

High-Density Lipoprotein at the Interface of Type 2 Diabetes Mellitus And Cardiovascular Disorders

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Abstract: Type 2 diabetes mellitus is associated with low high-density lipoprotein (HDL) cholesterol levels, which is an independent cardiovascular risk factor. Low HDL cholesterol concentrations reflect a dysregulation in HDL metabolism, which is determined by the concerted action of different proteins, including cholesterol ester transfer protein, lecithin: cholesterol acyltransferase, endothelial and lipoprotein lipase, phospholipid transfer protein, and hepatic lipase, as well as different receptors, including the scavenger receptor class B type I and ATP-binding cassette transporter A1 and G1. Type 2 diabetes mellitus is besides a dysregulation in HDL metabolism, also associated with dysfunctional HDL: HDL-mediated reverse cholesterol transport as well as the anti-oxidative and endothelial-protective features of HDL are impaired in type 2 diabetes mellitus. The first part of the present review gives an overview of how type 2 diabetes mellitus affects the expression and/or activity of receptors and proteins involved in HDL metabolism and how different diabetes-associated factors influence the functionality of HDL. The second part of the review focuses on describing the newest insights in the impact of HDL on glucose metabolism and on diabetes-associated cardiovascular complications.

Keywords: Diabetes mellitus, high-density lipoprotein, dysfunction, remodeling, glucose metabolism, endothelial dysfunction, diabetic cardiopathy.

1. INTRODUCTION

Consistent evidence of cross-sectional and prospective epidemiological studies have demonstrated that high-density lipoprotein (HDL) cholesterol is a strong independent inverse predictor of risk of ischemic cardiovascular diseases [1-3]. The cardiovascular-protective effects of HDL in this setting have mainly been attributed to its role in reverse cholesterol transport, i.e. the centripetal transport of excess cholesterol from peripheral tissue towards the liver for excretion into bile or to steroidogenic organs for steroid hormone synthesis. However, the effects of HDL are pleiotropic, including its direct anti-oxidative, anti-inflammatory, and anti-apoptotic features [4-6].

Type 2 diabetes mellitus is characterized by dyslipidemia, including increased low-density lipoprotein (LDL) cholesterol levels, low HDL cholesterol levels, and increased triglycerides. Since reduced levels of HDL are predictive of cardiovascular events in statin-treated patients who have low LDL cholesterol levels [7], sole statin treatment under type 2 diabetes mellitus is insufficient, and merges for the use of HDL-raising pharmacotherapy. However, HDL is heterogeneous and can alter from an anti-inflammatory, anti-atherogenic particle to a pro-inflammatory particle [8], especially in an inflammatory condition as diabetes mellitus. Therefore, HDL-raising strategies/pharmacotherapy have to be evaluated not only in how much they increase HDL cholesterol, but - even more important - how they affect HDL functionality.

This review summarizes how type 2 diabetes mellitus alters HDL metabolism and functionality. On the other hand, it demonstrates the cardiovascular properties of HDL in the context of diabetes mellitus, underscoring the importance of finding an appropriate HDL-raising strategy for clinical purposes.

2. HDL AND HDL METABOLISM

HDL are the smallest and most dense plasma lipoproteins ($d=1.063-1.21$ g/ml). Apolipoprotein (apo) A-I is the main apo of HDL and constitutes approximately 70% of the total HDL protein mass. Consequently, a strong correlation between apo A-I plasma

