



# HDL functionality in type 1 and type 2 diabetes: new insights

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## Purpose of review

To critically appraise new insights into HDL structure and function in type 1 diabetes (T1DM) and type 2 diabetes (T2DM).

## Recent findings

In young T1DM patients with early renal impairment and a high inflammatory score, both HDL antioxidative activity and endothelial vasodilatory function were impaired, revealing a critical link between HDL dysfunction, subclinical vascular damage, systemic inflammation and end organ damage. HDL may inhibit development of T2DM by attenuating endoplasmic reticulum (ER) stress and apoptotic loss of pancreatic  $\beta$ -cells, an effect due in part to ABC transporter-mediated efflux of specific oxysterols with downstream activation of the hedgehog signalling receptor, Smoothed. The apoM-sphingosine-1-phosphate complex is critical to HDL antidiabetic activity, encompassing protection against insulin resistance, promotion of insulin secretion, enhanced  $\beta$ -cell survival and inhibition of hepatic glucose production. Structure-function studies of HDL in hyperglycemic, dyslipidemic T2DM patients revealed both gain and loss of lipidomic and proteomic components. Such changes attenuated both the optimal protective effects of HDL on mitochondrial function and its capacity to inhibit endothelial cell apoptosis. Distinct structural components associated with individual HDL functions.

## Summary

Extensive evidence indicates that both the proteome and lipidome of HDL are altered in T1DM and T2DM, with impairment of multiple functions.

## Keywords

dyslipidemia, glycaemic burden, HDL subspecies, lipidome, pancreatic  $\beta$  cells, proteome

## INTRODUCTION

The potential causal relationships between HDL and the pathogenesis of vascular inflammation and premature atherosclerosis on the one hand, and between HDL and glucose homeostasis on the other, remain the subject of debate; these questions are of pertinence to both type 1 diabetes (T1DM) and type 2 diabetes (T2DM) given the high cardiovascular risk with which they are associated [1–3,4<sup>a</sup>,5–7,8<sup>a</sup>,9<sup>a</sup>]. Considering the paucity of evidence for causal links between HDL functionality and the development of T1DM or T2DM, the objectives of this review are to summarise progress in our understanding of the impact of perturbed glucose homeostasis and dyslipidaemia in T1DM and T2DM on the structure and functionality of HDL particles.

## FUNCTIONALITY OF HDL PARTICLES: THE PROTEOME AND LIPIDOME

A wide spectrum of biological activities – which have been equated with diverse functions *in vivo* – and

which may all be of potential relevance to hyperglycaemia, inflammation and vascular dysfunction in diabetes depending on the metabolic and pathophysiological contexts – are exerted by HDL particles. Such activities include cellular efflux of cholesterol (CEC) in the context of reverse cholesterol transport,

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**Curr Opin Endocrinol Diabetes Obes** 2022, 29:112–123

DOI:10.1097/MED.0000000000000705

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## KEY POINTS

- HDL structure (proteome and lipidome) is integral to its biological activities.
- Abnormalities induced in HDL structure in both T1DM and T2DM which directly impair functionality involve both the lipidome and proteome, and include dysregulation of glucose and lipid metabolism, nonenzymatic glycation and oxidation of both proteins and lipids, and inflammation.
- Diabetic alterations in HDL functionality involve impairment of cellular cholesterol efflux and sterol homeostasis, of anti-inflammatory, antioxidative and antiapoptotic activities, of endothelial protection and vasodilatory activity, of antidiabetic functions, of rescue of mitochondrial function and of transport and cellular delivery of metabolically active miR species.
- Whether impaired HDL function plays a causal role in the pathophysiology of premature atherosclerotic cardiovascular disease (ASCVD) in T1DM and T2DM is indeterminate, requiring substantial further investigation.
- The development of 'smart' reconstituted HDL particles transporting the apoM/sphingosine-1-phosphate complex represents a novel therapeutic option for protection against development of insulin resistance and vascular damage in diabetes.

a spectrum of anti-inflammatory activities, antiprotease activity, antioxidative activity, antiapoptotic activity, antithrombotic and antiplatelet activity, endothelial protection and repair including endothelial cell progenitor homeostasis, proangiogenic activity, activity integral to innate and adaptive immunity, antiinfectious and antiparasitic activities as exemplified by implication in reverse lipopolysaccharide transport and by antitypanosomic activity, antidiabetic activity and regulation of glucose homeostasis, and transport and tissue delivery of small noncoding RNAs, respectively (Fig. 1) [9<sup>•</sup>,10,11,12<sup>•</sup>,13,14,15<sup>••</sup>,16<sup>•</sup>,17<sup>••</sup>,18<sup>•</sup>–20<sup>•</sup>,21]. These multifarious activities frequently involve cellular signal transduction, and are relevant to multiple cell types, including endothelial cells, immunoinflammatory cells such as monocytes and monocyte-derived macrophages, pancreatic  $\beta$  cells, hepatocytes, adipocytes and myocytes and smooth muscle cells [9<sup>•</sup>,10,11,12<sup>•</sup>,13,14,15<sup>••</sup>,16<sup>•</sup>,17<sup>••</sup>,18<sup>•</sup>–20<sup>•</sup>,21].

