Chapter 11
Lipoprotein Glycation in Diabetes Mellitus

Alicia J. Jenkins, Richard L. Klein, Andrea J. Semler, and Andrzej S. Januszewski

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

A. J. Jenkins
NHMRC Clinical Trials Centre, The University of Sydney, Sydney, NSW, Australia

Department of Medicine, St. Vincent's Hospital, The University of Melbourne, Melbourne, VIC, Australia

Diabetes and Vacular Medicine, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia
e-mail: alicia.jenkins@baker.edu.au

A. S. Januszewski (✉)
NHMRC Clinical Trials Centre, The University of Sydney, Sydney, NSW, Australia

Department of Medicine, The University of Melbourne, St. Vincent's Hospital, Melbourne, VIC, Australia
e-mail: andrzej.januszewski@sydney.edu.au

R. L. Klein · A. J. Semler
Division of Endocrinology, Diabetes, and Medical Genetics, Medical University of South Carolina, Charleston, SC, USA
e-mail: kleinrl@musc.edu; semleraj@musc.edu

A. J. Jenkins, P. P. Toth (eds.), *Lipoproteins in Diabetes Mellitus*, Contemporary Diabetes, https://doi.org/10.1007/978-3-031-26681-2_11

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.

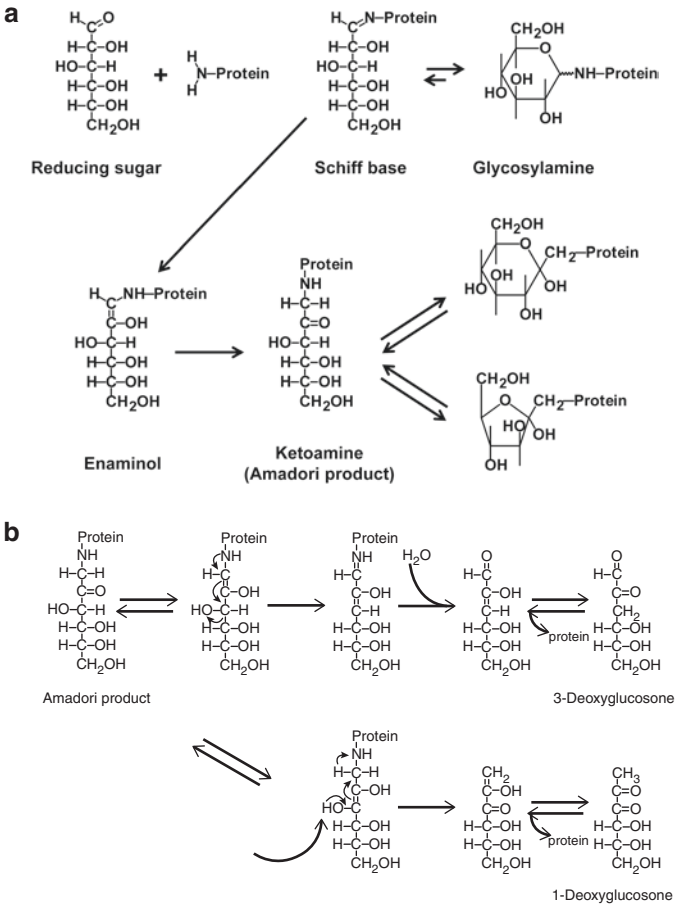

Fig. 11.1 Biochemistry of early and late glycation. (**a**) Early steps of the Maillard reaction. The reducing sugars in open chain form reacting an amino group on proteins to form a reversible Schiff base. The Schiff base then forms a cyclic glycosylamine or can rearrange to an enaminol and then to 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)

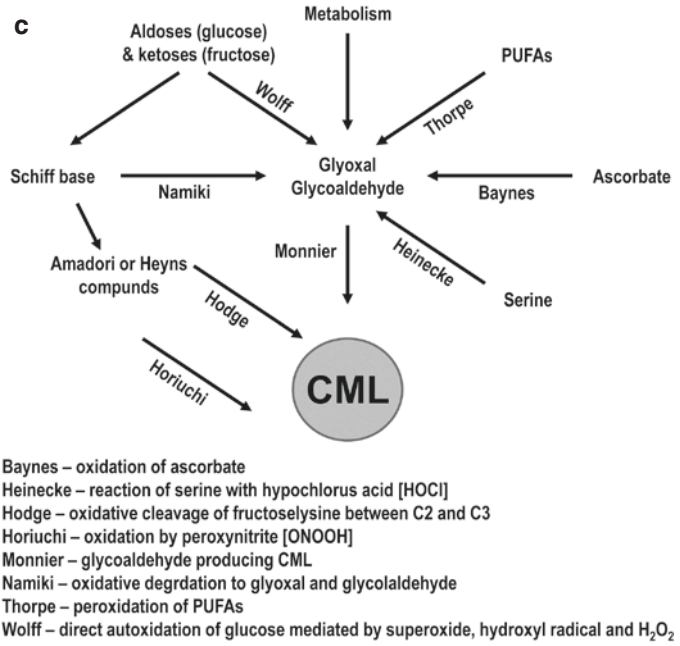

Fig. 11.1 (continued)

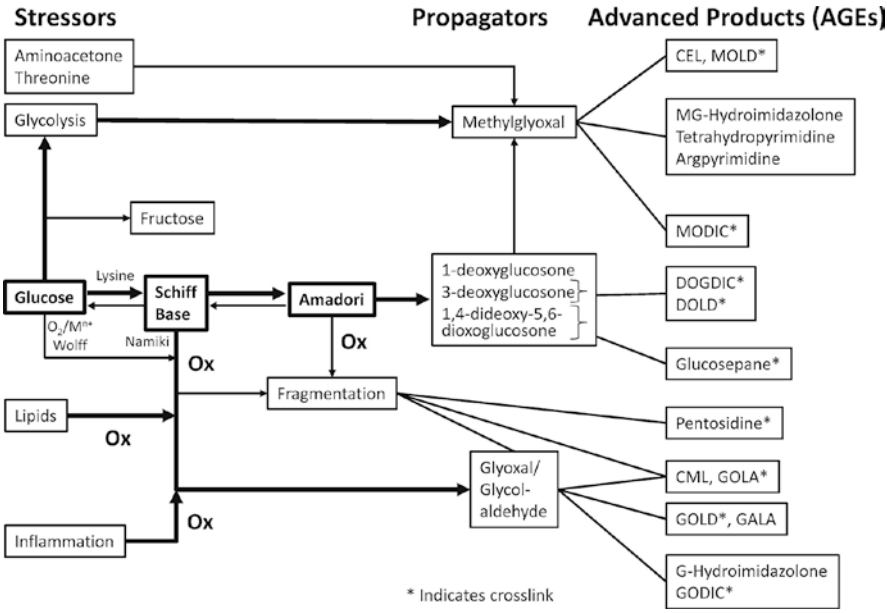

Fig. 11.2 Factors affecting AGEs formation and accumulation. (Reproduced with permission from: V.M. Monnier and X. Wu, "Enzymatic deglycation with amadoriase enzymes from Aspergillus sp. as a potential strategy against the complications of diabetes and aging" Biochem. Soc. Trans. (2003) 31, 1349–1353)

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.

Acknowledgments Grant support for studies discussed herein was provided by the Juvenile Diabetes Research Foundation, American Diabetes Association, National Institutes of Health, Department of Veterans Affairs Merit Review (RK), the Diabetes Research and Wellness Foundation, Lions SightFirst Diabetic Retinopathy Research Program, the National Health and Medical Research Foundation, and National Heart Foundation (Australia). The authors also acknowledge their collaborators, including Professors Timothy Lyons, John Baynes, Susan Thorpe, Maria Lopes-Virella, Gabe Virella, Kristian Hanssen, Bente Kilhovd, David O'Neal, Kerry-Anne Rye, Philip Barter, Michael Davies, Drs Craig Nelson, Andrew Wilson Estelle Nobecourt, Kwok Leung Ong and the DCCT/EDIC Research Group and the FIELD Trial, Ms. Connie Karschimkus and all study participants and site staff.

References

1. International Diabetes Federation (IDF) Atlas, 10th ed. https://diabetesatlas.org/atlas/tenth-edition/. Accessed 7 Jun 2022.
2. American Diabetes Association. Diabetes vital statistics. Alexandria, VA: American Diabetes Association; 1996.
3. Jay RH, Betteridge DJ. The heart and macrovascular disease in diabetes mellitus. In: Pickup JC, Williams G, editors. Chronic complication of diabetes. Melbourne: Blackwell Press; 1994.
4. Jenkins AJ, Rowley KG, Lyons TJ, Best JD, Hill MA, Klein RL. Lipoproteins and diabetic microvascular complications. Curr Pharm Des. 2004;10:3395–418.
5. Betteridge DJ. Risk factors for arterial disease in diabetes: dyslipidaemia. In: Tooke JE, editor. Diabetic angiopathy. Sydney: Arnold; 1999. p. 65–92.
6. Reckless JPD. Diabetes. In: Dunitz M, editor. Diabetes and lipids: pocketbook, Martin Dunitz medical pocket books. 2nd ed. London: Publisher Taylor and Francis; 2011. p. 26–37.
7. Howard BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res. 1987;28(6):613–28.