concentrations and HDL cholesterol levels exists [9]. Proteomic studies have extended the list of identified apo and associated proteins present in HDL and its subclasses. The lipid fraction of HDL consists of a great variety of compounds including phospholipids, free and esterified fatty acids, and different ceramides and sphingolipids, including especially sphingosine-1-phosphate (S1P) [10], which has recently been shown to be a potent anti-inflammatory agent [11]. The relevance of sphingolipids in the pleiotropic effects of HDL is in detail reviewed by Tölle *et al.* [12] in the same Current Pharmaceutical Design HDL volume. Unlike other lipoproteins, HDL are not formed as mature lipoproteins, but appear in plasma as precursor particles. These particles have a discoidal shape and consist of bilayers of phospholipids and unesterified cholesterol that are stabilized by apo [13]. HDL exists as several subpopulations that differ in size (in order of decreasing size: HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, HDL_{3c}), density (HDL₂ $d=1.063-1.125$ g/ml and HDL₃ $d=1.125-1.21$ g/ml), charge (α - versus pre β -migrating HDL), lipid composition and apo content (Lp A-I containing apo A-I but not apo A-II and Lp A-I: A-II containing both apo A-I and apo A-II), as well as in their capacity to accept cellular cholesterol [14]. These subpopulations undergo remodeling and interconversion by the addition and removal of their neutral lipids, phospholipids, and apo components due to the action of several proteins. Enzymes involved in HDL metabolism are lecithin: cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), hepatic, endothelial and lipoprotein lipase and phospholipid transfer protein (PLTP). In type 2 diabetes mellitus, the action of proteins involved in HDL metabolism and the receptor-mediated uptake or efflux of free cholesterol / cholesterol esters towards/from HDL is modified, leading to altered HDL remodeling and low HDL cholesterol levels. An overview of how the regulation of proteins and receptors involved in HDL metabolism is changed under type 2 diabetes mellitus is given in the subsequent paragraph and in Table 1.

3. IMPACT OF TYPE 2 DIABETES MELLITUS ON HDL METABOLISM

3.1. Impact of Type 2 Diabetes Mellitus on ABCA1/ABCG1 Expression

The ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) are known regulators of macrophage cholesterol

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Table 1. Impact of Diabetes Mellitus on High-Density Lipoprotein Metabolism

Receptor/Enzyme	Effect	Diabetes Mellitus-Associated Factor
ABCA1 hepatocytes / macrophages	↓ mRNA and destabilization unchanged mRNA expression impaired ABCA1-dependent cholesterol removal ↓ mRNA	unsaturated FA [23] insulin, glucose, saturated FA [23] AGE [27] inflammation [28]
ABCG1 macrophage	↓ mRNA and function	glucose [21, 22]
SR-BI macrophages peripheral cells liver	↑ mRNA + shift towards cholesterol influx inhibit cholesterol efflux to HDL block SR-BI and depress plasma clearance of HDL	not specified [29] AGE [30] AOPP [32]
CETP	↑ CE transfer unchanged activity	triglycerides [43] not specified [44-47]
LCAT	↓ activity	AGE [49]
LPL Muscle	↓ activity	triglycerides [53]
EL	↑ serum levels	not specified [58]
HL	↑ mRNA ↓ activity	glucose [59] adiponectin [61]
PLTP	↑ activity	not specified [66]

homeostasis and are key players in reverse cholesterol transport [15]. The importance of ABCA1 in reverse cholesterol transport and HDL metabolism follows from the pathogenesis of Tangier disease, a disorder caused by mutations in the *ABCA1* gene and characterized by severely reduced HDL levels [16]. ABCA1 and ABCG1 are both expressed in multiple tissues including lung, heart, and spleen, and their expression is upregulated in macrophages upon lipid loading [17,18]. Whereas ABCA1 is responsible for cholesterol and phospholipid efflux towards lipid-poor or lipid-free apo A-I [19], ABCG1 mediates cholesterol and phospholipid efflux to mature HDL [20]. Although the role of ABCA1 and ABCG1 in macrophage foam cell formation and the development of atherosclerosis is well documented, little is known about how ABCA1- and ABCG1-mediated cholesterol metabolism is altered in the setting of type 2 diabetes mellitus. Mauldin *et al.* [21] could demonstrate that macrophage ABCG1 expression and function are decreased in mouse models of type 2 diabetes mellitus and that the elevated glucose environment was partially responsible for this reduction in ABCG1 in diabetic macrophages. Recently, the same investigators showed that ABCG1 expression is decreased in macrophages isolated from patients with type 2 diabetes mellitus, leading to decreased cholesterol efflux to HDL and resulting in increased macrophage cholesterol ester content [22]. More studies have been focused on investigating the regulation of ABCA1 expression and ABCA1-dependent cholesterol removal. *ABCA1* gene expression is severely decreased in the liver and peritoneal macrophages of diabetic mice compared with euglycemic control mice, a deficit which can be restored by treatment with insulin. Incubation of cultivated HepG2 hepatocytes and RAW264.7 macrophages with unsaturated fatty acids, but not with insulin, glucose, saturated fatty acids, or hydroxybutyrate, downregulates ABCA1 mRNA and protein [23]. Moreover, unsaturated fatty acids

