing from five to ten million persons with diabetes (Fig. 31.2b) [17]. The National Health and Nutrition Examination Surveys (NHANES) in the United States diagnosing diabetes from HbA1c and fasting glucose show an increase in diagnosed

diabetes from 7% and in total diabetes from 10% in 1999–2000 to 11% and 14%, respectively, in 2017–2018, while undiagnosed diabetes was stable at around 3% [18]. A different NHANES analysis of the smaller number of persons with full OGTT (including the 2-h OGTT value), however, suggested that total diabetes prevalence may not be increasing, with stable total diabetes levels from 2005 to 2016, albeit with prevalence of diagnosed diabetes increasing from 7.6% in 2005–2006 to 10.1% in 2015–2016, while undiagnosed prevalence levels decreased from 5.4% to 4.6%, respectively [19]. Diabetes prevalence projections in North America from 2015 to 2040 are for increases from 8.5 to 11.7% in Houston, from 9.1 to 11.9% in Mexico City, and from 7.2 to 11.3% in Vancouver [20].

Relationships Between Diabetes and Obesity

Obesity measurement has generally been standardized with the body mass index (BMI), the weight in kilograms divided by the square of the height in meters, with "normal" BMI considered to be 18.5–25, overweight >25 to <30, and obesity 30 kg/m^2 and over. The prevalence of obesity among adults in the United States was 20.9% in 2001, accompanied by diabetes prevalence of 7.9% [21], with dramatic increase in obesity prevalence to 42.4% in 2017–2018 [22], accompanied by a 10.5% diabetes prevalence [23]. Comparing BMI in multiple countries, among >50,000 persons with diabetes and >550,000 persons with normoglycemia, a recent analysis using these cutoff levels showed normal weight in 45% of those with diabetes, but in 61% of those with normal glycemic status, while 34% and 22% were overweight and 14% and 6% were obese, respectively [24]. Optimal BMI, however, varies from region to region and among different ethnicities. In fact, a confusing issue has been the use of BMI criteria derived from Western populations to define obesity in the Asian populations [25]. To account for the differences, the WHO established a lower criterion of BMI of 23 as cutoff for overweight in East Asia and the Asian Pacific region [26]. For example, in a study analyzing the association between BMI and diabetes, the cutoff for optimal sensitivity and specificity for diabetes ranged, in men, from 23.8 in East, South, and Southeast Asia to 28.1 in Oceania, and, in women, from 23.9 in East, South, and Southeast Asia to 28.3 in Latin America and the Caribbean, and in the Middle East and North Africa [27].

Central obesity with increased abdominal circumference is often, although not always, related to greater levels of visceral adiposity and is seen even more commonly than obesity based on BMI criteria. Abdominal obesity, defined by waist circumference >102 cm for men and >88 cm for women, increased in prevalence in the NHANES of 1999/2000 through 2013/2014 from 47 to 57% in association with increase in diabetes prevalence from 9 to 12% [28]. A meta-analysis of 13.2 million persons from a European population reported 31% abdominal obesity prevalence in 1985–1999, increasing to 48% of the adult population in 2010–2014 (Fig. 31.3) [29].

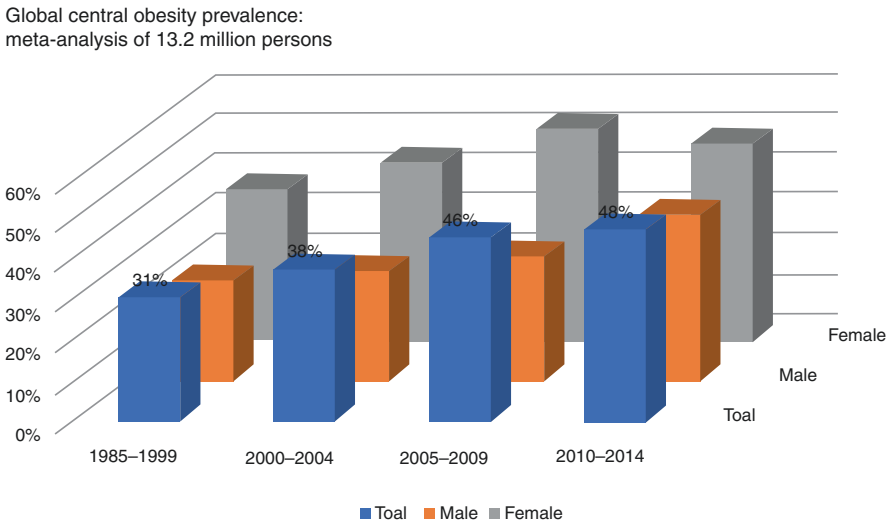

Fig. 31.3 Global prevalence of central obesity, 1985–2014. Redrawn from data in [29]. Wong et al. European Journal of Epidemiology 2020;35:673–683

Relationships Between Diabetes and Aging

The global prevalence of diabetes in high- and middle-income countries increases with age, from 5% at age 35–39 to 10% at age 45–49, to 15% at age 50–54, and to the highest level, 20%, at age ≥65 [30]. Furthermore, the prevalence of diabetes is particularly increasing in the elderly. In a previous review [31], we noted that the elderly group has considerably higher diabetes prevalence than younger adults, implying that this group will account for ever-increasing numbers of people with diabetes. Studies of this point include a US survey of nursing home residents aged 55+ years finding that the prevalence of diabetes increased over the period 1995–2004, from 16.9 to 26.4% among men and from 16.1 to 22.2% among women [32]. Among noninstitutionalized US adults aged 65+ years, the prevalence of diabetes increased over the period 1994–2003 from 15.3 to 24.8% [33]. In Australia, the prevalence of diabetes among people aged 60–79 years increased over the period 1991–2003 from 8.1 to 15.2%, with a projection of a further increase to 22.4% in 2016 [34]. In Taiwan, diabetes prevalence among people aged 60–79 years increased over the period 2000–2007 from 17.6 to 25.9% [35]. A study based on data from the US National Health Interview Survey has suggested that among those aged 65–74 years, the prevalence of diabetes will increase from 16.0% in 2005 to 32.7% in 2050 [36]. Another study showed that diabetes prevalence among US Medicare beneficiaries aged ≥68 years increased from 23% in 2001 to 31.6% in 2015 [37].

Recent studies further support the importance of aging in the growth of diabetes. Over the past two decades, the incidence of diabetes in Denmark increased by 22%, in part due to changes in the population age distribution, with the proportion of patients with diabetes over age 70 years predicted to increase from 43 to 46% for women and from 38 to 45% for men by 2030 [4]. The projected worldwide prevalence of diabetes will be approximately 20% at age 65–99 years in 2030 and 2045 [38], so that, as this population subset grows, the number of persons with diabetes will follow. The most rapidly growing subgroup of the world's population is of persons over age 64 years, projected to increase from around 0.5 billion in 2010 to 1 billion in 2030, 1.5 billion in 2050, and 2 billion in 2070; by the year 2100, there will be approximately 2.5 billion persons aged >64 years, approximately the same number as those aged 45–64 years and those aged <20 years [31]. Both in China and in the United States, by 2100, those aged ≥65 years will be the most prevalent age subgroup [31].