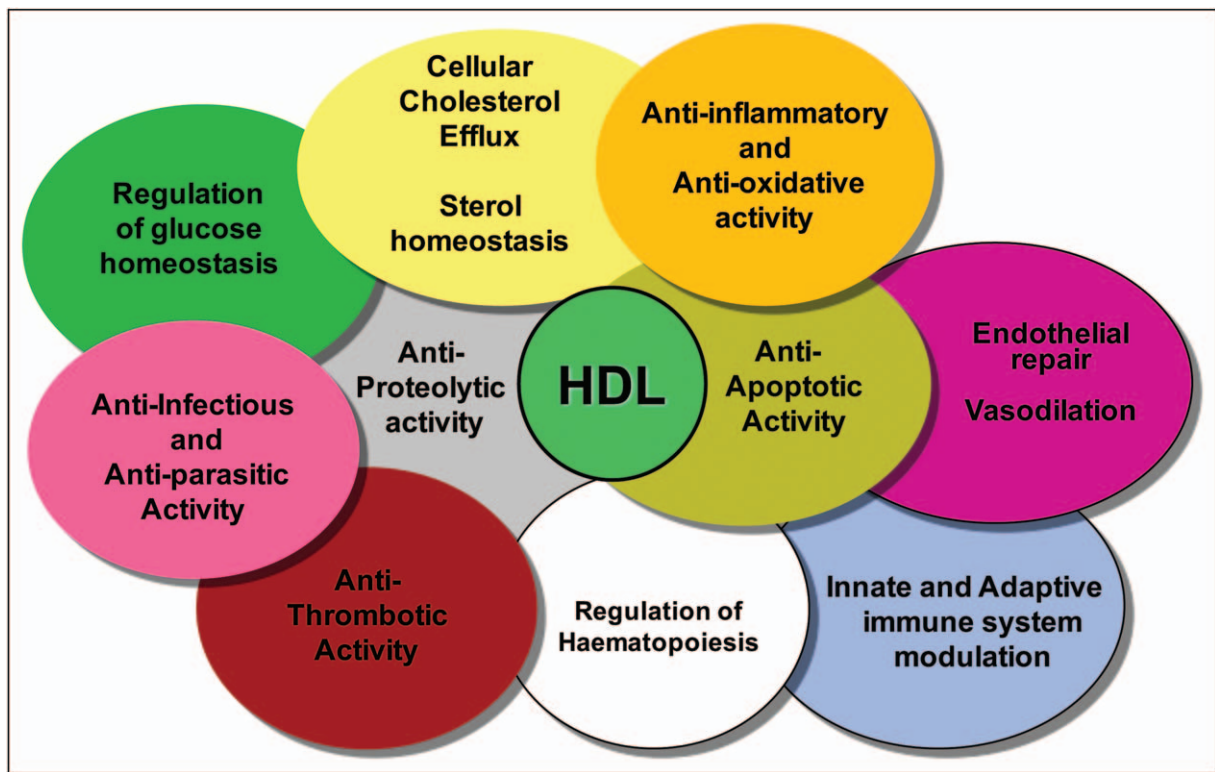
HDL particles equally function as a transport platform for key biologically active molecules, and notably sphingosine-1-phosphate (S1P), hormones such as oestradiol and thyroxine, carotenoid precursors of vitamin A, vitamin E, potent antioxidative lipids including plasmalogens and lycopene, and miRNAs,

among which miRNA-223 may act as a coordinator of cholesterol homeostasis [12<sup>•</sup>,22,23,24<sup>••</sup>].

Consideration of the nature of the starting biological material and the methodologies employed for isolation of total HDL, or HDL subfractions and / or their subspecies, is critical to evaluation of their function [25–27]. HDLs are frequently isolated as lipoproteins in the density interval 1.063–1.21 g/ml by flotation ultracentrifugation; as in some precipitation techniques, this multistep procedure exposes HDL to high ionic strength [26]. Partial loss of small apolipoproteins and oxidative modification of lipid and protein components may occur; rigorous quality control and characterisation of the isolated HDL fraction(s) is a prerequisite [26]. Moreover, HDL subfractions prepared by differing methods may not contain the same populations of particle subspecies [25,26].

In the context of the marked heterogeneity of HDL particles in diabetes, substantial evidence supports the contention not only that both the proteome and lipidome are integral to the overall biological activity expressed by a given HDL particle, but also that the plasma pool of HDL consists of a highly diverse spectrum of individual particle species, each defined by distinct lipid and protein components (Fig. 2) [25–27,28<sup>••</sup>,29–32,33<sup>••</sup>,34]. Some 95 proteins (and potentially up to 200) constitute the HDL proteome; the plasma abundance of many of these proteins is insufficient to permit one copy per HDL particle, thereby suggesting that specific proteins may be bound to distinct particle species, which may then exert specific functions [31,32,33<sup>••</sup>]. Thus, the capacity of an HDL particle to integrate several proteins may be limited by size and surface constraints, as in the case of small dense HDL3 [25,26,30–32,33<sup>••</sup>]. The HDL lipidome is equally highly complex [34], state-of-the-art mass spectrometric analyses revealing that more than 400 molecular lipid species are detectable (Meikle PJ and Chapman MJ, unpublished data). The working hypothesis that a specific biological function of HDL might be mediated by a distinct particle subspecies defined by a specific cluster(s) of bound proteins and lipids is now plausible; the possibility that a single particle species might exert more than one function cannot be excluded [18<sup>•</sup>,30–32,33<sup>••</sup>]. In a similar manner to the proteome, the lipid composition of HDL particles differs between subfractions and between subspecies and is an intimate feature of their functionality [28<sup>••</sup>,29,34].

The specific function(s) with which an HDL particle is endowed may well relate to processes involved in its formation. Whether particles constituting the HDL spectrum in human plasma result primarily by maturation through progressive lipidation of a pre $\beta$ ,



**FIGURE 1.** Schematic summary of the wide spectrum of biological activities displayed by human HDL particles. This schematic does not imply that all such activities are transported by one and the same HDL particle; indeed, HDL particles are highly heterogeneous in terms of their structure, metabolism and functionality.

lipid-poor particle as classically proposed, or whether individual HDL particle size species principally originate in the liver, are metabolically stable and undergo only minor remodelling to larger or smaller particles as recently reported, remains indeterminate; moreover, these processes may be altered in both T1DM and T2DM [1,11,35]. Thus, the enlarged hepatic pool of triglyceride in T2DM may result in direct production of HDL with elevated triglyceride content. Particle size stability in plasma does not however preclude dynamic exchange and transfer of both apolipoproteins and lipids; indeed, transfer of free cholesterol-rich and phospholipid-rich surface remnants from triglyceride-rich lipoproteins (TGRL) and remnants contribute significantly to the HDL lipid pool under normal conditions, although such transfer is diminished (–25%) in T2DM [35,36<sup>¶</sup>].