8. Maillard LC. Réaction générale des acides aminés sur les sucres: ses conséquences biologiques. Compte-rendu Société de Biologie. 1912;72(LXXII):599–601 (559–61).
9. Thorpe SR, Lyons TJ, Baynes JW. Oxidative stress and vascular disease. Boston, MA: Kluwer Academic Publisher; 2000. p. 259–85.
10. Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR. The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. J Biol Chem. 1996;271(17):9982–6.
11. Lyons TJ, Jenkins AJ. Glycation, oxidation, and lipoxidation in the development of the complications of diabetes: a carbonyl stress hypothesis. Diabetes Rev. 1997;5:365–91.
12. Lyons TJ. Glycation, oxidation, and glycoxidation reactions in the development of diabetic complications. Contrib Nephrol. 1995;112:1–10.
13. Cao Z, Cooper ME. Pathogenesis of diabetic nephropathy. J Diabetes Invest. 2011;2(4):243–7.
14. Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, Baynes JW. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? Kidney Int. 1998;54(4):1290–5.
15. http://www.imars.org/online/?page_id=655. 21 May 2012.
16. Niwa T. 3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. J Chromatogr B Biomed Sci Appl. 1999;731(1):23–36.
17. Jono T, Nagai R, Lin X, Ahmed N, Thornalley PJ, Takeya M, Horiuchi S. Nepsilon-(Carboxymethyl) lysine and 3-DG-imidazolone are major AGE structures in protein modification by 3-deoxyglucosone. J Biochem. 2004;136(3):351–8.
18. Goldberg T, Cai W, Peppa M, Dardaine V, Baliga BW, Uribarri J, Vlassara H. Advanced glycoxidation end products in commonly consumed foods. J Am Diet Assoc. 2004;104:1287–91.
19. Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, Heitmann K, Vlassara H. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci U S A. 1997;94:6474–9.
20. Uribarri J, Peppa M, Cai W, Goldberg T, Lu M, Baliga S, Vassalotti JA, Vlassara H. Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. Am J Kidney Dis. 2003;42:532–8.
21. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, Yong A, Striker G, Vlassara H. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc. 2010;110:911–6.
22. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. J Intern Med. 2002;251:87–101.
23. Cai W, He JC, Zhu L, Chen X, Wallenstein S, Striker GE, Vlassara H. Reduced oxidant stress and extended lifespan in mice exposed to a low glycotoxin diet: association with increased AGER1 expression. Am J Pathol. 2007;170(6):1893–902.
24. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, Peppa M, Rayfield EJ. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci U S A. 2002;99:15596–601.
25. Negrean M, Stirban A, Stratmann B, Gawlowski T, Horstmann T, Götting C, Kleesiek K, Mueller-Roesel M, Koschinsky T, Uribarri J, Vlassara H, Tschoepe D. Effects of low- and high-advanced glycation end product meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Clin Nutr. 2007;85:1236–43.
26. Baynes JW, Dominiczak H. Medical biochemistry. 2nd ed. New York: Elsevier Mosby; 2005.
27. Lund-Katz S, Ibdah JA, Letizia JY, Thomas MT, Phillips MC. A 13C NMR characterization of lysine residues in apolipoprotein B and their role in binding to the low density lipoprotein receptor. J Biol Chem. 1988;263(27):13831–8.
28. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, Brownlee M. Over-expression of glyoxalase-1 in bovine endothelial cells inhibits intracellular advanced glycation end-product formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. J Clin Invest. 1998;101(5):1142–7.

29. Lu J, Randell E, Han Y, Adeli K, Krahn J, Menq QH. Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. Clin Biochem. 2011;44(4):307–11.
30. Lyons TJ, Klein RL, Baynes JW, Stevenson HC, Lopes-Virella MF. Stimulation of cholesteryl ester synthesis in human monocyte-derived macrophages by lipoproteins from Type I diabetic subjects: the influence of non-enzymatic glycosylation of low-density lipoproteins. Diabetologia. 1987;30:916–23.
31. Hayashi Y, Okumura K, Matsui H, Imamura A, Miura M, Takahashi R, Murakami R, Ogawa Y, Numaguchi Y, Murohara T. Impact of low-density lipoprotein particle size on carotid intima-media thickness in patients with type 2 diabetes mellitus. Metabolism. 2007;56(5):608–13.
32. Januszewski AS, Karschimkus C, Davis KE, O'Neal D, Ward G, Jenkins AJ. Plasma 1,5 anhydroglucitol levels, a measure of short-term glycaemia: assay assessment and lower levels in diabetic vs. non-diabetic subjects. Diabetes Res Clin Pract. 2012;95(1):e17–9.
33. Ahmed MU, Thorpe SR, Baynes JW. Identification of N epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. J Biol Chem. 1986;261(11):4889–94.
34. Jenkins AJ, Thorpe SR, Alderson NL, Hermayer KL, Lyons TJ, King LP, Chassereau CN, Klein RL. In vivo glycated low-density lipoprotein is not more susceptible to oxidation than nonglycated low-density lipoprotein in type 1 diabetes. Metabolism. 2004;53(8):969–76.
35. Lopes-Virella MF, Klein RL, Lyons TJ, Stevenson HC, Witztum JL. Glycosylation of low-density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages. Diabetes. 1988;37(5):550–7.
36. Klein RL, Laimins M, Lopes-Virella MF. Isolation, characterization, and metabolism of the glycated and nonglycated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects. Diabetes. 1995;44(9):1093–8.
37. Tanaka A, Yui K, Tomie N, Baba T, Tamura M, Makita T, Numano F, Nakatani S, Kato Y. New assay for glycated lipoproteins by high-performance liquid chromatography. Ann N Y Acad Sci. 1997;811:385–94.
38. Cohen MP, Lautenslager G, Shea E. Glycated LDL concentrations in non-diabetic and diabetic subjects measured with monoclonal antibodies reactive with glycated apolipoprotein B epitopes. Eur J Clin Chem Clin Biochem. 1993;31(11):707–13.
39. Doucet C, Huby T, Ruiz J, Chapman MJ, Thillet J. Non-enzymatic glycation of lipoprotein(a) in vitro and in vivo. Atherosclerosis. 1995;118:135–43.
40. Makino K, Furbee JW, Scanu AM, Fless GM. Effect of glycation on the properties of lipoprotein(a). Arterioscler Thromb Vasc Biol. 1995;15:385–91.
41. Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, Baynes JW. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Invest. 1993;91(6):2463–9.
42. Virella G, Derrick MB, Pate V, Chassereau C, Thorpe SR, Lopes-Virella MF. Development of capture assays for different modifications of human low-density lipoprotein. Clin Diagn Lab Immunol. 2005;12(1):68–75.
43. Nagai R, Matsumoto K, Ling X, Suzuki H, Araki T, Horiuchi S. Glycolaldehyde, a reactive intermediate for advanced glycation end products, plays an important role in the generation of an active ligand for the macrophage scavenger receptor. Diabetes. 2000;49:1714–23.
44. Chen D, Scott E, Fulcher J, Jenkins A. Tailoring the treatment for Type 2 diabetes. One size does not fit all. In: Basu R, Basu A, editors. Precision medicine in diabetes. Springer Press; 2022.
45. Kim EJ, Ramachandran R, Wierzbicki AS. Lipidomics in diabetes. Curr Opin Endocrinol Diabetes Obes. 2022;29(2):124–30.
46. Sobczak AIS, Pitt SJ, Smith TK, Ajjan RA, Stewart AJ. Lipidomic profiling of plasma free fatty acids in type-1 diabetes highlights specific changes in lipid metabolism. Biochim Biophys Acta Mol Cell Biol Lipids. 2021;1866(1):158823.