Relationships Between Diabetes and Ethnicity

The heterogeneity in diabetes prevalence from region to region strongly suggests that different ethnic groups have differences in susceptibility to diabetes. Studies in specific regions support this as an additional factor of importance in determining diabetes prevalence. Diabetes prevalence among First Nations people in Ontario, Canada, from 1995 to 2014 is 28% greater than among other persons in the same region [39]. An analysis of nearly 500,000 persons from 2007 to 2010 in the UK Biobank showed diabetes prevalence around 5% in Whites and Chinese, 10% in Blacks, and 18% in South Asians [40]. In the United States, studies using self-reported telephone health survey data through 2012 show high diabetes rates associated with non-Hispanic African-American ethnicity [41]. Diabetes prevalence estimates in US Medicare populations from 2001 to 2015 consistently show ≥25% higher diabetes prevalence among Black, Hispanic, and Asian/Pacific Islander men and women than among White men and women [37].

Relationships Between Diabetes and Physical Activity, with Interactive Effect of Environmental Toxins

Among ~14,000 persons in the US NHANES, nearly two-thirds engaged in physical activity (PA) for least 2.5 h per week, in association with 19–32% lower likelihood of diabetes than that among those with lower levels of PA [42]. Among 3932 participants in the NHANES 2015–2016, the prevalence of diabetes was 29%, 34%, and 42% lower among participants in the second, third, and fourth quartiles of PA compared with those in the lowest PA quartile [43].

Although the effect of PA does not appear to be as great as that of optimal BMI, the interaction of PA with other risk factors is of interest. Using data from nearly

300,000 persons in the National Health Interview Survey (NHIS) dataset from 2004 to 2013, PA, though important, appeared to be a weaker predictor of diabetes than ethnicity (Black vs. White), BMI, and optimal sleep duration (7–8 h per night) [44]. Similarly, a study based on data from >8000 participants in the 2004 Joint Canada/U.S. Survey of Health showed that the higher prevalence of diabetes in the United States, controlling for age, sex, race, and education, was only modestly explained by differences in levels of PA [45].

Another factor contributing to the development of diabetes appears to be ambient air pollution, with greater levels of exposure to this associated with 11–23% increase in type 2 diabetes prevalence in a study of 7770 persons aged 50 years and over in China [46]. Potentiation of the association of low physical activity with diabetes by elevated levels of exposure to air pollution was found in a study of 156,314 adults in Taiwan followed in 2001–2016; those with high physical activity and low particulate matter had a 64% lower risk of type 2 diabetes than those who were inactive and had high particulate matter exposure [47].

Summary

This overview allows understanding of the factors involved in the diabetes epidemic, with a projected increase in the number of persons with diabetes from 537 million in 2021, by ~20%, to 644 million by 2030 and by an additional ~20% to 783 million by 2045 [9]. Beyond the progressive obesity epidemic, population growth emerges as the major explanation, responsible for some 60% of the growth in diabetes [9], with aging of the population further contributing to the diabetes prevalence. Furthermore, the projected decrease in diabetes mortality, primarily due to the improvement in the management of comorbidities, specifically atherosclerotic cardiovascular disease, appears to be another factor which will lead to increasing numbers of persons with diabetes. The areas in which intervention appears to offer promise in reducing diabetes development are improved lifestyle, focusing on reduced calorie and CHO diets, and increase in energy expenditure through exercise to attenuate the progressive increase in obesity and decrease in physical inactivity, along with initiatives to improve air pollution. By addressing these reversible factors, we should be able to use the insights from epidemiology to meaningfully intervene.

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Chapter 32
Epidemiology, Control, and Cardiovascular Outcomes of Dyslipidemia in Diabetes

Wenjun Fan and Nathan D. Wong

Diabetes mellitus (DM) is a well-established independent risk factor for atherosclerotic cardiovascular diseases (ASCVD) [1]. Compared with individuals without DM, DM patients have a two to four times increased risk for ASCVD events [1]. Dyslipidemia is a principal risk factor for ASCVD both in those with and without DM. Dyslipidemia in DM is frequently characterized by hypertriglyceridemia (HTG), reduced high-density lipoprotein cholesterol (HDL-C), and moderate elevations in low-density lipoprotein cholesterol (LDL-C) with a greater number of small dense LDL particles. These measures are all associated with increased risk of ASCVD. Insulin resistance, defined as the decreased ability of insulin to act effectively on peripheral target tissues (especially muscle, adipose tissue, and liver), results from a combination of genetic susceptibility and obesity and is a shared characteristic that almost certainly contributes to the distinctive lipid triad found in those with DM [2] (Central Illustration).

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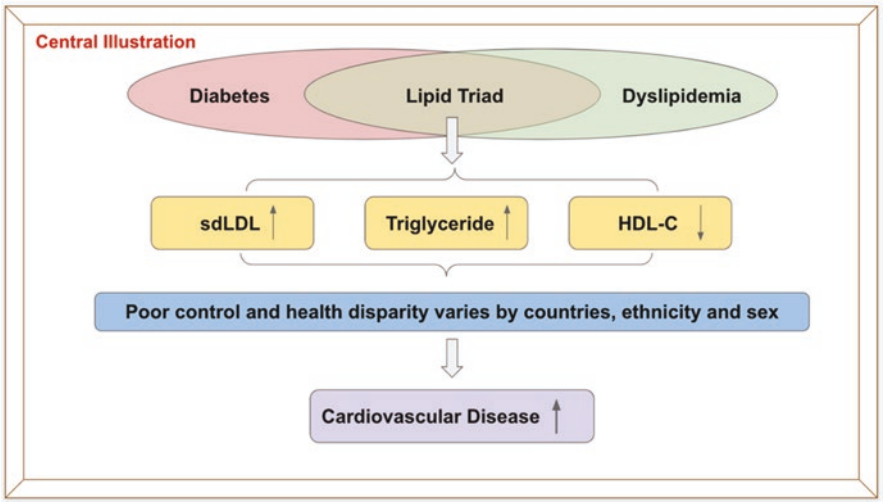

In this chapter, we will focus on the epidemiology of dyslipidemia, disparities in lipid control, and its association with ASCVD risk among DM patients.

Prevalence and Risk Factors for Dyslipidemia in Diabetes

Dyslipidemia is common in people with DM and in particular type 2 DM (T2DM) but the prevalence varies between different populations, the presence of the metabolic syndrome, and there is variation regarding how it is defined. Commonly, dyslipidemia is defined as elevated triglycerides (TG) (≥150 mg/dL), decreased HDL-C (<40 mg/dL in men and <50 mg/dL in women), or increased LDL-C (defined as ≥100 mg/dL) [3], although in those with T2DM two or even all three of these frequently occur together. In the United States, it is estimated that 35–50% of patients with T2DM have dyslipidemia [4–6], and over 40% have elevated TG regardless of statin use [7]. In European countries, the prevalence of elevated TG and decreased HDL-C has been reported to be approximately 15% regardless of lipid treatment, but higher than those without DM [8]. Results from the China National Nutrition and Health Survey (CNNHS) have shown the prevalence of dyslipidemia is 39.9%, 46.8%, and 59.3% in participants with normal glucose, prediabetes, and T2DM [9]. In Bangladesh, the prevalence of dyslipidemia among DM patients was 73% for men and 71% for women [10]. A cross-sectional survey among DM patients in Thailand has suggested that the prevalence of dyslipidemia is high as 88.9% [11].