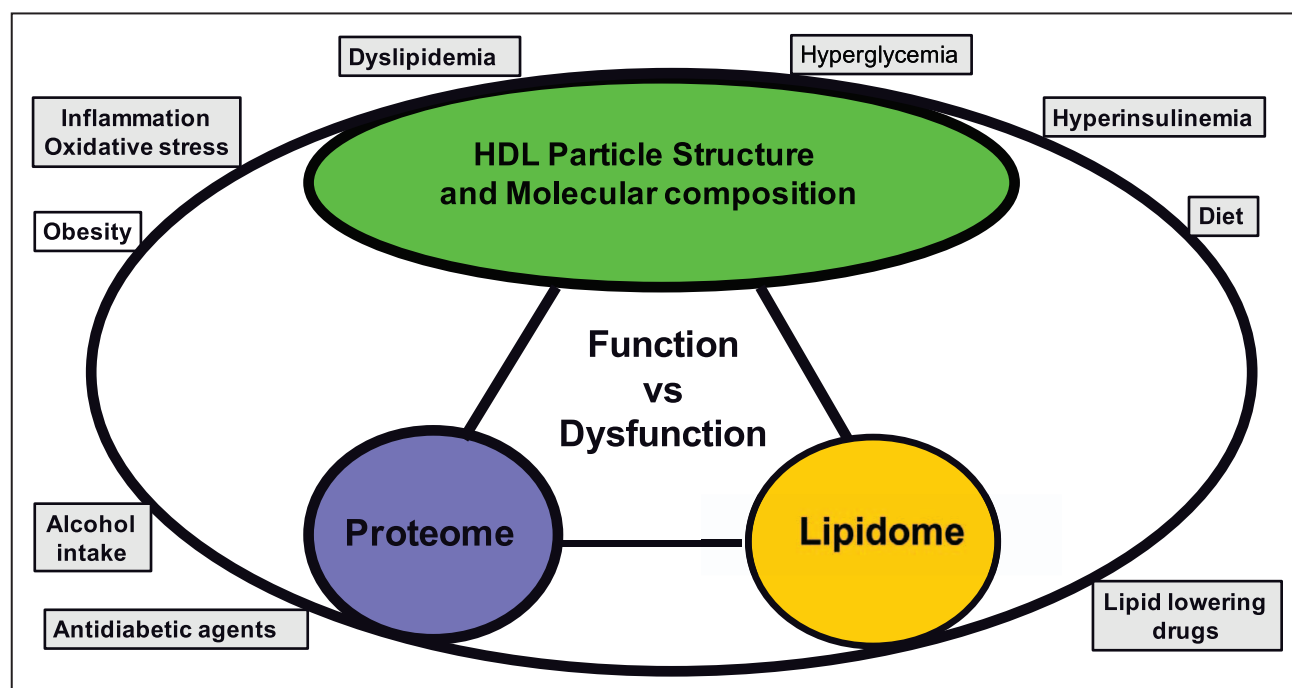
### DYNAMIC ASPECTS OF HDL METABOLISM, STRUCTURE AND FUNCTIONALITY IN DIABETES

The compositional and functional features of HDL particles in a single plasma sample are a ‘photo-shot’ in time of the summation of integrated processes of particle production, intravascular metabolism and remodelling by lipases and lipid transfer proteins,

and cellular interactions. Such features are directly impacted by the metabolic environment. Indeed, major factors which may significantly modify HDL structure and function in the context of diabetes include hyperglycaemia, insulin resistance, hyperinsulinemia, insulin therapy, dyslipidaemia, inflammation and oxidative stress, obesity, dietary components and alcohol intake; lipid-lowering pharmacotherapy – and potentially antidiabetic agents – may equally impact HDL metabolism, composition and function (Fig. 2) [7,17<sup>\*\*</sup>,19<sup>¶</sup>,22,26,36<sup>¶</sup>].

### HDL FUNCTIONALITY IN TYPE 1 DIABETES

T1DM, involving autoimmune-mediated destruction of pancreatic  $\beta$  cells, uniquely features insulin therapy from its inception, with the goal of controlling glucose homeostasis. Indeed, an excessive glycaemic burden is a major driver of systemic and tissular oxidative stress, endothelial dysfunction and vascular inflammation, end organ damage such as nephropathy and retinopathy, and ASCVD; its effects are exacerbated by synergism with concomitant risk factors [5]. Nonenzymatic glycation of plasma proteins by advanced glycosylation end-products in poorly controlled T1DM thus underlies covalent modification of major HDL apolipoproteins



**FIGURE 2.** The lipidome and proteome of HDL in normolipidemic, normoglycemic healthy subjects are integral to expression of the optimal biological activities of HDL particles which are equated with its functionality. The lipidome and proteome are however markedly altered in both type 1 diabetes and type 2 diabetes, leading to functional impairment, that is, dysfunctionality. The lipidome is composed of more than 400 molecular lipid species (PJ Meikle and MJ Chapman, unpublished observations) while the proteome is composed of at least 95 distinct protein species. Multiple distinct particle subfractions are now recognised with the circulating HDL pool, which now extends to distinct particle subspecies with defined protein and lipid components. Lipid–lipid, lipid–protein and protein–protein interactions ensure the integrity and stability of HDL particles; such interactions may confer conformational changes in major apolipoproteins such as apo-AI and apo-AII, which may in turn impact functionality. The lipidome, proteome and functionality of HDL are impacted by their metabolic environment, major factors including dyslipidaemia and dysglycaemia.

such as apo-AI, with subsequent altered metabolism and attenuation of function [8<sup>9</sup>,10,26]. Equally, elevated oxidative stress results in glycooxidation of HDL proteins and peroxidation of HDL lipids. Quantitatively, lipoprotein profiles in T1DM show variability as a function of the degree of glycaemic control and additional factors but may be quasi-normal when glycaemic status is well controlled [8<sup>9</sup>,9<sup>9</sup>,10]. Insulin therapy upregulates expression of lipoprotein lipase however, leading to efficient lipolysis of TGRL and supranormal levels of HDL-C.