47. Alesi S, Ghelani D, Rassie K, Mousa A. Metabolomic biomarkers in gestational diabetes mellitus: a review of the evidence. Int J Mol Sci. 2021;22(11):5512.
48. Baye E, Ukropec J, de Courten MP, Vallova S, Krumpolec P, Kurdiova T, Aldini G, Ukropcova B, de Courten B. Effect of carnosine supplementation on the plasma lipidome in overweight and obese adults: a pilot randomised controlled trial. Sci Rep. 2017;7(1):17458.
49. Kaplan M, Aviram M. Macrophage plasma membrane chondroitin sulfate proteoglycan binds oxidized low-density lipoprotein. Atherosclerosis. 2000;149(1):5–17.
50. Tsmikas S, Shortal BP, Witztum JL, Palinski W. In vivo uptake of radiolabeled MDA2, an oxidation specific monoclonal antibody, provides an accurate measure of atherosclerotic lesions rich in oxidized LDL and is highly sensitive to their regression. Arterioscler Thromb Vasc Biol. 2000;20:689–97.
51. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med. 1993;14(3):303–11.
52. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. J Physiol Biochem. 2001;57(2):43–56.
53. Jenkins AJ, Velarde V, Klein RL, Joyce KC, Phillips KD, Mayfield RK, Lyons TJ, Jaffa AA. Native and modified LDL activate extracellular signal-regulated kinases in mesangial cells. Diabetes. 2000;49(12):2160–9.
54. Song W, Barth JL, Yu Y, Lu K, Dashti A, Huang Y, Gittinger CK, Argraves WS, Lyons TJ. Effects of oxidized and glycated LDL on gene expression in human retinal capillary pericytes. Invest Ophthalmol Vis Sci. 2005;46(8):2974–82.
55. Song W, Barth JL, Lu K, Yu Y, Huang Y, Gittinger CK, Argraves WS, Lyons TJ. Effects of modified low-density lipoproteins on human retinal pericyte survival. Ann N Y Acad Sci. 2005;1043:390–5.
56. Barth JL, Yu Y, Song W, Lu K, Dashti A, Huang Y, Argraves WS, Lyons TJ. Oxidised, glycated LDL selectively influences tissue inhibitor of metalloproteinase-3 gene expression and protein production in human retinal capillary pericytes. Diabetologia. 2007;50(10):2200–8.
57. Tames FJ, Mackness MI, Arrol S, Laing I, Durrington PN. Non-enzymatic glycation of apolipoprotein B in the sera of diabetic and non-diabetic subjects. Atherosclerosis. 1992;93(3):237–44.
58. Younis N, Charlton-Menys V, Sharma R, Soran H, Durrington PN. Glycation of LDL in non-diabetic people: small dense LDL is preferentially glycated both in vivo and in vitro. Atherosclerosis. 2009;202(1):162–8.
59. Lyons TJ, Otvos JD, Klein RL, Zheng D, Garvey WT, Jenkins AJ, DCCT/EDIC Research Group. Nuclear magnetic resonance (NMR)-determined lipoprotein subclass profile: effects of hyperglycemia, lipoprotein glycation, and comparison with standard lipid profile. Diabetes. 2000;49(Suppl 1):A268.
60. Lyons TJ, Jenkins AJ, Zheng D, Klein RL, Otvos JD, Yu Y, Lackland DT, McGee D, MB MH, Lopes-Virella M, Garvey WT, DCCT/EDIC Research Group. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. Diabet Med. 2006;23(9):955–66.
61. Toma L, Stancu CS, Sima AV. Endothelial dysfunction in diabetes is aggravated by glycated lipoproteins; novel molecular therapies. Biomedicines. 2021;9:18.
62. Yegin A, Ozben T, Yegin H. Glycation of lipoproteins and accelerated atherosclerosis in non-insulin-dependent diabetes mellitus. Int J Clin Lab Res. 1995;25(3):157–61.
63. Mamo JC, Szeto L, Steiner G. Glycation of very low density lipoprotein from rat plasma impairs its catabolism. Diabetologia. 1990;33(6):339–45.
64. Klein RL, Wohltmann HJ, Lopes-Virella MF. Influence of glycemic control on interaction of very low- and low-density lipoproteins isolated from type I diabetic patients with human monocyte-derived macrophages. Diabetes. 1992;41(10):1301–7.
65. Klein RL, Lyons TJ, Lopes-Virella MF. Metabolism of very low- and low-density lipoproteins isolated from normolipidaemic type 2 (non-insulin-dependent) diabetic patients by human monocyte-derived macrophages. Diabetologia. 1990;33(5):299–305.

66. Klein RL, Lyons TJ, Lopes-Virella MF. Interaction of very-low-density lipoprotein isolated from type I (insulin-dependent) diabetic subjects with human monocyte-derived macrophages. Metabolism. 1989;38(11):1108–14.
67. Klein RL, Lopes-Virella MF. Metabolism by human endothelial cells of very low density lipoprotein subfractions isolated from type 1 (insulin-dependent) diabetic patients. Diabetologia. 1993;36(3):258–64.
68. Moro E, Alessandrini P, Zambon C, Pianetti S, Pais M, Cazzolato G, Bon GB. Is glycation of low density lipoproteins in patients with Type 2 diabetes mellitus a LDL pre-oxidative condition? Diabet Med. 1999;16(8):663–9.
69. Akanji AO, Abdella N, Mojiminiyi OA. Determinants of glycated LDL levels in nondiabetic and diabetic hyperlipidaemic patients in Kuwait. Clin Chim Acta. 2002;317(1–2):171–6.
70. Rabbani N, Chittari MV, Bodmer CW, Zehnder D, Ceriello A, Thornalley PJ. Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin. Diabetes. 2010;59(4):1038–45.
71. Chao PC, Huang CN, Hsu CC, Yin MC, Guo YR. Association of dietary AGEs with circulating AGEs, glycated LDL, IL-1α and MCP-1 levels in type 2 diabetic patients. Eur J Nutr. 2010;49(7):429–34.
72. Steinberg D, Witztum JL. Lipoproteins and atherogenesis. Current concepts. JAMA. 1990;264(23):3047–52.
73. Soran H, Durrington PN. Susceptibility of LDL and its subfractions to glycation. Curr Opin Lipidol. 2011;22(4):254–61.
74. Rabbani N, Godfrey L, Xue M, Shaheen F, Geoffrion M, Milne R, Thornalley PJ. Glycation of LDL by methylglyoxal increases arterial atherogenicity: a possible contributor to increased risk of cardiovascular disease in diabetes. Diabetes. 2011;60(7):1973–80.
75. Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes. 1994;43(8):1010–4.
76. Jenkins AJ, Klein RL, Chassereau CN, Hermayer KL, Lopes-Virella MF. LDL from patients with well-controlled IDDM is not more susceptible to in vitro oxidation. Diabetes. 1996;45(6):762–7.
77. Edwards IJ, Wagner JD, Litwak KN, Rudel LL, Cefalu WT. Glycation of plasma low density lipoproteins increases interaction with arterial proteoglycans. Diabetes Res Clin Pract. 1999;46(1):9–18.
78. Wang X, Bucala R, Milne R. Epitopes close to the apolipoprotein B low density lipoprotein receptor-binding site are modified by advanced glycation end products. Proc Natl Acad Sci U S A. 1998;95(13):7643–7.
79. Sima AV, Botez GM, Stancu CS, Manea A, Raicu M, Simionescu M. Effect of irreversibly glycated LDL in human vascular smooth muscle cells: lipid loading, oxidative and inflammatory stress. J Cell Mol Med. 2010;14(12):2790–802.
80. Lam MC, Tan KC, Lam KS. Glycoxidized low-density lipoprotein regulates the expression of scavenger receptors in THP-1 macrophages. Atherosclerosis. 2004;177(2):313–20.
81. Brown BE, Rashid I, van Reyk DM, Davies MJ. Glycation of low-density lipoprotein results in the time-dependent accumulation of cholesteryl esters and apolipoprotein B-100 protein in primary human monocyte-derived macrophages. FEBS J. 2007;274(6):1530–41.
82. Brown BE, Dean RT, Davies MJ. Glycation of low-density lipoproteins by methylglyoxal and glycolaldehyde gives rise to the in vitro formation of lipid-laden cells. Diabetologia. 2005;48(2):361–9.
83. Artwohl M, Graier WF, Roden M, Bischof M, Freudenthaler A, Waldhäusl W, Baumgartner-Parzer SM. Diabetic LDL triggers apoptosis in vascular endothelial cells. Diabetes. 2003;52(5):1240–7.
84. Sonoki K, Yoshinari M, Iwase M, Iino K, Ichikawa K, Ohdo S, Higuchi S, Iida M. Glycoxidized low-density lipoprotein enhances monocyte chemoattractant protein-1 mRNA expression in human umbilical vein endothelial cells: relation to lysophosphatidylcholine contents and inhibition by nitric oxide donor. Metabolism. 2002;51(9):1135–42.