There is also variation between gender across different epidemiological studies. One study suggests that women with T2DM more frequently have low HDL-C as compared to men ($p < 0.05$), while no significant difference is found regarding serum cholesterol, serum triglyceride, and serum LDL ($p > 0.05$) in DM patients [12]. Another study in China shows that women with DM have slightly lower

dyslipidemia prevalence than men (38.7% vs. 43.3%) while dyslipidemia is defined according to Chinese dyslipidemia prevention guideline based on having any one of the following: high total cholesterol (≥6.22 mmol/L), HTG (≥2.26 mmol/L), and/or high LDL-C (≥4.14 mmol/L) or low HDL-C (<1.04 mmol/L), use of antihyperlipidemic medications, or self-reported dyslipidemia [9]. Among those with concomitant coronary heart disease (CHD), women have higher levels of total cholesterol (4.98 vs. 4.46 mmol/L; $p < 0.001$), LDL-C (2.82 vs. 2.54 mmol/L; $p < 0.001$), and triglycerides (2.02 vs. 1.79 mmol/L; $p < 0.001$) compared to their male counterparts [13]. Sex disparities of dyslipidemia also exist due to fewer women receiving lipid-lowering therapy than men with DM (38.1% vs. 48.2%; $p < 0.001$) [13].

When comparing dyslipidemia across race/ethnicity, most minority subgroups have an increased prevalence of elevated TG compared to non-Hispanic white persons except African American persons (AAs). Most minority groups also have an increased prevalence of low HDL-C, except for Japanese and AA persons. The prevalence of high LDL-C is increased among Asian Indian, Filipino, Japanese, and Vietnamese persons compared with non-Hispanic white persons [14]. The prevalence of low HDL-C remained higher in Asian Indian DM patients (67.4%) than in central and northern European (37.3%), Japanese (34.1%), or Chinese (17.0%) persons, even in individuals with LDL-C of less than 3 mmol/L [15]. According to data from a community-based cohort study, 63.4% of Hispanic persons with diabetes have elevated LDL-C [16], compared to 36% among those without diabetes [17]. Compared with central and northern European persons, adjusted odds ratios (ORs) for having lower HDL-C are 3.07 (95%CI: 2.15–4.40) and 2.37 (95%CI: 1.67–3.35) in Asian Indian persons, but 0.11 (0.07–0.20) and 0.16 (0.08–0.32) in Chinese who had undiagnosed and diagnosed DM, respectively [15].

Patients with DM and obesity are at increased risk for long-term vascular outcomes [18]. There is significant variation in the rates of obesity in the DM population around the world. In the United States, 87% of DM patients are overweight or obese [19], while the prevalence is similar in United Kingdom (90%) [20] and African countries (85%) [21], and slightly lower in Asian countries (59.7%) [22]. An observational study has shown the prevalence of dyslipidemia (elevated TG, increased LDL-C, decreased HDL-C) is 53.4%, 60.3%, 44.8%, respectively, in obese T2DM patients, compared to 37.4%, 64.6%, 46.5%, respectively, in normal-weight T2DM. It also showed that obese T2DM patients are more likely to have elevated TG (OR = 2.11, 95%CI: 1.03–4.31, $p < 0.05$) while other lipid profile is similar to those with normal weight [9]. In obese people, there is an increase in total fat in the body including both subcutaneous fat and visceral fat. Visceral fat is more strongly associated with dyslipidemia than subcutaneous fat, although the coexistence of both lead to greater abnormalities in the lipid profile than the presence of either alone [23]. Higher TG and LDL-C levels and lower HDL-C levels are noted in those with visceral obesity among those with increased BMI. This is supported by observations made when comparing the average lipid levels between the four groups classified on the basis of having increased waist circumference and/or BMI [24]. Groups with normal waist circumference and normal BMI have the most

favorable lipid profile, whereas those with normal waist circumference but elevated BMI (or vice versa) show an intermediate lipid profile score, and those with elevations in both BMI and waist circumference have the most abnormal lipid profiles [25].

Dyslipidemia in Diabetes: LDL-C

The prevalence of the abnormal LDL-C remains high among DM patients worldwide. A global estimate is that 65% of patients with DM have LDL-C levels ≥100 mg/dL, which strongly points to the need for greater control efforts by lifestyle and pharmacotherapy [26, 27]. Of note, South and Southeast Asian populations, with approximately over a billion people who live in or come from India, Pakistan, Bangladesh, Sri Lanka, Nepal, Bhutan, Indonesia, Singapore, and Malaysia [28, 29], are disproportionally affected by dyslipidemia, which contributes to their greater risk of developing ASCVD. The prevalence of LDL-C ≥130 mg/dL has been reported to be 41.9% and 47.9% in Indonesia and Philippines general populations, respectively [30]. Meanwhile, the overall prevalence of T2DM in South Asia is high and has increased rapidly over the past four decades [28].

As one of the major independent risk factors for cardiovascular disease (CVD), numerous epidemiological studies have examined the association between LDL-C and ASCVD outcomes, especially among those at high risk such as persons with DM. An observational primary prevention cohort including 19,095 DM patients with statin use in the US reveals that compared to achieved LDL-C ≥100 mg/dL, LDL-C <50 mg/dL are 34% less likely to have the composite outcome of nonfatal MI, ischemic stroke, or CHD death [31]. A retrospective cohort study from the Korean National Health Insurance Service database of over two million DM patients without prior CVD has found that among non-statin users, LDL-C levels of 130–159 mg/dL and ≥160 mg/dL are significantly associated with the risk of myocardial infarction (MI) (HR = 1.19, 95%CI: 1.14–1.25; HR = 1.53, 95%CI: 1.46–1.62, respectively). Among statin users, LDL-C ≥70 mg/dL is significantly associated with an increased risk of stroke and MI [32].

Although high LDL-C predicts increased ASCVD risk in patients with DM, and its reduction has the most significant effect on lowering ASCVD risk, elevated LDL-C levels per se are not frequently characterized as part of the dyslipidemia profile in DM and the prevalence of high LDL-C levels in individuals with DM is similar to that in general population [2]. In the United Kingdom Prospective Diabetes Study (UKPDS), no differences in total cholesterol levels were reported between DM and non-DM subjects [33]. LDL-C levels have been reported to be comparable in men but slightly higher in women with T2DM compared to those without [33]. However, compared with non-DM persons, people with DM often have a different LDL particle size distribution, characterized by greater proportions of highly atherogenic, small dense LDL (sdLDL) particles [34].