Early findings in recent-onset T1DM indicated that HDL display both anti-inflammatory and immunoregulatory activities [10]. By contrast, HDL cholesterol efflux capacity (CEC) is significantly decreased early in the disease course independently of glucose control [9<sup>9</sup>]. Recently a shift to a larger mean HDL particle size (~10%) accompanied by lower HDL particle numbers (~30%) was reported in T1DM patients relative to matched controls; this latter parameter was independently associated with significant enhancement in total HDL-mediated CEC in recent-onset

T1DM, but not with ABCA1-independent CEC [37]. Discrepancies are frequent in reported findings for CEC in T1DM and may in part be explained by differences between the averaged activity of all particles in a total HDL fraction as compared with the activity in distinct subfractions or subspecies and preferably expressed on a per particle basis (see below), by differences in the cell systems used for in-vitro experimentation and in the biological and therapeutic status of the patients such as glycaemic burden. Study of HDL function in T1DM therefore requires strict definition of the temporal/longitudinal stage of the condition, of comorbidities and of biological, clinical and therapeutic status, conditions which have rarely been satisfied in published investigations.

The capacity of HDL to remove and detoxify lipid hydroperoxides associated with either modified LDL or cell membranes, a metric of antioxidative activity, is consistently impaired in T1DM patients, and is observed independently of the degree of glycaemic control [9<sup>9</sup>]. This effect appears to be linked to reduction in HDL-associated paraoxonase-1 (PON1) activity,

although the endogenous substrate of this enzyme is indeterminate.

In a novel study of HDL function in T1DM in subsets of individuals in two small cohorts with long-term T1DM with or without microvascular and macrovascular complications, individuals without vascular complications exhibited higher levels of medium-sized HDL particles (M-HDL); such protected individuals equally displayed higher levels of HDL-associated PON1 mass and activity, which were in part transported by M-HDL [38<sup>■</sup>]. No additional functional distinctions were however observed in the long-term protected T1DM individuals. Furthermore, evidence for alterations in the HDL proteome in young T1DM subjects versus matched controls in relation to the degree of glycaemic control has provided evidence to support the contention that the HDL proteome is altered [39]. A small number of specific HDL proteins were altered in T1DM independently of glycaemic control, whereas others were partially or totally corrected with optimal glycaemic control. While the relationship of these changes, principally involving enrichment in protease inhibitors, to HDL functionality remains speculative, nonetheless they highlight the impact of glycaemic burden on the HDL proteome in T1DM. Such proteomic alterations are accompanied by changes in the HDL lipidome, which frequently feature both triglyceride enrichment and elevation in ratios of free cholesterol/phosphatidylcholine and sphingomyelins/phosphatidylcholine, the latter (sphingomyelins/phosphatidylcholine) indicative of enhanced HDL surface rigidity; the former (free cholesterol/phosphatidylcholine) may result from enhanced transfer of cholesterol-rich TGRL surface fragments during lipolysis, or attenuated cholesteryl ester transfer protein (CETP)-mediated lipid transfer activity, or diminished cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT), or a combination of these [9<sup>■</sup>,10,36<sup>■</sup>]. In addition, significant depletion (up to 15%) in S1P cargo was detected in both HDL2 and HDL3 sub-fractions of long-term T1DM subjects; given the critical role of S1P in endothelial cell signalling and in stimulation of nitric oxide synthesis, such a reduction may contribute to impaired endothelial vasorelaxation (see discussion below) [9<sup>■</sup>].

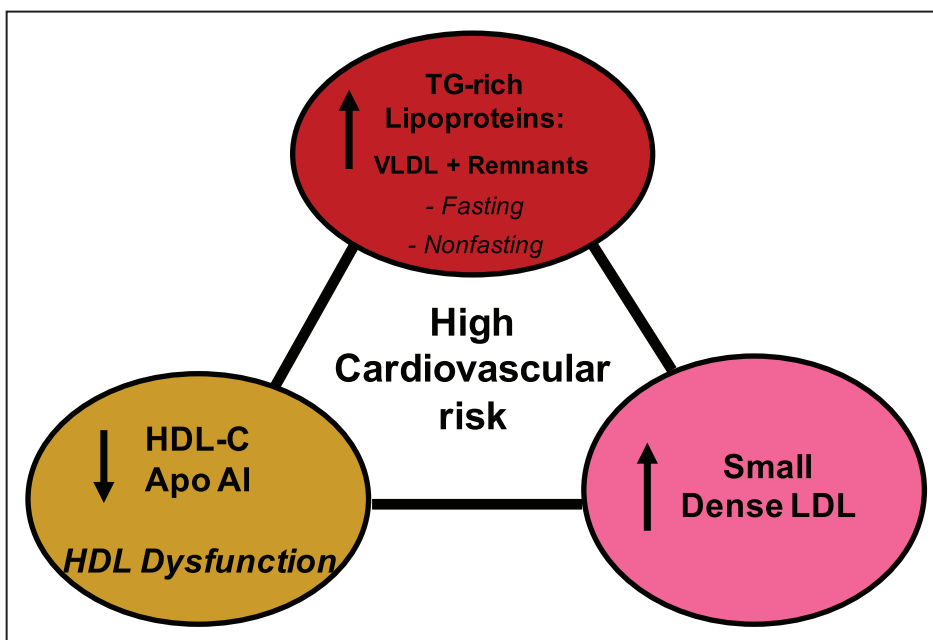
Young T1DM subjects with renal impairment represent a very high-risk group for future cardiovascular and renal events, and are characterised by dyslipidaemia, systemic inflammation perturbed vascular homeostasis which typically progresses to endothelial dysfunction and ultimately to premature ASCVD. Earlier studies established that HDL in T1DM exhibited an impaired anti-inflammatory activity [10]. In an innovative study, Chiesa *et al.*

[40] evaluated the interplay between the antioxidant activity of HDL and its capacity to protect nitric oxide bioavailability with inflammatory status and in-vivo endothelial function (as flow-mediated dilation of the brachial artery) in a cohort of adolescents with T1DM ( $n = 70$ ) and early renal dysfunction and compared them with matched control subjects. Using the albumin/creatinine ratio as a measure of renal function, an increased inflammatory risk score and HDL dysfunction were characteristic of patients with early renal impairment. HDL dysfunction was detected as a diminished capacity to inhibit in-vitro superoxide anion production and as reduction in PON-1 activity; in addition, reduction in HDL-mediated nitric oxide availability was observed. Importantly, endothelial dysfunction was limited to patients with renal impairment accompanied by both a high inflammatory risk score and elevated HDL-C level. Surprisingly, glycaemic burden was not a factor in contributing to the dysfunctional HDL phenotype. Despite limited insight into causality, this study nonetheless highlights the clinically significant link between HDL dysfunctionality, subclinical vascular damage, systemic inflammation and early renal impairment in T1DM [40].