85. Sonoki K, Iwase M, Iino K, Ichikawa K, Yoshinari M, Ohdo S, Higuchi S, Iida M. Dilazep and fenofibric acid inhibit MCP-1 mRNA expression in glycoxidized LDL-stimulated human endothelial cells. Eur J Pharmacol. 2003;475(1–3):139–47.
86. Lyons TJ, Li W, Wells-Knecht MC, Jokl R. Toxicity of mildly modified low-density lipoproteins to cultured retinal capillary endothelial cells and pericytes. Diabetes. 1994;43(9):1090–5.
87. Lyons TJ, Li W, Wojciechowski B, Wells-Knecht MC, Wells-Knecht KJ, Jenkins AJ. Aminoguanidine and the effects of modified LDL on cultured retinal capillary cells. Invest Ophthalmol Vis Sci. 2000;41(5):1176–80.
88. Gupta S, Rifici V, Crowley S, Brownlee M, Shan Z, Schlondorff D. Interactions of LDL and modified LDL with mesangial cells and matrix. Kidney Int. 1992;41(5):1161–9.
89. Schlondorff D. Cellular mechanisms of lipid injury in the glomerulus. Am J Kidney Dis. 1993;22(1):72–82.
90. Santini E, Lupi R, Baldi S, Madec S, Chimenti D, Ferrannini E, Solini A. Effects of different LDL particles on inflammatory molecules in human mesangial cells. Diabetologia. 2008;51(11):2117–25.
91. Ha H, Kamanna VS, Kirschenbaum MA, Kim KH. Role of glycated low density lipoprotein in mesangial extracellular matrix synthesis. Kidney Int Suppl. 1997;60:S54–9.
92. Fujii Y, Iwano M, Dohi K. Effect of lipids on glomerular fibrinolysis in vitro. Contrib Nephrol. 1997;120:140–5.
93. Zhang J, Ren S, Sun D, Shen GX. Influence of glycation on LDL-induced generation of fibrinolytic regulators in vascular endothelial cells. Arterioscler Thromb Vasc Biol. 1998;18(7):1140–8.
94. Ren S, Shen GX. Impact of antioxidants and HDL on glycated LDL-induced generation of fibrinolytic regulators from vascular endothelial cells. Arterioscler Thromb Vasc Biol. 2000;20(6):1688–93.
95. Ren S, Lee H, Hu L, Lu L, Shen GX. Impact of diabetes-associated lipoproteins on generation of fibrinolytic regulators from vascular endothelial cells. J Clin Endocrinol Metab. 2002;87(1):286–91.
96. Ma GM, Halayko AJ, Stelmack GL, Zhu F, Zhao R, Hillier CT, Shen GX. Effects of oxidized and glycated low-density lipoproteins on transcription and secretion of plasminogen activator inhibitor-1 in vascular endothelial cells. Cardiovasc Pathol. 2006;15(1):3–10.
97. Sangle GV, Zhao R, Mizuno TM, Shen GX. Involvement of RAGE, NADPH oxidase, and Ras/Raf-1 pathway in glycated LDL-induced expression of heat shock factor-1 and plasminogen activator inhibitor-1 in vascular endothelial cells. Endocrinology. 2010;151(9):4455–66.
98. Calzada C, Coulon L, Halimi D, Le Coquil E, Pruneta-Deloche V, Moulin P, Ponsin G, Véricel E, Lagarde MJ. In vitro glycoxidized low-density lipoproteins and low-density lipoproteins isolated from type 2 diabetic patients activate platelets via p38 mitogen-activated protein kinase. Clin Endocrinol Metab. 2007;92(5):1961–4.
99. Ferretti G, Rabini RA, Bacchetti T, Vignini A, Salvolini E, Ravaglia F, Curatola G, Mazzanti L. Glycated low density lipoproteins modify platelet properties: a compositional and functional study. J Clin Endocrinol Metab. 2002;87(5):2180–4.
100. Galle J, Schneider R, Winner B, Lehmann-Bodem C, Schinzel R, Münch G, Conzelmann E, Wanner C. Glycoxidized LDL impair endothelial function more potently than oxidized LDL: role of enhanced oxidative stress. Atherosclerosis. 1998;138(1):65–77.
101. Dong Y, Wu Y, Wu M, Wang S, Zhang J, Xie Z, Xu J, Song P, Wilson K, Zhao Z, Lyons T, Zou MH. Activation of protease calpain by oxidized and glycated LDL increases the degradation of endothelial nitric oxide synthase. J Cell Mol Med. 2009;13(9A):2899–910.
102. Posch K, Simecek S, Wascher TC, Jürgens G, Baumgartner-Parzer S, Kostner GM, Graier WF. Glycated low-density lipoprotein attenuates shear stress-induced nitric oxide synthesis by inhibition of shear stress-activated L-arginine uptake in endothelial cells. Diabetes. 1999;48(6):1331–7.
103. Nivoit P, Wiernsperger N, Moulin P, Lagarde M, Renaudin C. Effect of glycated LDL on microvascular tone in mice: a comparative study with LDL modified in vitro or isolated from diabetic patients. Diabetologia. 2003;46(11):1550–8.

104. Calvo C, Ponsin G, Berthezene F. Characterization of the non enzymatic glycation of high density lipoprotein in diabetic patients. Diabetes Metab. 1988;14(3):264–9.
105. Ferretti G, Bacchetti T, Marchionni C, Dousset N. Effect of non-enzymatic glycation on aluminium-induced lipid peroxidation of human high density lipoproteins (HDL). Nutr Metab Cardiovasc Dis. 2004;14(6):358–65.
106. Rashduni DL, Rifici VA, Schneider SH, Khachadurian AK. Glycation of high-density lipoprotein does not increase its susceptibility to oxidation or diminish its cholesterol efflux capacity. Metabolism. 1999;48(2):139–43.
107. Zhou H, Tan KC, Shiu SW, Wong Y. Increased serum advanced glycation end products are associated with impairment in HDL antioxidative capacity in diabetic nephropathy. Nephrol Dial Transplant. 2008;23(3):927–33.
108. Kalogerakis G, Baker AM, Christov S, Rowley KG, Dwyer K, Winterbourn C, Best JD, Jenkins AJ. Oxidative stress and high-density lipoprotein function in Type I diabetes and end-stage renal disease. Clin Sci (Lond). 2005;108(6):497–506.
109. Mastorikou M, Mackness B, Liu Y, Mackness M. Glycation of paraoxonase-1 inhibits its activity and impairs the ability of high-density lipoprotein to metabolize membrane lipid hydroperoxides. Diabet Med. 2008;25(9):1049–55.
110. Perségol L, Foissac M, Lagrost L, Athias A, Gambert P, Vergès B, Duvillard L. HDL particles from type 1 diabetic patients are unable to reverse the inhibitory effect of oxidised LDL on endothelium-dependent vasorelaxation. Diabetologia. 2007;50(11):2384–7.
111. Low H, Hoang A, Forbes J, Thomas M, Lyons JG, Nestel P, Bach LA, Sviridov D. Advanced glycation end-products (AGEs) and functionality of reverse cholesterol transport in patients with type 2 diabetes and in mouse models. Diabetologia. 2012;55(9):2513–21.
112. Passarelli M, Shimabukuro AF, Catanozi S, Nakandakare ER, Rocha JC, Carrilho AJ, Quintão EC. Diminished rate of mouse peritoneal macrophage cholesterol efflux is not related to the degree of HDL glycation in diabetes mellitus. Clin Chim Acta. 2000;301(1–2):119–34.
113. de Boer JF, Annema W, Schreurs M, van der Veen JN, van der Giet M, Nijstad N, Kuipers F, Tietge UJ. Type I diabetes mellitus decreases in vivo macrophage-to-feces reverse cholesterol transport despite increased biliary sterol secretion in mice. J Lipid Res. 2012;53(3):348–57.
114. Barter P, Rye K. Lecithin:cholesterol acyltransferase. In: Betteridge D, Illingworth D, Shepherd J, editors. Lipoproteins in health and disease. New York: Oxford University Press; 1999. p. 261–76.
115. Nobecourt E, Davies MJ, Brown BE, Curtiss LK, Bonnet DJ, Charlton F, Januszewski AS, Jenkins AJ, Barter PJ, Rye KA. The impact of glycation on apolipoprotein A-I structure and its ability to activate lecithin:cholesterol acyltransferase. Diabetologia. 2007;50(3):643–53.
116. Nelson CL, Karschimkus CS, Dragicevic G, Packham DK, Wilson AM, O'Neal D, Becker GJ, Best JD, Jenkins AJ. Systemic and vascular inflammation is elevated in early IgA and type 1 diabetic nephropathies and relates to vascular disease risk factors and renal function. Nephrol Dial Transplant. 2005;20(11):2420–6.
117. Sugimoto H, Shikata K, Hirata K, Akiyama K, Matsuda M, Kushiro M, Shikata Y, Miyatake N, Miyasaka M, Makino H. Increased expression of intercellular adhesion molecule-1 (ICAM-1) in diabetic rat glomeruli: glomerular hyperfiltration is a potential mechanism of ICAM-1 upregulation. Diabetes. 1997;46(12):2075–81.
118. Khalfaoui T, Lizard G, Beltaief O, Colin D, Ben Hamida J, Errais K, Ammous I, Zbiba W, Tounsi L, Zhioua R, Anane R, Ouertani-Meddeb A. Immunohistochemical analysis of cellular adhesion molecules (ICAM-1, VCAM-1) and VEGF in fibrovascular membranes of patients with proliferative diabetic retinopathy: preliminary study. Pathol Biol (Paris). 2009;57(7–8):513–7.
119. Albertini JP, Valensi P, Lormeau B, Aurousseau MH, Ferrière F, Attali JR, Gattegno L. Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM. Effect of intensive insulin treatment. Diabetes Care. 1998;21(6):1008–13.
120. Nobécourt E, Tabet F, Lambert G, Puranik R, Bao S, Yan L, Davies MJ, Brown BE, Jenkins AJ, Dusting GJ, Bonnet DJ, Curtiss LK, Barter PJ, Rye KA. Nonenzymatic glycation impairs