LDL particles in patients with DM may be atherogenic even at normal LDL-C concentrations. For example, glycosylated LDL can be taken up by macrophage

scavenger receptors in an unregulated manner, thereby contributing to foam cell formation [35]. In addition, HTG is associated with small, dense, and cholesterol ester-depleted LDL particles. Thus, individuals with T2DM and mild to moderate HTG exhibit the pattern of smaller, denser particles of LDL-C. These particles might be more susceptible to oxidative modification and catabolism via macrophage scavenger receptors than pattern regular LDL particles. High sdLDL plus DM is significantly associated with major adverse cardiovascular events (MACEs) after adjustment of confounding risk factors [36]. Overproduction of LDL apo B100 may also occur with T2DM even with mild degrees of hyperglycemia, especially if there is concomitant elevation of very low-density lipoprotein (VLDL), resulting in the atherogenic dyslipidemia triad. Each LDL particle, regardless of its density or cholesterol content, contains only a single tightly bound molecule of apoB. The often "normal" level of LDL-C seen in many patients with DM often masks an increased particle number (higher apoB) associated with slower clearance of small dense particles [37]. Thus, measurement of the serum concentration of apoB provides a more discriminating index of atherogenic risk or therapeutic response than LDL-C. The use of LDL-C in DM typically underestimates the atherogenic contribution of TG-rich particles, therefore non-HDL cholesterol or apoB are better measures of atherogenicity in patients with DM [38].

Dyslipidemia in Diabetes: Triglycerides

Elevated triglyceride levels are a key characteristic of dyslipidemia among DM patients, which may further exacerbate insulin resistance and β-cell dysfunction [39, 40]. Although the exact mechanisms are only partially understood, it seems that elevated concentrations of free fatty acids (FFAs) disrupt or modulate the cascade linking insulin receptors with glucose transporters and impair the normal function of the β-cell [41]. In addition, FFAs are important modulators of inflammation. Therefore, HTG may induce subclinical inflammation which then leads to insulin resistance and β-cell dysfunction. The fact that HTG can worsen glucose metabolism is clinically important as it explains why it is more difficult to control hyperglycemia in DM patients with HTG compared to those with normal triglyceride values. It also explains why patients usually require less intensive antidiabetic treatment once HTG has resolved.

Normal fasting levels of plasma TG are defined by current clinical guidelines as <1.69 mmol/L (<150 mg/dL) [3]. The definitions of elevated triglyceride levels vary but fasting TG levels of 1.69–2.25 mmol/L (150–199 mg/dL) are often considered moderately elevated and fasting TG ≥2.26 or 2.83 mmol/L (200 or 250 mg/dL) are considered high and ≥5.65 mmol/L (500 mg/dL) severely elevated [3]. In overall populations, the prevalence of HTG among middle and older aged persons are 12.3% and 11.9% in Taiwan [42], and 49.9% in Thailand [11]. In the United States, the prevalence of elevated TG (≥150 mg/dL) among non-statin users was 24.7% in the general population compared to 45.4% in diabetes patients, while a much smaller difference was seen in statin users (31.6% vs. 39.5%) [43]. Even among DM

statin users with LDL-C <70 mg/dL, borderline HTG (150–199 mg/dL) prevalence was 16.8% and HTG (>200 mg/dL) prevalence was 16.7% [7]. The prevalence of HTG was lowest among non-Hispanic Blacks DM patients (14.4%) and highest among Hispanics (32.2%), especially those without statin treatment, while gender differences in HTG prevalence have not been noted in the US DM patients [7].

Elevated plasma TG can be due to increased TG production, decreased lipolysis of TG, and/or reduced clearance of TG-rich lipoproteins (TRLs) [44]. Moderate HTG is common in persons at increased risk of CVD, including patients with T2DM. Moreover, HTG is also strongly associated with a host of other potential risk factors such as obesity, increased levels of all apolipoprotein (apo)B particles like remnant lipoproteins (RLPs), and sdLDL, and low levels of HDL-C [45]. Causes of HTG in DM include increased hepatic VLDL production and defective removal of chylomicrons and chylomicron remnants (CMRs) from serum, which often reflects poor glycemic control. Overproduction of VLDL, with increased secretion of both TG and apo B100, appears to be the central cause of increased plasma VLDL levels in patients with T2DM [46]. Increased assembly and secretion of VLDL is probably a direct result of both insulin resistance and increases in FFA flux to the liver as well as de novo hepatic lipogenesis (with increased TG synthesis). Lipoprotein lipase (LPL) levels are reduced in T2DM, resulting in less triglyceride lipolysis, and this may contribute significantly to elevated TG levels, particularly in severely hyperglycemic patients [47]. Insulin or oral antiglycemic agents only partly correct VLDL abnormalities in the majority of individuals with T2DM.

Abundant epidemiological evidence associates TG levels with CVD risk [48, 49] although the risk is often attenuated when adjusted for potential confounders, such as HDL-C [37]. One study examining participants from the Atherosclerosis Risk in Communities Study and Framingham Offspring Study free of CVD suggests that the risk for CVD increased as average TGs rose until an inflection point of ~100 mg/dL in men and ~200 mg/dL in women, above which this risk association plateaued. The interaction term between TG and HDL-C is significant, for those with low HDL-C, the association between the TG level and risk is steepest until around 100 mg/dL, whereas in those with higher HDL-C, the CVD risk increased with increasing TG levels [50]. In DM patients, a retrospective cohort study based on electronic health records (EHRs) from an integrated healthcare delivery system involving 27, 953 patients with controlled LDL-C with prior CVD or at least one other CVD risk factor has shown that comparing high TG (200–499 mg/dL) versus normal TG (<150 mg/dL) the rate ratio (RR) = 1.30 (95%CI: 1.08–1.58, $p = 0.006$) for nonfatal MI, RR = 1.23 (95%CI: 1.01–1.49, $p = 0.037$) for nonfatal stroke, and RR = 1.21 (95%CI: 1.02–1.43, $p = 0.027$) for coronary revascularization after adjusting for age, sex, race/ethnicity, smoking status, blood pressure, HbA1c, serum creatinine, presence of ischemic heart disease, and study site [51]. Meta-analysis among 132,044 T2DM patients shows pooled unadjusted risk ratios of CVD for an increase in baseline TG, log TG of 1-mmol/L, and comparing the highest vs. the lowest TG in T2DM were 1.06 (95%CI: 1.02–1.09), 1.30 (95%CI: 1.18–1.42) and 1.30 (95%CI: 1.16–1.46), respectively, but these were attenuated after adjusting for other lipids parameters [52]. Recently, more research has focused on a new marker, triglyceride-glucose (TyG) index that reflects insulin resistance, as an independent predictor of

CVD in both the general [53] and DM population [54]. In particular, multivariate Cox hazards regression analysis revealed that the TyG index was an independent predictor of MACE (95%CI 1.201–1.746; $p < 0.001$) after adjusting for known CVD risk factors among DM patients with acute coronary syndrome [54] (Table 32.1).