## HDL FUNCTIONALITY IN TYPE 2 DIABETES

A marked dyslipidaemic phenotype is typical of T2DM in both the fasting and postprandial states, featuring hypertriglyceridemia and subnormal levels of triglyceride-rich HDL (as HDL-C) with defective function (Fig. 3); the metabolism of the circulating TGRL, LDL and HDL pools is intimately linked via the action of lipases [lipoprotein lipase (LPL), hepatic lipase (HL) and endothelial lipase (EL)], lipid transfer proteins [CETP and phospholipid transfer protein (PLTP)], LCAT and apolipoprotein exchange and transfer. The recent finding that free cholesterol transfer from TGRL during lipolysis is attenuated may further enhance the elevated ratio of triglyceride to free cholesterol in T2DM HDL; the latter represents a biomarker of HDL dysfunction [1,7,26,36<sup>■</sup>,41–44]. Despite the classical paradigm that insulin resistance causes dyslipidaemia however, it remains conjectural as to whether components of the atherogenic dyslipidaemia themselves favour development of insulin resistance, as glucose intolerance, hyperinsulinemia and beta cell dysfunction evolve concomitantly with perturbation of lipid metabolism [41].

Profound HDL dysfunction is typical of T2DM patients, and is exacerbated primarily by the degree of hypertriglyceridemia, hyperglycaemic burden, and chronic systemic and tissular inflammation with



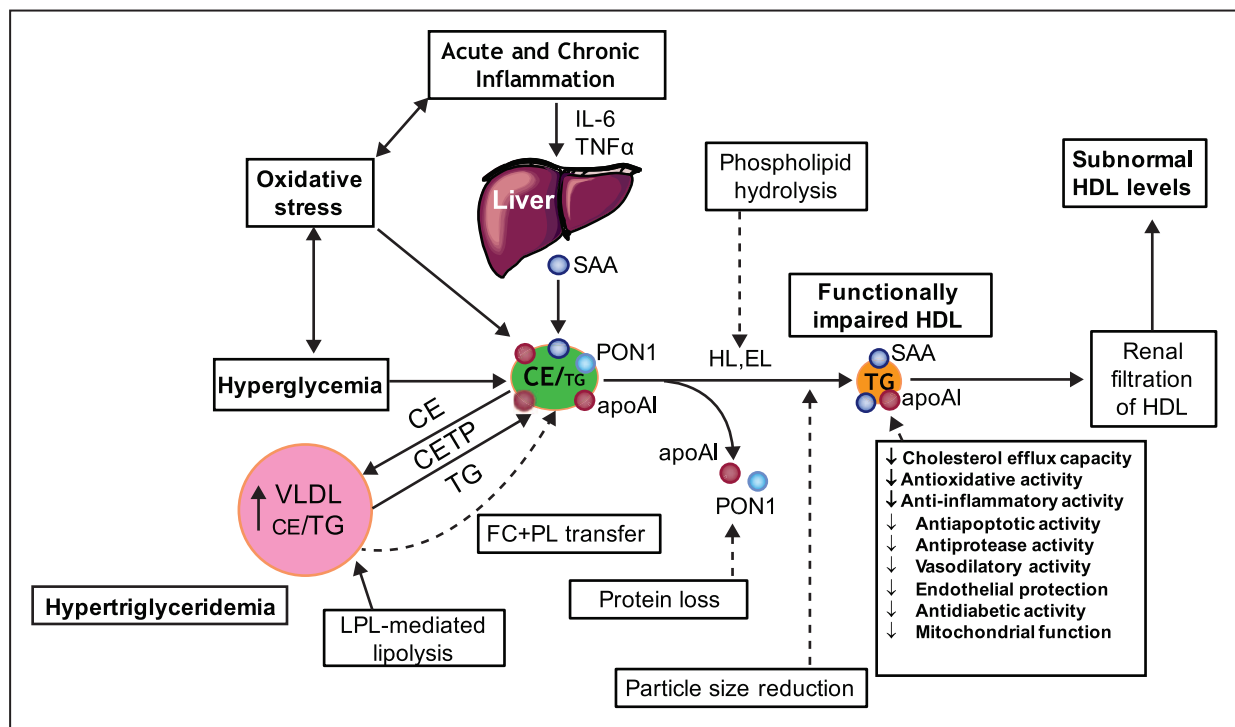
**FIGURE 3.** Schematic representation of the major components of the atherogenic dyslipidemia typical of patients displaying type 2 diabetes with poor glycemic control. This dyslipidemia features hypertriglyceridemia involving elevated levels of VLDLs and remnants, a predominance of small, cholesterol-poor dense LDL in the LDL particle profile and subnormal levels of HDL and apoAI which exhibit impaired functionality. Elevated flux of free fatty acids from adipose tissue to the liver as a result of peripheral insulin resistance in type 2 diabetes are a major metabolic driver of hepatic VLDL production and thus of this dyslipidemic phenotype.

concomitant elevation in oxidative stress (Fig. 4) [17<sup>••</sup>,26,42–44,45<sup>••</sup>]. Nonenzymatic glycation of HDL proteins resulting from poorly controlled glycaemia, together with oxidative modification of both proteins and lipids, are prominent as potentially causal factors in inducing HDL dysfunction; such dysfunction is extensively documented, and involves attenuated CEC and impaired antioxidative, anti-inflammatory, antiapoptotic, vasoprotective and vasodilator activities [17<sup>••</sup>,19<sup>•</sup>,42–44,45<sup>••</sup>]. Again, activities monitored in the total HDL fraction may differ significantly from those in HDL subfractions. Alterations equally occur in small noncoding RNA transport by HDL in T2DM, which results from altered export from pancreatic beta cells [24<sup>••</sup>]. Indeed, the export process for miR-375-3p to HDL is inversely regulated by glucose-stimulated insulin secretion and appears to be independent of cholesterol homeostasis [24<sup>••</sup>,46<sup>•</sup>]. Given the potential action of HDL-associated miRNAs in modulating gene expression in target cells, with for example impact on vascular function, it is of relevance that diabetic nephropathy specifically attenuated HDL-mediated delivery of proangiogenic miR-132-3p to endothelial cells [24<sup>••</sup>,47].