the antiinflammatory properties of apolipoprotein A-I. Arterioscler Thromb Vasc Biol. 2010;30(4):766–72.
121. Hedrick CC, Thorpe SR, Fu MX, Harper CM, Yoo J, Kim SM, Wong H, Peters AL. Glycation impairs high-density lipoprotein function. Diabetologia. 2000;43(3):312–20.
122. Pan B, Ren H, He Y, Lv X, Ma Y, Li J, Huang L, Yu B, Kong J, Niu C, Zhang Y, Sun WB, Zheng L. HDL of patients with type 2 diabetes mellitus elevates the capability of promoting breast cancer metastasis. Clin Cancer Res. 2012;18(5):1246–56.
123. Tabet F, Lambert G, Cuesta Torres LF, Hou L, Sotirchos I, Touyz RM, Jenkins AJ, Barter PJ, Rye KA. Lipid-free apolipoprotein A-I and discoidal reconstituted high-density lipoproteins differentially inhibit glucose-induced oxidative stress in human macrophages. Arterioscler Thromb Vasc Biol. 2011;31(5):1192–200.
124. Liu D, Ji L, Zhang D, Tong X, Pan B, Liu P, Zhang Y, Huang Y, Su J, Willard B, Zheng L. Nonenzymatic glycation of high-density lipoprotein impairs its anti-inflammatory effects in innate immunity. Diabetes Metab Res Rev. 2012;28(2):186–95.
125. Klaya F, Durlach V, Bertin E, Monier F, Monboisse JC, Gillery P. Evaluation of serum glycated lipoprotein(a) levels in noninsulin-dependent diabetic patients. Clin Biochem. 1997;30(3):227–30.
126. Galle J, Winner B, Conzelmann E, Wanner C. Impairment of endothelial function induced by glycoxidized lipoprotein a [Lp(a)]. Cell Mol Biol (Noisy-le-Grand). 1998;44(7):1035–45.
127. Shen GX. Impact and mechanism for oxidized and glycated lipoproteins on generation of fibrinolytic regulators from vascular endothelial cells. Mol Cell Biochem. 2003;246(1–2):69–74.
128. Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. Atherosclerosis. 2000;150:413–9.
129. Prescott S, Zimmerman G, Stafforinie D, McIntryre T. Platelet activating factor and related Lipid mediators. Annu Rev Biochem. 2000;69:419–45.
130. de Castro SH, Faria Neto HC, Gomes MB. Platelet-activating factor acetylhydrolase (PAF-AH) activity in patients with type 1 diabetes mellitus. Arq Bras Cardiol. 2007;88(2):179–84.
131. Gomes MB, Cobas RA, Nunes E, Nery M, Castro-Faria-Neto HC, Tibiriçá E. Serum platelet-activating factor acetylhydrolase activity: a novel potential inflammatory marker in type 1 diabetes. Prostaglandins Other Lipid Mediat. 2008;87(1–4):42–6.
132. Gomes MB, Cobas RA, Nunes E, Castro-Faria-Neto HC, da Matta MF, Neves R, Tibiriçá E. Plasma PAF-acetylhydrolase activity, inflammatory markers and susceptibility of LDL to in vitro oxidation in patients with type 1 diabetes mellitus. Diabetes Res Clin Pract. 2009;85(1):61–8.
133. Serban M, Tanaseanu C, Kosaka T, Vidulescu C, Stoian I, Marta DS, Tanaseanu S, Moldoveanu E. Significance of platelet-activating factor acetylhydrolase in patients with non-insulin-dependent (type 2) diabetes mellitus. J Cell Mol Med. 2002;6(4):643–7.
134. Yamashita S, Matsuzawa Y. Cholesteryl ester transfer protein. In: Betteridge D, Illingworth D, Shepherd J, editors. Lipoproteins in health and disease. New York: Oxford University Press; 1999. p. 277–300.
135. Wegner M, Araszkiewicz A, Zozulińska-Ziółkiewicz D, Wierusz-Wysocka B, Pioruńska-Mikołajczak A, Pioruńska-Stolzmann M. The relationship between concentrations of magnesium and oxidized low density lipoprotein and the activity of platelet activating factor acetylhydrolase in the serum of patients with type 1 diabetes. Magnes Res. 2010;23(2):97–104.
136. Précourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, Levy E. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis. 2011;214(1):20–36.
137. Mackness B, McElduff P, Mackness MI. The paraoxonase-2-310 polymorphism is associated with the presence of microvascular complications in diabetes mellitus. J Intern Med. 2005;258(4):363–8.
138. Mackness MI, Mackness B, Durrington PN, Connelly PW, Hegele RA. Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins. Genet Mol Biol. 1996;7(2):69–76.

139. Mackness B, Durrington PN, Mackness MI. The paraoxonase gene family and coronary heart disease. Curr Opin Lipidol. 2002;13(4):357–62.
140. Tavori H, Khatib S, Aviram M, Vaya J. Characterization of the PON1 active site using modeling simulation, in relation to PON1 lactonase activity. Bioorg Med Chem. 2008;16(15):7504–9.
141. Kelso GJ, Stuart WD, Richter RJ, et al. Apolipoprotein J is associated with paraoxonase in human plasma. Biochemistry. 1994;33:832–9.
142. Gaidukov L, Tawfik DS. High affinity, stability, and lactonase activity of serum paraoxonase PON1 anchored on HDL with ApoA-I. Biochemistry. 2005;44:11843–54.
143. Jakubowski H. Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation. J Biol Chem. 2000;275:3957–62.
144. Costa LG, Cole TB, Furlong CE. Paraoxonase (PON1): from toxicology to cardiovascular medicine. Acta Biomed. 2005;76(Suppl 2):50–7.
145. Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-density lipoprotein loses its antiinflammatory properties during acute influenza A infection. Circulation. 2001;103(18):2283–8.
146. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res. 2005;46(6):1239–47.
147. Chen Q, Reis SE, Kammerer CM, et al. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study. Am J Hum Genet. 2003;72(1):13–22.
148. Ruiz J, Blanche H, James RW, et al. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet. 1995;346:869–72.
149. Pfohl M, Koch M, Enderele MD, et al. Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery disease, and myocardial infarction in Type 2 diabetes. Diabetes. 1999;48:623–7.
150. Kao YL, Donaghue K, Chan A, Knight J, Silink M. A variant of paraoxonase (PON1) gene is associated with diabetic retinopathy in IDDM. J Clin Endocrinol Metab. 1998;83:2589–92.
151. Jenkins AJ, Klein RL, Zheng D, et al. Paraoxonase genotype (192 Gln-Arg) and serum paraoxonase arylesterase activity: relationship with Type I diabetes and nephropathy. Diabetes. 2000;49(Suppl 1):643P.
152. Kao Y, Donaghue KC, Chan A, Bennetts BH, Knight J, Silink M. Paraoxonase gene cluster is a genetic marker for early microvascular complications in type 1 diabetes. Diabet Med. 2002;19(3):212–5.
153. Leviev I, Kalix B, Brulhart Meynet MC, James RW. The paraoxonase PON1 promoter polymorphism C(−107)T is associated with increased serum glucose concentrations in non-diabetic patients. Diabetologia. 2001;44(9):1177–83.
154. Mackness B, Durrington PN, Boulton AJ, Hine D, Mackness MI. Serum paraoxonase activity in patients with type 1 diabetes compared to healthy controls. Eur J Clin Investig. 2002;32(4):259–64.
155. Kordonouri O, James RW, Bennetts B, et al. Modulation by blood glucose levels of activity and concentration of paraoxonase in young patients with type 1 diabetes mellitus. Metabolism. 2001;50(6):657–60.
156. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by HDL against LDL oxidative modification. FEBS Lett. 1998;423:57–60.
157. Ferretti G, Bacchetti T, Marchionni C, Caldarelli L, Curatola G. Effect of glycation of high density lipoproteins on their physicochemical properties and on paraoxonase activity. Acta Diabetol. 2001;38(4):163–9.
158. Inoue M, Suehiro T, Nakamura T, Ikeda Y, Kumon Y, Hashimoto K. Serum arylesterase/diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. Metabolism. 2000;49(11):1400–5.