In contrast to the increasing awareness of the importance of HDL-C, acceptance of the role of raised TGs in CVD is hampered because they serve as a marker for

Table 32.1 Recent epidemiological studies on the association between dyslipidemia and cardiovascular outcomes among diabetes patients

	Author/ reference	Study/cohort	Participants	Key findings
LDL-C	Jin JL, et al. (2020) [36]	Prospective observational cohort study in China	– 4148 patients with stable CAD – mean age: 59.7 ± 9.8 – median follow-up of 5.1 years	– High sdLDL plus DM was significantly associated with MACEs after adjustment of confounding risk factors (HR = 1.83, 95%CI: 1.24–2.70, $p < 0.05$)
	Kim MK, et al. (2019) [32]	Retrospective cohort study based on Korean National Health Insurance Service database	– 2,077,135 T2DM patients without prior CVD – Mean age 58.3 ± 10.5 years – Median follow-up 7.1 years	– Among non-statin users, LDL-C levels of 130–159 mg/dL and ≥160 mg/dL were significantly associated with the risk of MI: HR = 1.19 (95%CI: 1.14–1.25) and 1.53 (1.46–1.62), respectively – Among statin users, LDL--C ≥70 mg/dL were significantly associated with increased risk of stroke and MI
	Rana JS, et al. (2020) [31]	Observational cohort study from an integrated healthcare delivery system in the US	– 19,095 statin-treated adults with T2DM without prior ASCVD – Mean age 63.4 years – Mean follow-up of 5.9 years	– Relative to achieved LDL-C ≥100 mg/dL, LDL-C <50 mg/dL had a hazard ratio of 0.66 (95%CI: 0.52–0.82) for composite outcome of nonfatal MI, ischemic stroke, or CHD death

(continued)

Table 32.1 (continued)

	Author/ reference	Study/cohort	Participants	Key findings
TG	Ye X, et al. (2019) [52]	Meta-analysis of 31 prospective studies	– 132,044 T2DM patients – Mean age 59.8 years – Follow-up length varied between 1 and 13 years	– Pooled RR (95%CI) of CVD for an increase in baseline TG, log TG by 1-mmol/L and categorized in the highest vs. the lowest TG in T2DM were 1.06 (1.02–1.09), 1.30 (1.18–1.42), and 1.30 (1.16–1.46), not significant after adjusting for other lipids parameters
	Wang L, et al. (2020) [54]	A single center, retrospective observational cohort study in China	– 2531 DM patients with ACS – Mean age 66.3 ± 6.8 years – Mean follow-up of 3 years	– TyG index calculated as ln (fasting TG level [mg/dL] × FBG level [mg/dL]/2) was an independent predictor of MACE (HR = 1.46, 95%CI 1.21–1.75; $p < 0.001$)
	Nichols GA, et al. (2019) [51]	Retrospective study based on EHR from an integrated healthcare delivery system	– 27,953 DM patients with controlled LDL-C with prior ASCVD or at least one other CVD risk factor – Mean age 65.3 ± 9.0 years – Mean follow-up of 5.37 years	– Comparing high TG (200–499 mg/dL) versus normal TG (<150 mg/dL); RR = 1.30 (95%CI: 1.08–1.58, $p = 0.006$) for nonfatal MI; RR = 1.23 (95%CI: 1.01–1.49, $p = 0.037$) for nonfatal stroke; RR = 1.21 (95%CI: 1.02–1.43, $p = 0.027$) for coronary revascularization

(continued)

Table 32.1 (continued)

	Author/ reference	Study/cohort	Participants	Key findings
HDL-C	Wu Z, et al. (2021) [55]	Prospective community-based study	– 8244 participants with DM free of CVD and without use of lipid-lowering medication – Mean age of 55.8 ± 10.6 – Mean follow-up of 10.4 years	– Adjusted HR = 1.62 (95%CI: 1.19, 2.20) for HDL cholesterol concentrations >2.07 mmol/L, relative to HDL cholesterol concentrations of 1.30–1.42 mmol/L – Low HDL cholesterol concentrations failed to predict future CVD
	Shen Y, et al. (2019) [56]	LEAD cohort study (Louisiana experiment assessing diabetes outcomes)	– 27,113 African Americans and 40,431 whites with T2DM – Mean age of 66.5 ± 12.1 years – Mean follow-up of 3.0 years	– Multivariable-adjusted HRs across levels of HDL-C at baseline (<30 [reference group], 30–39.9, 40–49.9, 50–59.9, 60–69.9, 70–79.9, and ≥80 mg/dL) were 1.00, 0.89, 0.82, 0.75, 0.78, 0.76, and 0.75 (p-trend <0.001) for ischemic stroke
	Fanni G, et al. (2020) [57]	Retrospective population-based cohort study in Italy	– 2133 T2DM patients - mean aged 66 + 11 years – Mean follow-up of 14 years	– No associations between lower and upper HDL cholesterol tertiles for cardiovascular mortality in men and women (HR = 0.97; 95%CI: 0.77–1.23; HR = 0.94; 95%CI: 0.75–1.18), respectively

Abbreviation: *CAD* coronary artery disease; *sdLDL* small dense low-density lipoprotein; *MACE* major adverse cardiac events; *HR* hazard ratio; *95%CI* 95% confidence interval; *CVD* cardiovascular disease; *LDL-C* low-density lipoprotein cholesterol; *TG* triglycerides; *HDL-C* high-density lipoprotein cholesterol; *MI* myocardial infarction; *ASCVD* atherosclerotic cardiovascular disease; *RR* relative risk; *CHD* coronary heart disease; *ACS* acute coronary syndrome; *TyG* triglyceride glucose; *FBG* fasting blood glucose

other atherogenic factors (e.g., low HDL-C concentrations and elevated sdLDL levels). However, in a meta-analysis of prospective studies in the general population, the contribution of TG to CVD is independent of HDL-C [58]. After adjusting for HDL-C and other risk factors, the increased risks associated with a difference in TG levels of 1 mmol/L were 12% in men and 37% in women [58]. Moreover, approximately 77.5% of those with DM and HTG had an estimated 10-year ASCVD risk of ≥7.5%, with almost 40% of statin users having ASCVD risk ≥20%, which left them with significant residual CVD risk [7]. In another multivariate analysis, the element of the risk attributable to TG themselves appears less significant, but the risk associated with HTG is still substantial with fasting levels of 2.6–4.5 mmol/L associated with a twofold excess of CHD risk and levels of 4.5–9.0 mmol/L with up to a ninefold elevation risk [59, 60].

Therefore, there is a need to better define the relationship between plasma TG levels, the apoB lipoprotein particles that carry, TG and cholesterol (chylomicrons, VLDL, IDL, and RLPs), and the relative atherogenicity of those lipoproteins versus LDL. Increased understanding of these difficult issues will offer critical insights needed to facilitate our search for additional approaches to CVD prevention and treatment options for hypertriglyceridemia patients, especially those with diabetes.

Dyslipidemia in Diabetes: HDL-C

HDL-C is generally considered to be a cardioprotective lipoprotein, and its importance is illustrated by epidemiological studies that demonstrate an inverse relationship between HDL-C levels and CVD risk for both sexes [61]. For example, in the Framingham study, the CVD risk is increased nearly six-fold in women with HDL-C levels <1.2 mmol/L compared with women with HDL-C levels >1.7 mmol/L [61]. In individuals with DM, the CVD risk that accompanies a low HDL-C level is demonstrated by the UKPDS, with the relative risk of CVD increasing by 1.15 for every 0.1 mmol/L decrement in HDL-C [62]. Results from the Louisiana Experiment Assessing Diabetes (LEAD) cohort study involving 27,113 AAs and 40,431 White DM patients have shown that multivariable-adjusted HRs across levels of HDL-C at baseline (<30 [reference group], 30–39, 40–49, 50–59, 60–69, 70–79, and ≥80 mg/dL) are 1.00, 0.89, 0.82, 0.75, 0.78, 0.76, and 0.75 (p-trend < 0.001) for ischemic stroke [56]. In contrast, many recent epidemiological studies have found no association or even inverse association between lower HDL-C and higher CVD risk among DM population. A retrospective cohort study among 2133 DM patients in Italy found no relationship between lower or upper HDL-C tertiles for CV mortality in men and women, respectively [57]. A prospective community-based cohort study in 8244 participants with DM but free of CVD and without lipid-lowering medication suggests a 62% higher CVD risk among those with HDL-C concentration over 2.07 mmol/L compared to 1.30–1.42 mmol/L (adjusted HR = 1.62, 95%CI: 1.19–2.20), while lower HDL-C concentration fails to predict future CVD [55] (Table 32.1).