have been shown to phosphorylate and destabilize ABCA1 through a phospholipase D2 pathway [24]. As the functional consequence, unsaturated fatty acids inhibit cholesterol efflux from macrophages which may contribute to low HDL cholesterol and increased cardiovascular risk of diabetic patients.

Advanced glycation end products (AGEs), which can be formed via metabolic and oxidative pathways, are elevated in diabetes [25,26]. AGEs have been implicated in the pathogenesis of diabetic vascular disease [25,26]. AGE precursors impair ABCA1-dependent cholesterol removal from cells [27]. In addition, AGEs have also been detected on lipoproteins isolated from diabetic subjects [24], raising the possibility that carbonyl modifications of apo A-I contribute to abnormal HDL metabolism. Finally, a recent study demonstrated that inflammation retards numerous components of reverse cholesterol transport and HDL efflux function, independent of HDL cholesterol levels *in vivo* [28]. Particularly cholesterol flux through liver to bile and feces is impaired by inflammation, which is consistent with inflammation-induced downregulation of hepatic expression of ABCG5, ABCG8, and ABCB11 biliary transporters. *In vitro*, lipopolysaccharide impaired [³H]-cholesterol efflux from human macrophages to apo A-I and serum coincides with reduced expression of the cholesterol transporter ABCA1.

3.2. Impact of Type 2 Diabetes Mellitus on Scavenger Receptor Class B Type I Expression

Diabetes mellitus stimulates the expression of scavenger receptor class B type I (SR-BI) in macrophages and leads to a shift in its activity from HDL-mediated cholesterol efflux to HDL-mediated cholesterol influx, which may lead to increased foam cell formation and atherosclerosis development [29]. Ohgami *et al.* [30] previously demonstrated that AGE proteins, as ligands for SR-BI,

effectively inhibit both SR-BI-mediated selective uptake of HDL cholesterol esters and cholesterol efflux from peripheral cells to HDL. This takes place without having an effect on HDL binding to SR-BI, indicating that the binding domain(s) of AGE proteins and HDL are not identical. Furthermore, it has been recently shown that the affinity of AGE proteins to SR-BI strictly depends on the extent of modification by AGEs [31]. Only highly modified AGE-albumin is significantly recognized by SR-BI, whereas mildly, more physiological modified AGE-albumin shows no ligand activity. This is in contrast to advanced oxidation protein products (AOPPs), which are carried by oxidized plasma proteins, especially albumin, and accumulate in subjects with renal disease, coronary artery disease, and diabetes mellitus. Competition studies clearly showed that the SR-BI binding domain(s) of *in vitro*-generated AOPP-albumin, but not AGE-albumin, are identical and overlap with binding domains for native HDL [32]. AOPPs effectively block SR-BI and depress plasma clearance of HDL cholesterol and may contribute to the abnormal composition of HDL and the high cardiovascular risk observed in patients with chronic renal failure. It has recently been demonstrated that a decreased clearance of HDL, as shown in SR-BI-deficient mice, renders HDL dysfunctional and pro-inflammatory [33]. Moreover, hepatic expression of SR-BI has been shown to be a positive regulator of cholesterol efflux from macrophages [34], i.e. mice overexpressing SR-BI in the liver had markedly reduced plasma HDL cholesterol levels and yet considerably increased macrophage-derived [³H]-sterol excretion in the feces. Conversely, mice lacking SR-BI had markedly increased plasma levels of HDL cholesterol and yet greatly reduced macrophage-derived [³H]-sterol excretion in the feces. Hence, blockade of SR-BI (by AOPP-albumin), might directly contribute to foam cell formation in the arterial wall. These experiments directly indicate that the rate of macrophage reverse cholesterol transport is not simply a function of steady-state plasma concentrations of HDL cholesterol and cannot be predicted simply based on measurement of plasma HDL cholesterol.