159. Mackness B, Abuashia B, Boulton A, Mackness M. Low PON activity in Type 2 diabetes mellitus com plicated by retinopathy. Clin Sci. 2000;98:355–63.
160. Ikeda Y, Suehiro T, Inoue M, et al. Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. Metabolism. 1998;47(5):598–602.
161. Rajkovic MG, Rumora L, Barisic K. The paraoxonase 1, 2 and 3 in humans. Biochem Med (Zagreb). 2011;21(2):122–30.
162. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352(9131):837–53.
163. Peelman F, Vandekerckhove J, Rosseneu M. Structure and function of lecithin cholesterol acyl transferase: new insights from structural predictions and animal models. Curr Opin Lipidol. 2000;11(2):155–60.
164. Bieliciki JK, Forte TM, McCall MR. Minimally oxidized LDL is a potent inhibitor of LCAT activity. J Lipid Res. 1996;37:1012–21.
165. McCall M, van den Berg J, Kuypers F. Modification of LCAT activity and HDL structure. New links between cigarette smoking and coronary artery disease risk. Arterioscler Thromb. 1994;114:248–53.
166. Nakhjavani M, Asgharani F, Khalilzadeh O, Esteghamati A, Ghaneei A, Morteza A, Anvari M. Oxidized low-density lipoprotein is negatively correlated with lecithin-cholesterol acyltransferase activity in type 2 diabetes mellitus. Am J Med Sci. 2011;341(2):92–5.
167. Chang CK, Tso TK, Snook JT, Huang YS, Lozano RA, Zipf WB. Cholesteryl ester transfer and cholesterol esterification in type 1 diabetes: relationships with plasma glucose. Acta Diabetol. 2001;38(1):37–42.
168. Gillett MP, Obineche EN, El-Rokhaimi M, Lakhani MS, Abdulle A, Sulaiman M. Lecithin: cholesterol acyltransfer, dyslipoproteinaemia and membrane lipids in uraemia. J Nephrol. 2001;14(6):472–80.
169. Nestel P, Hoang A, Sviridov D, Straznicky N. Cholesterol efflux from macrophages is influenced differentially by plasmas from overweight insulin-sensitive and -resistant subjects. Int J Obes (Lond). 2012;36(3):407–13.
170. Nakhjavani M, Esteghamati A, Esfahanian F, Ghanei A, Rashidi A, Hashemi S. HbA1c negatively correlates with LCAT activity in type 2 diabetes. Diabetes Res Clin Pract. 2008;81(1):38–41.
171. Iizuka T. Effect of anti-diabetic treatment on high density lipoprotein-composition and lecithin:cholesterol acyltransferase activity—a comparison between insulin, sulfonylurea and diet alone treatments. Jpn J Med. 1989;28(4):457–61.
172. Akyuz F, Tekin N, Aydın O, Temel HE, Isikli B. The effect of metformin and exercise on serum lipids, nitric oxide synthase and liver nitric oxide levels in streptozotocin-nicotinamide induced diabetic rats. Afr J Pharm Pharmacol. 2012;6(5):336–42.
173. Fournier N, Myara I, Atger V, Moatti N. Reactivity of lecithin-cholesterol acyl transferase (LCAT) towards glycated high-density lipoproteins (HDL). Clin Chim Acta. 1995;234(1–2):47–61.
174. Zhang Z, Yamashita S, Hirano K, Nakagawa-Toyama Y, Matsuyama A, Nishida M, Sakai N, Fukasawa M, Arai H, Miyagawa J, Matsuzawa Y. Expression of cholesteryl ester transfer protein in human atherosclerotic lesions and its implication in reverse cholesterol transport. Atherosclerosis. 2001;159(1):67–75.
175. Ishikawa Y, Ito K, Akasaka Y, Ishii T, Masuda T, Zhang L, Akishima Y, Kiguchi H, Nakajima K, Hata Y. The distribution and production of cholesteryl ester transfer protein in the human aortic wall. Atherosclerosis. 2001;156(1):29–37.
176. Kakko S, Tamminen M, Päivänsalo M, Kauma H, Rantala AO, Lilja M, Reunanen A, Kesäniemi YA, Savolainen MJ. Variation at the cholesteryl ester transfer protein gene in relation to plasma high density lipoproteins cholesterol levels and carotid intima-media thickness. Eur J Clin Investig. 2001;31(7):593–602.

177. Bagdade JD, Kelley DE, Henry RR, Eckel RH, Ritter MC. Effects of multiple daily insulin injections and intraperitoneal insulin therapy on cholesteryl ester transfer and lipoprotein lipase activities in NIDDM. Diabetes. 1997;46:414–20.
178. Passarelli M, Cantanozi S, Nakandakare ER, et al. Plasma lipoproteins from patients with poorly controlled diabetes mellitus and "in vitro" glycation of lipoproteins enhance the transfer rate of cholesteryl ester from HDL to apo-B-containing lipoproteins. Diabetologia. 1997;40:1085–93.
179. Lemkadem B, Loiseau D, Larcher G, Malthiery Y, Foussard F. Effect of the nonenzymatic glycosylation of high density lipoprotein-3 on the cholesterol ester transfer protein activity. Lipids. 1999;34(12):1281–6.
180. Connelly MA, Parry TJ, Giardino EC, Huang Z, Cheung WM, Chen C, Cools F, Van der Linde H, Gallacher DJ, Kuo GH, Sarich TC, Demarest KT, Damiano BP. Torcetrapib produces endothelial dysfunction independent of cholesteryl ester transfer protein inhibition. J Cardiovasc Pharmacol. 2010;55(5):459–68.
181. Simic B, Hermann M, Shaw SG, Bigler L, Stalder U, Dörries C, Besler C, Lüscher TF, Ruschitzka F. Torcetrapib impairs endothelial function in hypertension. Eur Heart J. 2012;33(13):1615–24.
182. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.
183. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353(25):2643–53.
184. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854–65.
185. Scott R, Kesaniemi A, Wu L, Colagiuri S, Keech A, Jenkins AJ. Long-term glycemic variability and vascular complications in type 2 diabetes: post hoc analysis of the FIELD study. J Clin Endocrinol Metab. 2020;105(10):dgaa361.
186. Ceriello A, Prattichizzo F. Variability of risk factors and diabetes complications. Cardiovasc Diabetol. 2021;20(1):101.
187. Papachristoforou E, Lambadiari V, Maratou E, Makrilakis K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. J Diabetes Res. 2020;2020:7489795.
188. Zhou Z, Sun B, Huang S, Zhu C, Bian M. Glycemic variability: adverse clinical outcomes and how to improve it? Cardiovasc Diabetol. 2020;19(1):102.
189. International Hypoglycaemia Study Group. Hypoglycaemia, cardiovascular disease, and mortality in diabetes: epidemiology, pathogenesis, and management. Lancet Diabetes Endocrinol. 2019;7(5):385–96.
190. Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. Nat Rev Endocrinol. 2014;10(12):711–22.
191. Rutter MK, Prais HR, Charlton-Menys V, Gittins M, Roberts C, Davies RR, Moorhouse A, Jinadev P, France M, Wiles PG, Gibson JM, Dean J, Kalra PA, Cruickshank JK, Durrington PN. Protection against nephropathy in diabetes with atorvastatin (PANDA): a randomized double-blind placebo-controlled trial of high- vs. low-dose atorvastatin. Diabet Med. 2011;28(1):100–8.
192. Beisswenger PJ, Howell SK, Touchette AD, Lal S, Szwergold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. Diabetes. 1999;48(1):198–202.
193. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Furtado RHM, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine MS. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet. 2019;393(10166):31–9.