The distribution of HDL-C varies by age, sex, ethnic groups in both the general and DM populations, together with a significant trend over the past decade. Overall, mean HDL-C levels are higher among women compared to men, and there is a slight increase for both sexes over time [63]. Based on National Health and Nutrition Examination Survey (NHANES) data in the United States, the prevalence of low HDL-C (defined as <40 mg/dL) declined from 22.2% during 2007–2008 to 16.0% during 2017–2018 [63], and the proportion of low HDL-C was over three times higher among men (26.6%) than among women (8.5%) [63]. Although the prevalence is higher among men than among women in all age groups, the trend of HDL-C level across the life span is different between genders. The prevalence of low HDL-C among adults aged 20 and over in US adults was 17.2% in 2015–2018 and is higher among those aged 20–39 (17.6%) and 40–59 (18.5%) than among those aged 60 and over (14.6%) [63]. Among men, the prevalence of low HDL-C was higher among those aged 40–59 (29.6%) than those aged 60 and over (24.6%). While the difference in prevalence between men aged 20–39 (25.0%) and 40–59 was similar to the difference between men aged 60 and over and 40–59, it is not statistically significant. In women, the prevalence of low HDL-C declined with age, from 10.3% among those aged 20–39 to 7.8% among those aged 40–59 and 6.4% among those aged 60 and over. For patients with DM, over half of the men and over two-thirds of women have low levels of HDL-C (≤40 mg/dL in men or ≤50 mg/dL in women) [64] A more recent study has indicated a similar percentage among DM patients, with almost 60% of women and 47.6% men affected by abnormal HDL-C (<40 mg/dL) [65]. In some developing countries, this proportion ranges from 23.6% [66] to as high as almost 75% [67].

Although environmental factors play a role, variations in HDL-C levels are at least 50% genetically determined [68] and there are significant differences in serum levels across ethnic groups. The prevalence of low HDL-C is lowest in non-Hispanic black (11.9%) adults compared with non-Hispanic whites (16.6%), non-Hispanic Asian (15.8%), and Hispanic (21.9%) adults [63]. Similarly, the prevalence of low HDL-C was consistently lower among women than among men across all race and Hispanic-origin groups. In the Northern Manhattan Study, participants from local communities have a mean HDL-C of 43.9, 52.3, and 49.3 mg/dL among Hispanics, AAs, and non-Hispanic White, with the corresponding proportion of decreased HDL-C being 64.1%, 40.5%, and 46.9%, respectively for these three ethnic groups ($p < 0.0001$) [69]. Among DM patients, mean HDL-C is significantly lower than those with normal glucose levels (44.84 ± 12.93 vs. 53.79 ± 16.25, $p < 0.0001$) according to data from NHANES 1999–2018 [70] A similar pattern is observed among ethnic groups, with AAs having significantly higher HDL-C level compared to their Whites counterparts [71]. In patients with type 1 diabetes mellitus (T1DM), 76% and 66% of Hispanic men and women are not in optimal control of HDL-C, compared to 57% and 58% among Whites, respectively [72].

More recently it has been shown that HDL may also directly affect glucose metabolism. In a study evaluating the cholesteryl ester transfer protein inhibitor torcetrapib, it was observed that higher HDL-C concentrations were associated with less hyperglycemia [73]. Also, it has been demonstrated that the infusion of

recombinant HDL can improve glucose metabolism in patients with T2DM [73]. Since then, a number of studies have examined the underlying pathophysiology, and several mechanisms were identified. HDL induces reverse cholesterol transport, and the altered intracellular lipid environment is believed to reduce micro-inflammation. Furthermore, direct anti-inflammatory properties of HDL may also play a role. In some clinical situations, a, however, high HDL-C level might be unfavorable. For example, in the acute phase response as seen after surgery, or in the setting of chronic inflammation or DM, the proteome of HDL may undergo some changes that affect its physiological quality even though its level remains within the normal range [74]. DM is associated with increased oxidative stress resulting in greater production of the reactive forms of oxygen (e.g., superoxide anion, hydroxyl ions, peroxynitrite) which induce tissue damage. The expression of "dysfunctional HDL" has been proposed in the literature to describe HDL particles that lose their antioxidative and anti-inflammatory properties, that is, HDL-C that loses its basic cardioprotective functions [75]. Recent observational studies have confirmed that the atheroprotective activity of properly functioning HDL-C is frequently impaired in clinical situations associated with oxidative stress such as DM [75]. However, the mechanism underlying this transformation is still unclear, and there are no widely accepted methods for determining HDL function in selected groups of patients. It has been suggested that these transformed HDL particles with reduced antioxidative activity may become a more useful biomarker of cardiovascular risk than the "old" HDL.

Other Lipid Measures: Lipoprotein(a)

Lipoprotein(a) is an LDL particle with an apolipoprotein(a) moiety covalently attached to apoB, and elevations in lipoprotein(a) are genetically determined a proven risk factor for ASCVD [76]. Levels of lipoprotein(a) of >50 mg/dL are defined as a risk enhancing factor by the 2018 ACC/AHA/Multisociety guidelines [3] and are prevalent in approximately 20% of the population. Persons with DM have similar lipoprotein(a) levels to those without DM [77]. While higher lipoprotein(a) levels have been shown to protect against developing DM [78], among those with DM, higher lipoprotein(a) levels are associated with worse outcomes among a large cohort of Chinese adults with DM both with [79] and without [80] prior CVD. One study examines patients with ASCVD and diabetes who are on statin treatment, suggests that Lp(a) is an independent risk factor for subsequent ASCVD events [81]. However, further studies should be done in other populations to confirm the prognostic significance of elevated lipoprotein(a) and specific cut-points of lipoprotein(a) that best identify increased ASCVD risk in persons with DM.

Control of Dyslipidemia in Diabetes

Optimal control of lipids, in particular LDL-C is the key approach to lower lipid-related ASCVD risk among DM patients. According to the 2023 American Diabetes Association (ADA) Standards in Medical Care, while optimal lipid levels for adults and children with T2DM are LDL-C <100 mg/dL, HDL-C ≥40 mg/dL for men and ≥50 mg/dL for women, and TG < 150 mg/dL, there is a focus on achieving LDL-C <70 mg/dL in primary prevention higher risk persons with DM and <55 mg/dL for those with both DM and ASCVD [82]. Many studies have shown that poor glycemic control is associated with increased cholesterol levels in patients with T2DM [83]. Among them, 35% had high total cholesterol (TC) (≥200 mg/dL), 27% had high LDL-C (≥130 mg/dL), and 12% had high TG (≥200 mg/dL) [84]. It has been found that dyslipidemia remains largely undiagnosed and undertreated in high-risk populations such as patients with T2DM. Although HTG and low HDL-C may be the dominant abnormality, patients benefit most from lowering LDL-C [85].