Moreover, it has been recently shown that phosphatidylinositol-3-kinase (PI3K) regulates SR-BI subcellular localization and selective lipid uptake in hepatocytes [35]. SR-BI-dependent HDL selective cholesterol ester uptake in human HepG2 hepatoma cells was decreased (approximately 50%) by the PI3K inhibitors wortmannin and LY294002, whereas under conditions of PI3K activation by insulin, and to a lesser extent by the SR-BI ligand HDL, cell surface expression of SR-BI is promoted, resulting in increased SR-BI-mediated HDL selective lipid uptake. This implies that decreased hepatocyte PI3K activity in insulin-resistant states [36], such as type 2 diabetes, obesity, or metabolic syndrome, may impair reverse cholesterol transport by reducing cell surface expression of SR-BI.

However, one should be careful in extrapolating the SR-BI findings from mice to humans since there is a big difference in HDL-mediated cholesterol ester uptake *via* SR-BI between both species. In mice, HDL-mediated cholesterol ester uptake is the major pathway in reverse cholesterol transport [37], whereas in humans it is limited, accounting for only 5% of the total reverse cholesterol transport [38,39], whereas CETP plays an important role in reverse cholesterol transport in humans. For detailed reviewing of differences in reverse cholesterol transport between species, refer to Van Craeyveld *et al.* [40].

Recently, the importance of SR-BI expression in adipose tissue for cholesterol homeostasis has been demonstrated [41]. Insulin injection in wild-type mice induced a decrease in circulating HDL and was associated with the translocation of SR-BI from intracellular sites to the plasma membrane of adipose tissue. Refeeding upregulated adipose HDL selective cholesterol esters uptake and SR-BI proteins through transcriptional and post-transcriptional mechanisms. This occurred along with a decrease in serum HDL and an increase in adipose cholesterol content. These results sug-

gest that adipose tissue SR-BI translocation might be a link between adipose tissue lipid storage and HDL clearance. Furthermore, the results suggest that in case of insulin resistance, the uptake of cholesterol into adipose tissue might be impaired leading to excessive cholesterol in non-adipose tissue.

Finally, a novel SR-BI inhibitor, ITX5061, a molecule initially characterized as a p38 MAPK inhibitor, has recently been shown to increase HDL cholesterol levels by 20% in a human population of hypertriglyceridemic subjects with low HDL levels and to increase also moderately apo A-I, without affecting very low-density lipoprotein (VLDL)/LDL cholesterol or plasma triglyceride concentrations [42]. In addition, ITX5061 decreased the fractional catabolic rate of HDL cholesterol esters and reduced its hepatic uptake. In LDL^{r/-} mice with or without CETP expression, ITX5061 treatment resulted in reductions of early atherosclerotic lesions in the aortic arch by 40%. Since increased HDL cholesterol levels do not *per se* imply improved reverse cholesterol transport, preclinical and clinical studies testing the potential of ITX5061 in the context of (type 2) diabetes mellitus are further requested.

3.3. Impact of Type 2 Diabetes Mellitus on Cholesterol Ester Transfer Protein

CETP action results in transfer of cholesterol esters from HDL towards triglyceride-rich lipoproteins, and reciprocal transfer of triglycerides from triglyceride-rich lipoproteins to HDL. Elevated triglycerides in insulin resistance and type 2 diabetes mellitus increase the cholesterol ester transfer process out of HDL, leading to cholesterol ester depletion and triglyceride enrichment of HDL and subsequent increased HDL catabolism [43]. Increased plasma cholesterol ester transfer is therefore considered to be a determinant of low HDL cholesterol. In general, there is no difference in CETP activity between type 2 diabetic and non-diabetic subjects [44-46] and between insulin-sensitive and insulin-resistant subjects [47].