194. Zou CY, Liu XK, Sang YQ, Wang B, Liang J. Effects of SGLT2 inhibitors on cardiovascular outcomes and mortality in type 2 diabetes: a meta-analysis. Medicine (Baltimore). 2019;98(49):e18245.
195. Butler J, Usman MS, Khan MS, Greene SJ, Friede T, Vaduganathan M, Filippatos G, Coats AJS, Anker SD. Efficacy and safety of SGLT2 inhibitors in heart failure: systematic review and meta-analysis. ESC Heart Fail. 2020;7(6):3298–309.
196. Nespoux J, Vallon V. Renal effects of SGLT2 inhibitors: an update. Curr Opin Nephrol Hypertens. 2020;29(2):190–8.
197. Scheen AJ. Beneficial effects of SGLT2 inhibitors on fatty liver in type 2 diabetes: a common comorbidity associated with severe complications. Diabetes Metab. 2019;45(3):213–23.
198. Mirarchi L, Amodeo S, Citarrella R, Licata A, Soresi M, Giannitrapani L. SGLT2 inhibitors as the most promising influencers on the outcome of non-alcoholic fatty liver disease. Int J Mol Sci. 2022;23(7):3668.
199. Modi A, Agrawal A, Morgan F. Euglycemic diabetic ketoacidosis: a review. Curr Diabetes Rev. 2017;13(3):315–21.
200. Musso G, Saba F, Cassader M, Gambino R. Diabetic ketoacidosis with SGLT2 inhibitors. BMJ. 2020;371:m4147.
201. Colhoun HM, Thomason MJ, Mackness MI, Maton SM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Fuller JH, Collaborative AtoRvastatin Diabetes Study (CARDS). Design of the Collaborative AtoRvastatin Diabetes Study (CARDS) in patients with type 2 diabetes. Diabet Med. 2002;19(3):201–11.
202. Collins R, Armitage J, Parish S, Sleight P, Peto R, Heart Protection Study Collaborative Group. Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20536 people with cerebrovascular disease or other high-risk conditions. Lancet. 2004;363(9411):757–67.
203. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M, FIELD Study Investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005;366(9500):1849–61.
204. Davis TM, Ting R, Best JD, Donoghoe MW, Drury PL, Sullivan DR, Jenkins AJ, O'Connell RL, Whiting MJ, Glasziou PP, Simes RJ, Kesäniemi YA, Gebski VJ, Scott RS, Keech AC, Fenofibrate Intervention and Event Lowering in Diabetes Study Investigators. Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. Diabetologia. 2011;54(2):280–90.
205. Ting RD, Keech AC, Drury PL, Donoghoe MW, Hedley J, Jenkins AJ, Davis TM, Lehto S, Celermajer D, Simes RJ, Rajamani K, Stanton K, FIELD Study Investigators. Benefits and safety of long-term fenofibrate therapy in people with type 2 diabetes and renal impairment: the FIELD Study. Diabetes Care. 2012;35(2):218–25.
206. Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E, Merrifield A, Laatikainen LT, d'Emden MC, Crimet DC, O'Connell RL, Colman PG, FIELD Study Investigators. Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. Lancet. 2007;370(9600):1687–97.
207. ACCORD Study Group, ACCORD Eye Study Group, Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC Jr, Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med. 2010;363(3):233–44.
208. Zhu L, Hayen A, Bell KJL. Legacy effect of fibrate add-on therapy in diabetic patients with dyslipidemia: a secondary analysis of the ACCORDION study. Cardiovasc Diabetol. 2020;19(1):28.
209. Trialists CT. Efficacy of cholesterol-lowering therapy in 18 686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet. 2008;371:117–25.

210. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR, FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713–22.
211. Ballantyne CM, Bays H, Catapano AL, Goldberg A, Ray KK, Saseen JJ. Role of bempedoic acid in clinical practice. Cardiovasc Drugs Ther. 2021;35(4):853–64.
212. Wu H, Shang H, Wu J. Effect of ezetimibe on glycemic control: a systematic review and meta-analysis of randomized controlled trials. Endocrine. 2018;60(2):229–39.
213. Preiss D, Sattar N. Statins and the risk of new-onset diabetes: a review of recent evidence. Curr Opin Lipidol. 2011;22(6):460–6.
214. Younis NN, Soran H, Sharma R, Charlton-Menys V, Greenstein A, Elseweidy MM, Durrington PN. Small-dense LDL and LDL glycation in metabolic syndrome and in statin-treated and non-statin-treated type 2 diabetes. Diab Vasc Dis Res. 2010;7(4):289–95.
215. Suzuki T, Oba K, Futami S, Suzuki K, Ouchi M, Igari Y, Matsumura N, Watanabe K, Kigawa Y, Nakano H. Blood glucose-lowering activity of colestimide in patients with type 2 diabetes and hypercholesterolemia: a case-control study comparing colestimide with acarbose. J Nippon Med Sch. 2006;73(5):277–84.
216. Goldberg RB, Jacobson TA. Effects of niacin on glucose control in patients with dyslipidemia. Mayo Clin Proc. 2008;83(4):470–8.
217. Kobayashi S, Moriya H, Negishi K, Maesato K, Ohtake T. LDL-apheresis up-regulates VEGF and IGF-I in patients with ischemic limb. J Clin Apher. 2003;18(3):115–9.
218. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. Circulation. 1997;95(1):76–82.
219. Pares MN, D'Amico EA, Kutner JM, Chamone Dde A, Bydlowski SP. Platelet aggregation and lipoprotein levels in a patient with familial hypercholesterolemia after selective LDL-apheresis. Sao Paulo Med J. 1997;115(3):1448–51.
220. Kobayashi S, Oka M, Moriya H, Maesato K, Okamoto K, Ohtake T. LDL-apheresis reduces P-Selectin, CRP and fibrinogen—possible important implications for improving atherosclerosis. Ther Apher Dial. 2006;10(3):219–23.
221. Xi M, Hai C, Tang H, Chen M, Fang K, Liang X. Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. Phytother Res. 2008;22(2):228–37.
222. Motomura K, Fujiwara Y, Kiyota N, Tsurushima K, Takeya M, Nohara T, Nagai R, Ikeda T. Astragalosides isolated from the root of astragalus radix inhibit the formation of advanced glycation end products. J Agric Food Chem. 2009;57(17):7666–72.
223. Panagiotopoulos S, O'Brien RC, Bucala R, Cooper ME, Jerums G. Aminoguanidine has an anti-atherogenic effect in the cholesterol-fed rabbit. Atherosclerosis. 1998;136(1):125–31.
224. Ulrich P, Zhang X. Pharmacological reversal of advanced glycation end-product-mediated protein crosslinking. Diabetologia. 1997;40(Suppl 2):S157–9.
225. Thorpe SR, Baynes JW. Role of the Maillard reaction in diabetes mellitus and diseases of aging. Drugs Aging. 1996;9(2):69–77.
226. Philis-Tsimikas A, Parthasarathy S, Picard S, Palinski W, Witztum JL. Aminoguanidine has both pro-oxidant and antioxidant activity toward LDL. Arterioscler Thromb Vasc Biol. 1995;15(3):367–76.
227. Sing R, Barden A, Mori T, Beilin L. Advanced glycation end products. Diabetologia. 2001;44:129–46.
228. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP, ACTION I Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am J Nephrol. 2004;24(1):32–40.
229. Yamagishi S, Amano S, Inagaki Y, Okamoto T, Koga K, Sasaki N, Yamamoto H, Takeuchi M, Makita Z. Advanced glycation end products-induced apoptosis and overexpression of vas-