In real practice, however, individual and composite dyslipidemia is poorly controlled in DM patients. Earlier data in the United States has shown that less than one-third of men and only one-fifth of women with DM are in control for LDL-C, defined as <2.6 mmol/L (<100 mg/dL) while over 70% are not at goal [64]. Over half of men and over two-thirds of women have low levels of HDL-C (≤1.0 mmol/L [<40 mg/dL] in men or ≤1.3 mmol/L [≤50 mg/dL] in women), and over half have elevated levels of TG (≥1.7 mmol/L [150 mg/dL]) [64]. Low HDL-C is more common in white (70.1%) than in Hispanic (58.8%) or black (41.5%) persons ($p < 0.001$), and only 28.2% of subjects with DM were on lipid-lowering treatment. Control of LDL-C did not differ by treatment status, and only 3% of subjects are controlled to target levels for all lipids [64]. From more recent diabetes registry data, only 48.6% of DM patients have controlled LDL-C (defined as <100 mg/dL) [86]. Similar results are found in recent NHANES data reporting around half of the US DM patients have target control of LDL-C (defined as <100 mg/dL without prior CVD or <70 mg/dL with prior CVD) [87].

Internationally, suboptimal lipid control without appropriate treatment is also very common in countries across the world, especially in Asian and Middle East countries. One study conducted in multiple endocrinology clinics among top-ranked hospitals in China has suggested that the prevalence of dyslipidemia was 67.1% in T2DM subjects. In those with dyslipidemia, the proportion of awareness and treatment was 68.7% and 55.9%. Among participants with lipid-lowering therapy, only 40% of subjects achieved the LDL-C control less than 2.60 mmol/L. In those patients with previous CVD, the percentage of participants who achieved the LDL-C goal (1.80 mmol/L) was only 15.3% [88]. Prospective analysis from the first nationwide diabetes report of the national program for prevention and control of diabetes in Iran shows that around 13.2%, 11.9%, and 43.3% of patients with DM had controlled hyperglycemia, hyperlipidemia, and hypertension, respectively. In India, the

prevalence of hyperlipidemia is 40% among individuals with DM, 27% of those have received statin therapy, with over 57% receiving the target LDL-C control [89].

Efforts to address the health disparities in lipid control among minorities have made significant improvements in recent years. A large DM cohort with a median of 10 years follow-up has indicated that LDL-C testing increased from 48% at baseline to 70% at follow-up among AA patients and from 61% to 77% among white patients. Treatment with lipid-lowering drugs increased from 23% to 56% among AA patients and 33% to 61% among white patients. The proportion at goal increased from 24% to 59% and 35% to 76% among AA and white patients, respectively. AA patients are less likely to be tested for LDL-C (OR = 0.79; 95%CI: 0.73–0.86), treated with lipid-lowering agents (OR = 0.72; 95%CI: 0.65–0.80), have their medication dosage altered (OR = 0.65; 95%CI 0.59–0.73), or attain LDL-C goal (OR = 0.59; 95%CI 0.56–0.63) compared with white patients [90]. Disparities in access to healthcare and healthcare-seeking behavior may explain why lipid management impact is better among whites than among AAs. These disparities have been attributed to difficulties in accessing healthcare among uninsured minorities, and lower socioeconomic status has been associated with an inferior quality of care received [90]. Even among insured AAs, quality of care, particularly lipid treatment and control, is inferior to that received by other racial groups [91], although some findings suggest that patients of differing race and ethnic groups receive equal benefits when treated appropriately [92]. Another cohort study among DM patients has found that the percentage of statin use is 66.0%, 57.8%, 55.0%, and 53.6%, respectively, among white men (WM), black men (BM), white women (WW), and black women (BW) ($p < 0.001$). LDL-C control among those taking statins for WM, BM, WW, and BW was 75.3%, 62.7%, 69.0%, and 56.0%, respectively ($p < 0.001$) [93]. Healthcare provider awareness of these race-sex disparities may help to close the observed race-sex gaps in statin use and LDL-C control among people with DM. For the medication use, females are less likely to be on rosuvastatin (7.6% vs. 12%; $p < 0.001$), atorvastatin (41% vs. 46%; $p = 0.005$) but more likely to be on simvastatin (51% vs. 39%; $p < 0.001$) and combination hypolipidemic therapy (5.6% vs. 2.8%, $p < 0.001$) than males [94]. Females, especially those with very high ASCVD risk status, are also less likely to achieve LDL-cholesterol (adjusted OR = 0.58; 95%CI: 0.40–0.86; $p = 0.006$], non-HDL cholesterol (adjusted OR = 0.68; 95%CI: 0.46–0.99; $p = 0.048$], and apolipoprotein B (adjusted OR = 0.64; 95%CI: 0.44–0.92; $p = 0.016$) lipid targets [94].

As in any form of dyslipidemia, the primary target is the achievement of risk-stratified LDL-C levels. The causal relationship between LDL-C and ASCVD is well established, and drugs like statins that lower LDL-C levels by increasing LDL receptor activity are widely used to prevent CVD. However, despite the benefit of statins and marked reduction of circulating LDL-C levels, patients with diabetes continue to have more CVD events than patients without diabetes, indicating significant residual CVD risk. There are likely to be a number of reasons why cholesterol reduction in patients with diabetes is not sufficient to reduce CVD events to the levels found in patients without diabetes. Atherosclerotic lesions in patients with diabetes tend to be more inflamed, i.e., have greater numbers of macrophages [95]. Moreover, after cholesterol reduction, intravascular ultrasound studies show less regression of lesions in

people with diabetes [96]. Residual CVD risk appears to be at least partly linked to elevated plasma TG and abnormal metabolism of TRLs [97, 98], which are conventionally considered to consist of chylomicrons, VLDLs, and their respective RLPs and many of which are present in intermediate-density lipoproteins (IDLs).

Dyslipidemia Control and Cardiovascular Risk Reduction in Diabetes

There is strong evidence both from epidemiologic studies and clinical trial data documenting the importance of lipid control for reducing ASCVD risks in those with DM. Estimates obtained from simulating changes in total cholesterol and HDL-C in NHANES adults with DM to the UKPDS risk engine suggest that achieving optimal/aggressive total cholesterol levels would prevent 35.1% of CHD events and HDL-C levels would prevent 8.5% of CHD events, with composite aggressive management (total and HDL-C, systolic blood pressure, glycated hemoglobin, and smoking) estimated to prevent 54.8% of CHD events [99]. Furthermore, in a pooling project of the Multiethnic Study of Atherosclerosis, Jackson Heart Study, and Atherosclerosis in Risk in Communities Study subjects with DM, target control of LDL-C was associated with 33% lower CVD and 41% lower CHD risk (and if LDL-C, blood pressure, and HbA1c were at target together, 62% and 60% lower risks, respectively, compared to neither being at target) [99]. Moreover, in the BARI 2D cohort of persons with DM and angiographically stable CHD, not being at non-HDL-C target was associated with approximately 40–50% higher myocardial infarction, stroke, and death rates [100].