Current investigations of the impact of T2DM on HDL functionality are focussed on alterations in the lipidomic and proteomic components of

distinct HDL subfractions or subspecies and their impact on function. With respect to CEC, isolation of large, medium and small HDL particles from T2DM patients and matched controls revealed that, on a per particle basis, ABCA1-mediated efflux to small HDL was selectively attenuated (–23%) [48<sup>•</sup>]. This finding likely was explained by reduced content of SERPINA1 (serpin family member 1, equally termed  $\alpha$ 1-antitrypsin), an antiprotease and phospholipid-binding protein, in small HDL in T2DM, as in-vitro SERPINA1 enrichment of small HDL partially compensated for its deficient efflux activity [48<sup>•</sup>]. As covalent modification of apo-AI or apo-AII or other HDL apolipoproteins by glycation, carbamylation, nitration or oxidation leads to marked attenuation of biological activity, the potential contribution of posttranslational apolipoprotein modification to these experimental findings is indeterminate; a similar qualification frequently applies to studies of HDL functionality in diabetes [17<sup>••</sup>,46<sup>•</sup>,48<sup>•</sup>,49].

In an integrated approach, Cardner *et al.* [50<sup>••</sup>] defined the relationship between the structure (lipidomic and proteomic profiles) of ultracentrifugally isolated HDL from obese adult T2DM patients without coronary heart disease ( $n = 46$ ) and five distinct functions: CEC, inhibition of endothelial cell apoptosis, inhibition of beta cell apoptosis, rescue of



**FIGURE 4.** Altered metabolism of HDL in type 2 diabetes with hyperglycaemia and insulin resistance, hypertriglyceridemia and acute and/or chronic systemic inflammation associated with elevated oxidative stress. These factors act in a synergistic manner to modify the lipidome and proteome of HDL particles, resulting in impaired functionality. Chronic inflammation characteristic of type 2 diabetes is associated with elevated plasma levels of IL-6. As a result, the liver and other tissues may produce serum amyloid A, which binds to HDL, displaces apoA-I, paraoxonase-1 and other protein components. Oxidative stress may lead to oxidation of both lipids and proteins. Hyperglycaemia, through the formation of advanced glycosylation end products, results in glycation of HDL proteins, including apoA-I, with impaired biological activity. Elevated lipid transfer activity of CETP in hypertriglyceridemia enhances the triglyceride content of HDL with depletion of cholesteryl esters. Attenuated transfer of surface lipolytic fragments of VLDL to the HDL pool occurs in T2DM; these fragments consist mainly of free cholesterol and phospholipids. Lipolysis of HDL lipids by hepatic and endothelial lipases produces small, dense HDL with impaired functionality. Small HDLs are filtered by the kidney with subsequent fall in circulating HDL levels.

mitochondrial membrane potential and mitochondrial respiration, with either reference plasma (for CEC) or reconstituted HDL as calibrators; data were compared with those in a group of healthy control subjects. Importantly, this cohort was hyperglycaemic (HbA1c,  $54 \pm 11$  nmol/mol), mildly hypertriglyceridemia (triglycerides,  $2 \pm 1.5$  mmol) and exhibited subnormal HDL-C levels ( $1.2 \pm 0.3$  mmol). A high prevalence of both lipid-lowering (61% statin use) and antidiabetic medications was evident; the effects of such polymedication on HDL structure and function in this T2DM cohort are therefore indeterminate. The HDL particle profile showed a loss of large particles and preferential increase in small, triglyceride-rich HDL. These findings are not inconsistent with those of Mora *et al.* [51] who, in a prospective study in a cohort of 1687 women with 13-year follow-up involving nuclear magnetic spectroscopic analysis, observed that small HDL particles were correlated positively with

future risk of T2DM, and that large HDL were inversely associated after adjustment for established risk factors. By contrast, only a minor fall occurred in total HDL particle numbers ( $<5\%$ ;  $P < 0.001$ ).

Overall, HDL in the T2DM cohort of Cardner *et al.* [50<sup>\*\*\*</sup>] showed loss of specific lipids and proteins with gain of others; a net loss of proteins was observed. Thus, proteome depletion in apo-AIV, PON1, PON3, apo-D, apo-E, apo-F, apo-J and apo-M was associated with increase in contents of serum amyloid SAA1 (serum amyloid A) and SAA2, apo-CII, apo-CIII and fibrinogen. In this context, it is relevant that loss of some beneficial functional proteins may be magnified by gain of others with deleterious action; for example, apo-AIV enhances insulin secretion, whereas apo-CIII adversely induces pancreatic islet inflammation and promotes  $\beta$ -cell death [17<sup>\*\*\*</sup>]. In the lipidome, enrichment in phosphatidylethanolamine species occurred at the expense of depletion in ether-phosphatidylcholines,