cular endothelial growth factor in bovine retinal pericytes. Biochem Biophys Res Commun. 2002;290(3):973–8.
230. Whittier F, Spinowitz B, Wuerth JP, et al. Pimagedine safety profile in patients with Type 1 diabetes. J Am Soc Nephrol. 1999;10:184A.
231. Nilsson BO. Biological effects of aminoguanidine: an update. Inflamm Res. 1999;48:509–15.
232. Picard S, Parthasarathy S, Fruebis J, Witztum JL. Aminoguanidine inhibits oxidative modification of low-density lipoprotein protein and the subsequent increase in uptake by macrophage scavenger receptors. Proc Natl Acad Sci U S A. 1992;89(15):6876–80.
233. Jedidi I, Thérond P, Zarev S, Cosson C, Couturier M, Massot C, Jore D, Gardès-Albert M, Legrand A, Bonnefont-Rousselot D. Paradoxical protective effect of aminoguanidine toward low-density lipoprotein oxidation: inhibition of apolipoprotein B fragmentation without preventing its carbonylation. Mechanism of action of aminoguanidine. Biochemistry. 2003;42(38):11356–65.
234. Oak JH, Youn JY, Cai H. Aminoguanidine inhibits aortic hydrogen peroxide production. VSMC NOX activity and hypercontractility in diabetic mice. Cardiovasc Diabetol. 2009;8:65.
235. Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, McDaniel ML, Williamson JR, Currie MG. Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. Eur J Pharmacol. 1993;233(1):119–25.
236. Wu G. Nitric oxide synthesis and the effect of aminoguanidine and NG-monomethyl-L-arginine on the onset of diabetes in the spontaneously diabetic BB rat. Diabetes. 1995;44(3):360–4.
237. Khalifah RG, Baynes JW, Hudson BG. Amadorins: novel post-Amadori inhibitors of advanced glycation reactions. Biochem Biophys Res Commun. 1999;257(2):251–8.
238. Onorato JM, Jenkins AJ, Thorpe SR, Baynes JW. Pyridoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. Mechanism of action of pyridoxamine. J Biol Chem. 2000;275(28):21177–84.
239. Metz TO, Alderson NL, Thorpe SR, Baynes JW. Pyridoxamine, an inhibitor of advanced glycation and lipoxidation reactions: a novel therapy for treatment of diabetic complications. Arch Biochem Biophys. 2003;419(1):41–9.
240. Alkhalaf A, Klooster A, van Oeveren W, Achenbach U, Kleefstra N, Slingerland RJ, Mijnhout GS, Bilo HJ, Gans RO, Navis GJ, Bakker SJ. A double-blind, randomized, placebo-controlled clinical trial on benfotiamine treatment in patients with diabetic nephropathy. Diabetes Care. 2010;33(7):1598–601.
241. Wang Y, Li Y, Yang Z, Wang Z, Chang J, Zhang T, Chi Y, Han N, Zhao K. Pyridoxamine treatment of HK-2 human proximal tubular epithelial cells reduces oxidative stress and the inhibition of autophagy induced by high glucose levels. Med Sci Monit. 2019;25:1480–8.
242. Karachalias N, Babaei-Jadidi R, Rabbani N, Thornalley PJ. Increased protein damage in renal glomeruli, retina, nerve, plasma and urine and its prevention by thiamine and benfotiamine therapy in a rat model of diabetes. Diabetologia. 2010;53(7):1506–16.
243. Balakumar P, Rohilla A, Krishan P, Solairaj P, Thangathirupathi A. The multifaceted therapeutic potential of benfotiamine. Pharmacol Res. 2010;61(6):482–8.
244. Katare RG, Caporali A, Oikawa A, Meloni M, Emanueli C, Madeddu P. Vitamin B1 analog benfotiamine prevents diabetes-induced diastolic dysfunction and heart failure through Akt/Pim-1-mediated survival pathway. Circ Heart Fail. 2010;3(2):294–305.
245. Du X, Edelstein D, Brownlee M. Oral benfotiamine plus alpha-lipoic acid normalises complication-causing pathways in type 1 diabetes. Diabetologia. 2008;51(10):1930–2.
246. Beltramo E, Berrone E, Tarallo S, Porta M. Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications. Acta Diabetol. 2008;45(3):131–41.
247. Waanders F, van den Berg E, Nagai R, van Veen I, Navis G, van Goor H. Renoprotective effects of the AGE-inhibitor pyridoxamine in experimental chronic allograft nephropathy in rats. Nephrol Dial Transplant. 2008;23(2):518–24.
248. Degenhardt TP, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW, Thorpe SR, Baynes JW. Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. Kidney Int. 2002;61(3):939–50.

249. Stitt A, Gardiner TA, Alderson NL, Canning P, Frizzell N, Duffy N, Boyle C, Januszewski AS, Chachich M, Baynes JW, Thorpe SR. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. Diabetes. 2002;51(9):2826–32.
250. Alderson NL, Chachich ME, Youssef NN, Beattie RJ, Nachtigal M, Thorpe SR, Baynes JW. The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. Kidney Int. 2003;63(6):2123–33.
251. Lyons TJ, Jenkins AJ. Glycation, oxidation, and lipoxidation in the development of the complications of diabetes: a carbonyl stress hypothesis. Diabet Rev. 1997;5:365–91.
252. Stitt AW, Jenkins AJ, Cooper ME. Advanced glycation end products and diabetic complications. Expert Opin Investig Drugs. 2002;11(9):1205–23.
253. Brown SM, Smith DM, Alt N, Thorpe SR, Baynes JW. Tissue-specific variation in glycation of proteins in diabetes: evidence for a functional role of amadoriase enzymes. Ann N Y Acad Sci. 2005;1043:817–23.
254. Monnier VM, Wu X. Enzymatic deglycation with amadoriase enzymes from Aspergillus sp. as a potential strategy against the complications of diabetes and aging. Biochem Soc Trans. 2003;31:1349–53.
255. Monnier VM, Sell DR. Prevention and repair of protein damage by the Maillard reaction in vivo. Rejuvenation Res. 2006;9(2):264–73.
256. Cohen MP, Sharma K, Jin Y, Hud E, Wu VY, Tomaszewski J, Ziyadeh FN. Prevention of diabetic nephropathy in db/db mice with glycated albumin antagonists. A novel treatment strategy. J Clin Invest. 1995;95(5):2338–45.
257. Mitsuhashi T, Li YM, Fishbane S, Vlassara H. Depletion of reactive advanced glycation endproducts from diabetic uremic sera by a lysozyme-linked matrix. J Clin Invest. 1997;100:847–54.
258. Zheng F, Cai W, Mitsuhashi T, Vlassara H. Lysozyme enhances renal excretion of advanced glycation endproducts in vivo and suppresses adverse age-mediated cellular effects in vitro: a potential AGE sequestration therapy for diabetic nephropathy? Mol Med. 2001;7(11):737–47.
259. Li YM, Tan AX, Vlassara H. Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of advanced glycation-modified proteins to a con served motif. Nat Med. 1995;1:1057–61.
260. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, Cao R, Hori O, Stern D, Schmidt AM. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. J Clin Invest. 1996;97(1):238–43.
261. Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. Circulation. 2002;106(22):2827–35.
262. Santilli F, Vazzana N, Bucciarelli LG, Davì G. Soluble forms of RAGE in human diseases: clinical and therapeutical implications. Curr Med Chem. 2009;16(8):940–52.
263. Yan SF, Ramasamy R, Schmidt AM. Soluble RAGE: therapy and biomarker in unraveling the RAGE axis in chronic disease and aging. Biochem Pharmacol. 2010;79(10):1379–86.
264. Zhang H, Tasaka S, Shiraishi Y, Fukunaga K, Yamada W, Seki H, Ogawa Y, Miyamoto K, Nakano Y, Hasegawa N, Miyasho T, Maruyama I, Ishizaka A. Role of soluble receptor for advanced glycation end products on endotoxin-induced lung injury. Am J Respir Crit Care Med. 2008;178(4):356–62.
265. Zieman SJ, Melenovsky V, Clattenburg L, Corretti MC, Capriotti A, Gerstenblith G, Kass DA. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. J Hypertens. 2007;25(3):577–83.
266. Steppan J, Tran H, Benjo AM, Pellakuru L, Barodka V, Ryoo S, Nyhan SM, Lussman C, Gupta G, White AR, Daher JP, Shoukas AA, Levine BD, Berkowitz DE. Alagebrium in combination with exercise ameliorates age-associated ventricular and vascular stiffness. Exp Gerontol. 2012;47(8):565–72.
267. Gurbuz N, Sagdic G, Sanli A, Ciftcioglu A, Bassorgun I, Baykal A, Usta MF. Therapeutic effect of combination of alagebrium (ALT-711) and sildenafil on erectile function in diabetic rats. Int J Impot Res. 2012;24(3):114–21.

268. Hartog JW, Willemsen S, van Veldhuisen DJ, Posma JL, van Wijk LM, Hummel YM, Hillege HL, Voors AA, BENEFICIAL Investigators. Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. Eur J Heart Fail. 2011;13(8):899–908.
269. Kiland JA, Gabelt BT, Tezel G, Lütjen-Drecoll E, Kaufman PL. Effect of the age cross-link breaker alagebrium on anterior segment physiology, morphology, and ocular age and rage. Trans Am Ophthalmol Soc. 2009;107:146–58.
270. Coughlan MT, Thallas-Bonke V, Pete J, Long DM, Gasser A, Tong DC, Arnstein M, Thorpe SR, Cooper ME, Forbes JM. Combination therapy with the advanced glycation end product cross-link breaker, alagebrium, and angiotensin converting enzyme inhibitors in diabetes: synergy or redundancy? Endocrinology. 2007;148(2):886–95.
271. Freidja ML, Tarhouni K, Toutain B, Fassot C, Loufrani L, Henrion D. The AGE-breaker ALT-711 restores high blood flow-dependent remodeling in mesenteric resistance arteries in a rat model of type 2 diabetes. Diabetes. 2012;61(6):1562–72.
272. Krautwald M, Leech D, Horne S, Steele ML, Forbes J, Rahmadi A, Griffith R, Münch G. The advanced glycation end product-lowering agent ALT-711 is a low-affinity inhibitor of thiamine diphosphokinase. Rejuvenation Res. 2011;14(4):383–91.
273. Toma L, Sanda GM, Niculescu LS, Deleanu M, Stancu CS, Sima AV. Caffeic acid attenuates the inflammatory stress induced by glycated LDL in human endothelial cells by mechanisms involving inhibition of AGE-receptor, oxidative, and endoplasmic reticulum stress. Biofactors. 2017;43(5):685–97.