For LDL-C specifically, data from the Cholesterol Treatment Trialists' Collaboration suggests that those with DM have a similar approximately 20% risk reduction per mmol/L reduction (approximately 40 mg/dL) in LDL-C [101]. In the Heart Protection Study, the 2912 patients with DM without pre-existing vascular disease who were randomized to receive simvastatin showed a significant reduction in cardiovascular endpoints compared to those allocated to placebo [102]. Similarly, in the Collaborative Atorvastatin Diabetes Study, where the mean pretreatment LDL-C is 3.0 mmol/L (<2.5 mmol/L in 25% of patients), the dramatic risk reduction from atorvastatin resulted in early termination of the trial [103]. In both studies, there was no threshold of LDL-C below which statin therapy ceased to be beneficial. A meta-analysis of 18,686 people with DM from 14 randomized trials (1466 with type 1 and 17,220 with type 2) demonstrated a 9% reduction in all-cause mortality for every 1 mmol/L reduction in LDL-C [104]. The effects of statin therapy are similar irrespective of baseline characteristics and prior history of vascular disease. These trials have demonstrated that statins decrease the risk of CVD in people with DM with and without pre-existing cardiovascular conditions [104]. While the relative risk reduction achieved with statin treatment is similar in patients with or without DM, because those with DM have higher baseline absolute risk, the number needed to treat (NNT) will be lower in patients with DM compared to those without DM.

The risk factor reduction brought about by statins occurs in a dose-dependent fashion, with higher dose statins associated with a greater lowering of cardiovascular events [105]. It should also be noted that in diabetes, higher dosages of statins may be needed to slow or halt progression of carotid intima-media thickness or intravascular ultrasound-measured atheroma volume [106]. The American Diabetes Association 2022 guidelines recommend that all patients with DM with ASCVD or patients with a 10-year atherosclerotic cardiovascular risk ≥20% should be treated with high-intensity statins (goal of 50% reduction in LDL-cholesterol) in addition to lifestyle modification [82]. DM patients aged <40 with additional atherosclerotic cardiovascular risk factors (LDL-C ≥100 mg/dL, hypertension, CKD, smoking, albuminuria, and familial hypercholesterolemia (FH) of premature ASCVD), DM patients aged 40–75 years without ASCVD or 10-year ASCVD risk <20% and DM patients ≥75 years old should be treated with moderate-intensity statins with a goal of 30–49% LDL-C reduction [82]. Most recently, the new ACC/AHA/Multisociety guidelines indicate DM as a high-risk condition for ASCVD. In addition, they provided DM specific risk enhancers which included: DM duration of >10 years in T2DM and ≥20 years duration for T1DM, albuminuria ≥30 mg/G creatinine, an estimated glomerular filtration rate (eGFR) <60 mL/min /1.73 m^2, retinopathy, neuropathy, and an ankle-brachial index (ABI) < 0.9 to help inform the treatment decision, especially in those with DM < 40 years of age. In adults 40–75 years with DM regardless of 10-year risk should initiate moderate-intensity statin. In adults with diabetes with ASCVD or multiple ASCVD risk factors, it is reasonable to prescribe high-intensity statin to lower LDL-C by 50% or more [3]. These guidelines focus on statin intensity, instead of a specific LDL-C target.

In clinical practice, the primary strategy is to achieve optimal levels of LDL-C. The goal recommended by the American Diabetes Association is <70 mg/dL (<1.8 mmol/L) in DM patients with additional cardiovascular risk factors or atherosclerotic disease and is <100 mg/dL (<2.3 mmol/L) in all other DM patients [82]. While statins are first line therapy, if the maximally tolerated dose cannot achieve these targets, combination therapy with ezetimibe should be used [82]. The recently published data on the IMPROVE-IT (IMProved Reduction of Outcomes: Vytorin Efficacy International Trial) trial indicate that those with a recent acute coronary syndrome (ACS) who also have DM are in fact those who benefit from the statin-ezetimibe combination, as opposed to those with only a recent ACS without diabetes. Whether and how HTG is often observed in DM patients should be treated with adjuvant fibrates or omega-3-fatty acids is still a matter of debate [107]. In the FIELD clinical trial among T2DM individuals, 5.9% of patients on placebo and 5.2% of those on fenofibrate had a coronary event (relative risk reduction of 11%; HR = 0.89, 95%CI: 0.75–1.05; p = 0.16). It concluded that fenofibrate did not significantly reduce the risk of the primary outcome of coronary events, although there was a reduction of total cardiovascular events, mainly due to fewer nonfatal myocardial infarctions and revascularizations [108]. A secondary analysis of the ACCORDION lipid study suggested that the incidence rates in the fenofibrate group were lower with respect to all-cause mortality, CVD mortality, nonfatal myocardial infarction, congestive heart failure, and major CHD than those in the placebo group over a post-trial follow-up. Allocation to the combined fibrate-statin treatment arm during the trial

period has a beneficial legacy effect on all-cause mortality (adjusted HR = 0.65, 95%CI 0.45–0.94; $p = 0.02$) [109]. This legacy effect supports re-evaluation of fibrate as an add-on strategy to statins in order to reduce residual ASCVD risk. The recently completed PROMINENT trial involving pemafibrate given to patients with T2DM with high TG and low HDL-C failed to show a benefit in cardiovascular outcomes, with an observed increase in LDL-C and apolipoprotein B in the pemafibrate arm [110, 111]. Recently, the REDUCE-IT trial has suggested that icosapent ethyl is associated with 25% relative risk reduction for composite CV outcomes among individuals with elevated TG, with no evidence of heterogeneity in effect according to DM status; the risk reduction was similar in those with (23%) versus without (27%) DM [112]. While icosapent ethyl does modestly lower TG levels, the effects on ASCVD risk were similar in those with baseline TG levels ≥150 versus <150 mg/dL, indicating other TG-independent effects may be responsible for the risk reduction. Many novel therapies are in development to treat dyslipidemia, but none have been studied specifically in patients with diabetes [113]. In particular, proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors which show 50% or greater LDL-C reductions beyond statin therapy [114] show similar benefits for reducing ASCVD risk in those with and without DM among persons with ASCVD [115].

Conclusions

The prevalence of dyslipidemia remains high worldwide among persons with DM, with suboptimal LDL-C, low HDL-C, and elevated TG common in those with DM. Insulin resistance and obesity in particular contribute to the likelihood and severity of dyslipidemia in persons with DM. Reduction of LDL-C in particular, and more recently TG as well, can result in reduced risk of ASCVD in those with DM. Treatment and target control of dyslipidemia, which has focused on LDL-C control is often seen in less than half of those with DM with significant disparities among females and ethnic minorities. Control of dyslipidemia in those with DM has the greatest effect on ASCVD risk reduction and those who are at recommended targets for LDL-C or non-HDL-C are at lower risk of subsequent ASCVD events.

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Index

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