lysophosphatidylcholines, phosphatidylinositols, sphingomyelins and the 18:2 species of cholesteryl ester. T2DM HDL was dysfunctional in displaying decreased capacity to lower the mitochondrial potential of myotubes (MMM), an inability to promote maximal respiration of brown adipocytes (MRBA) and an attenuated ability to inhibit starvation-induced apoptosis of human aortic endothelial cells (SIAP). No change was seen in CEC in T2DM HDL as compared with control subjects, apo-AI concentration representing the principal determinant. Furthermore, CEC clustered poorly with other HDL functions and did not constitute a surrogate for them. Moreover, individual functions of HDL were poorly correlated with each other, suggesting that they may be determined by distinct structural components. Significantly, non-CEC functions were typically associated with minor HDL proteins, and notably apo-CIII and S100A9 for MMM, apo-F and cathepsin D for MRBA, apoL1 for endoplasmic reticulum-stress-induced apoptosis, and glycosylphosphatidyl-inositol specific phospholipase D1 (GLPD1) with SIAP. Three novel determinants of HDL function were identified, the sphingadienine-based sphingomyelin sphingomyelins 42.3 and GLPD1 for inhibition of SIAP, and apo-F, a lipid transfer inhibitor, for MRBA. Nonetheless, their precise functional roles remain speculative. Overall, these studies are seminal in demonstrating that (i) several biological activities of HDL are independent of each other, and are notably independent of CEC, (ii) lipidomic and proteomic components of HDL are modified in T2DM and underlie impaired functionality and (iii) HDL dysfunctionality in T2DM involves diverse biological activities [50<sup>\*\*\*</sup>].

The gain of SAA by HDL in T2DM is noteworthy; the origin of this acute phase protein lies in both hepatic and nonhepatic tissues [50<sup>\*\*\*</sup>,52<sup>\*\*\*</sup>]. As HDL-bound SAA appears to be biologically inert, HDL may serve as a transport vehicle to sequester SAA secreted from diverse tissues and thus protect the host from uncontrolled inflammation and tissue damage. Indeed, free SAA may stimulate multiple inflammatory processes during tissue injury or acute infection [52<sup>\*\*\*</sup>]. Binding of SAA to HDL displaces apo-AI and other apolipoproteins; nonetheless, the potential for SAA to modify CEC and the antioxidative and anti-inflammatory actions of HDL is subject to debate [52<sup>\*\*\*</sup>]. Definition of mechanisms regulating the dissociation of SAA from HDL in chronic diseases such as T2DM then becomes paramount to understanding of its pathological effects.

In-vivo turnover studies of HDL proteins may provide insight into mechanisms underlying alterations in the proteome of HDL in T2DM as observed above [53]. Thus, in a recent in-vivo kinetic investigation using <sup>2</sup>H<sub>2</sub>O enrichment of body water

coupled with mass spectrometry in new-onset, insulin-naïve, diet-controlled T2DM subjects ( $n=9$ ) with mild hyperglycaemia, the fractional catabolic rates of apo-AII, apo-J, apo-AIV, complement C3, transthyretin and vitamin D-binding protein were significantly increased; by contrast the half-life of PON1 was prolonged, but associated with reduced activity and mass and suggestive of a lower production rate [53]. Significantly, the proinflammatory index of HDL was a strong determinant of apo-AII flux. This proof-of-concept study clearly indicates that the in-vivo dynamics of HDL proteins are perturbed at a very early stage of T2DM, with potential consequences for their steady state concentrations.

### ANTIDIABETIC FUNCTIONS OF HDL: EFFECTS OF TYPE 2 DIABETES

Is HDL function relevant to glycaemic control and to lipotoxicity in pancreatic  $\beta$  cells in T2DM?

The antidiabetic properties of HDL include inhibition of pancreatic  $\beta$ -cell apoptosis induced by endoplasmic reticulum stress or by inhibition of native-induced or oxidised LDL-induced activation of the c-Jun N-terminal kinase pathway, improvement in glycaemic control due to enhanced glucose-stimulated insulin synthesis and secretion from  $\beta$  cells, improved insulin sensitivity, improved glucose uptake into heart and skeletal muscle and enhanced proliferation of  $\beta$  cells [11,15<sup>\*\*\*</sup>,17<sup>\*\*\*</sup>,26,45<sup>\*\*\*</sup>,46<sup>\*\*\*</sup>,54<sup>\*\*\*</sup>]. Apo-AI is intimately involved in these activities, which are independent of the ATP-binding cassette transporters ABCA1 and ABCG1 [54<sup>\*\*\*</sup>,55<sup>\*\*\*</sup>,56]. Whether these antidiabetic actions of HDL may afford  $\beta$ -cell protection at early stages of T1DM or T2DM remains conjectural.

A key mechanistic question concerns the potential relevance of HDL-mediated CEC via ABC transporters to these findings, or whether effects of HDL on intracellular cholesterol metabolism or receptor-mediated cell signalling are implicated, or both [15<sup>\*\*\*</sup>,46<sup>\*\*\*</sup>,54<sup>\*\*\*</sup>,55<sup>\*\*\*</sup>,56]. First, improvement in beta cell function by apo-AI occurred by a cholesterol-independent mechanism and was equally independent of ABCA1 and ABCG1 transporters in a genetically modified mouse model [56]. Second, the innovative study of Yalcinkaya *et al.* [15<sup>\*\*\*</sup>] in the immortalised rat  $\beta$ -cell line INS-1e revealed that both native human plasma HDL and reconstituted HDL prevented endoplasmic reticulum stress-dependent apoptosis induced by thapsigargin (TPSG), an inhibitor of the sarco/endoplasmic reticulum. This effect involved cellular efflux of oxysterols, notably 24-hydroxycholesterol (24-OHC), to HDL; moreover, supplementation of HDL with 24-OHC increased its cytoprotective action. Indeed, sterol efflux was essential, as silencing of ABC transporters abrogated the capacity of HDL to



prevent TPSG-induced cell death. Employing siRNA or pharmacologically mediated inhibition, the role of the hedgehog signalling receptor, smoothed (SMO), was equally evaluated. SMO inhibition attenuated the antiapoptotic effects of HDL. Finally, nuclear translocation of the SMO-activated transcription factor GL-1 was inhibited by endoplasmic reticulum stress but restored by both 24-OHC and HDL. Together, these insightful findings indicate that the cytoprotective effect of HDL on model  $\beta$  cells involves the mobilisation of oxysterols for direct activation of the hedgehog signalling receptor SMO. Furthermore, the capacity of HDL to ensure pancreatic  $\beta$ -cell homeostasis of both cholesterol and oxysterols is integral to HDL-mediated protection of  $\beta$ -cell function.

Do HDL subfractions differ in their capacity to positively impact insulin metabolism in pancreatic  $\beta$  cells? In the insulinoma MIN-6 cell line, hydrated density-defined human HDL subfractions HDL2a-3c were similar in their capacity to induce insulin secretion or to maintain insulin content under experimental conditions involving low, normal or high glucose levels [57]. Importantly therefore, all HDL subfractions may act as both insulin secretagogues (at low glucose) and insulin secretion enhancers (at high glucose) in MIN-6 cells.

Might defined lipid/protein complexes within HDL particles be involved in its antidiabetic action?

S1P, a sphingolipid mediator, regulates diverse functions in a wide range of cell types via the high-affinity G protein-coupled receptor system, involving receptors S1P1–5, and is anchored to HDL particles by its chaperone, apolipoprotein M [58]. A substantial body of evidence supports a direct role for the S1P/apo-M complex in exerting antidiabetic actions; such actions may extend to its role in regulating diverse inflammatory processes [16<sup>■</sup>,26,58,59<sup>■</sup>,60]. The actions of the S1P/apo-M complex in HDL encompass protection against insulin resistance in the liver, adipose tissue and skeletal muscle, promotion of insulin secretion, enhanced pancreatic  $\beta$ -cell survival and inhibition of hepatic glucose production [16<sup>■</sup>,26,58,59<sup>■</sup>]. Protective action against development of insulin resistance appears to involve activation of insulin signalling pathways, including the AKT and AMPK pathways, and equally upregulation of SIRT1 protein levels to improve mitochondrial function [16<sup>■</sup>,58,59<sup>■</sup>,60]. To what degree the 'antidiabetic tandem' of apo-M and S1P can exert protection against development of insulin resistance in man remains subject to debate [16<sup>■</sup>].

Plasma apo-M levels are moderately reduced in T2DM (<10%;  $P < 0.001$ ) [61<sup>■</sup>]. Most importantly however, apo-M activity is altered at high glucose levels due to glycation and polymerisation *in vitro*,

reducing its capacity to bind S1P [61<sup>■</sup>]. Such an effect can be anticipated to attenuate the pleiotropic activities of the apo-M/S1P complex, including antiapoptotic, cell proliferative and vasorelaxant actions as well as maintenance of vascular permeability. Apo-M polymers were not however detected in diabetic subjects [61<sup>■</sup>].

Finally, established evidence clearly indicates impairment of several additional HDL functions in T2DM of relevance to  $\beta$ -cell survival and insulin homeostasis, including CEC, antioxidative and anti-inflammatory actions (Fig. 4) [44,45<sup>■</sup>,46<sup>■</sup>,47,48<sup>■</sup>,49,50<sup>■</sup>,52<sup>■</sup>,54<sup>■</sup>,61<sup>■</sup>,62,63].

## CONCLUSION

Despite the contrasting quantitative changes in HDL (as HDL-C) in T1DM as compared with T2DM, current data attest to the impaired functionality of HDL particles in both these chronic cardiometabolic disorders. Glycaemic burden is a principal factor in such dysfunction in both T1DM and T2DM; this effect is amplified in T2DM – and to a lesser degree in T1DM – by atherogenic dyslipidaemia (Fig. 3) [63]. HDL dysfunctionality is therefore gaining traction as an innovative therapeutic target for prevention of the premature, accelerated vascular disease typical of both T1DM and T2DM, and thus for attenuation or even correction of the complex pathophysiological processes which underlie microangiopathy and macroangiopathy in these chronic conditions.

The current critical review has highlighted new insights into the relationships between HDL structural components and defined functions. Indeed, the preparative fractionation of HDL subspecies, the characterisation of their proteome and lipidome, and the definition of their functionality is becoming a reality. Furthermore, we have initial insights into alterations in the proteolipidome which are associated with impairment in HDL functionality in both T1DM and T2DM. Accumulating evidence in these disorders highlights the functional impairment of small dense HDL. It is therefore of significance that apoAI-rich, small dense HDL (HDL3c) were identified earlier as particles with potent capacity for ABCA1 transporter-mediated cellular cholesterol efflux and antioxidative, anti-inflammatory, antiapoptotic and antithrombotic activities in healthy normolipidaemic, nondiabetic, nonobese subjects [13,21,29,33<sup>■</sup>,58,60,64,65]. The phosphosphingolipidome of these particles is distinct, featuring phosphatidylserine and S1P enrichment for example [29,64].

These insights prompt novel therapeutic approaches to future targeting of HDL dysfunctionality. To initiate such a strategy, further intense

research is urgently needed to define both the individual structure of subspecies within the HDL3c subfraction and their specific functions. The in-vivo characteristics of each of these subspecies, such as plasma residence time, would provide insight into their intravascular metabolism and stability in a suitable animal model, such as the rabbit with enhanced hepatic lipase activity [66]. On the basis of such knowledge, reconstitution of proteo-lipidomically and functionally defined HDL particles becomes feasible. ApoAI-containing HDL particles transporting the apoM-S1P complex could be prioritised, particularly given that their functions are multiple, extending to antidiabetic and anti-inflammatory actions and endothelial maintenance and vasodilation.

The infusion of such reconstituted HDL featuring recombinant proteins and a defined lipidome portends the normalisation of specific aspects of HDL dysfunctionality in diabetes, potentially with beneficial clinical sequelae such as stabilisation or even regression of vascular disease or pancreatic  $\beta$ -cell protection, or both. Effective background treatment of dyslipidaemic patients with diabetes at high cardiovascular risk with antidiabetic and lipid-lowering agents will be essential in order to optimise the clinical benefit of such innovative therapy.

## Acknowledgements

None.

## Financial support and sponsorship

The cited studies of the Author were supported by a research grant from Pfizer (Grant WS285961-6027/ID #53234871) and by continuous research funding from the National Institute of Health and Medical Research (INSERM), Sorbonne/Pierre and Marie Curie University, Fondation de la Recherche Medicale, the City of Paris, ARLA (Association for Research on Lipoproteins and Atherogenesis), and NSFA (Nouvelle Societ  Francaise de l'Ath rosclerose).

## Conflicts of interest

There are no conflicts of interest for the preparation of this review.

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