3.4. Impact of Type 2 Diabetes Mellitus on Lecithin: Cholesterol Acyltransferase

LCAT transfers the sn-2-acyl group of lecithin to cholesterol, which requires the presence of apo A-I as its coactivator. LCAT activity therefore results in the generation of cholesterol esters, which are retained in the core of HDL particles, leading to the conversion of discoidal nascent HDL into spherical particles. Glycated hemoglobin levels negatively correlate with LCAT activity in type 2 diabetes [48]. Indeed, glycation of apo A-I alters the conformation of apo A-I in regions critical for LCAT activation and reduces LCAT activity in proportion to the extent of apo A-I glycation [49]. LCAT activity seems to be increased in type 2 diabetic patients, but this difference disappears after controlling for triglyceridemia [50].

3.5. Impact of Type 2 Diabetes Mellitus on Lipoprotein Lipase

Lipoprotein lipase (LPL) is the rate-limiting enzyme for the hydrolysis of the triglyceride core of triglyceride-rich lipoproteins, chylomicrons and VLDL, producing chylomicron remnants and intermediate low-density lipoprotein (IDL), respectively [51]. LPL is also important in HDL metabolism by contributing to the transfer of surface lipids after lipolysis to small HDL. It is a multifunctional enzyme produced by many tissues including adipose tissue, macrophages, pancreatic islets, and cardiac and skeletal muscle. Plasma LPL activity has been reported to be decreased in insulin-resistant subjects without diabetes [52]. In skeletal muscle, LPL activity is negatively related to plasma triglycerides and to the degree of insulin resistance [53].

3.6. Impact of Type 2 Diabetes Mellitus on Endothelial Lipase

Endothelial lipase (EL) is 44% identical to lipoprotein lipase and 41% identical to hepatic lipase. In contrast to lipoprotein lipase and hepatic lipase, which preferentially hydrolyze triglyceride-rich

lipoproteins, EL acts primarily as phospholipase and is more active against HDL [54]. The importance of endothelial lipase in HDL metabolism follows from experimental studies demonstrating that hepatic overexpression of EL after adenoviral gene transfer results in decreased HDL cholesterol and phospholipid levels [55] and transgenic overexpression of EL under control of an endogenous promoter results in moderately reduced HDL cholesterol levels [56]. In contrast, antibody inhibition of mouse EL activity results in significant increased HDL cholesterol and phospholipid levels [57].

Recently, it has been demonstrated that serum EL concentrations are increased in type 2 diabetic patients and associated with the degree of subclinical inflammation [58]. Insulin therapy could reduce EL levels in diabetic patients. At present, further studies are required to determine the impact of changes in EL on lipoprotein metabolism in diabetic patients.

3.7. Impact of Type 2 Diabetes Mellitus on Hepatic Lipase

Hepatic lipase (HL) reduces HDL particle size by hydrolysing its triglycerides and phospholipids. A decreased post-heparin plasma LPL/HL ratio is a determinant of low HDL₂ cholesterol in insulin resistance. Glucose has been shown to increase HL expression in HepG2 liver cells [59]. HL hepatocyte mRNA, mass, and plasma enzymatic activity increase concomitantly with the induction of an insulin-resistant state [60]. Moreover, there exists a significant inverse association between adiponectin - with hypo-adiponectinemia known to be associated with insulin resistance, diabetes, and obesity - and post-heparin plasma HL activity that is independent of other factors such as markers of insulin resistance or inflammation [61]. Therefore, adiponectin, rather than insulin, may represent an important factor contributing to the regulation of HL activity in both non-diabetic individuals and patients with type 2 diabetes mellitus. The effect of adiponectin on HL activity may also help to explain the HDL cholesterol-elevating action of adiponectin. The increased HL activity results in remnant HDL (HDL enriched with triglycerides followed by HL hydrolysis). Recently, Xiao *et al.* [62] demonstrated that remodeling of triglyceride-enriched HDL by HL results in enhanced binding, internalization, and degradation in tissues involved in HDL catabolism, contributing to rapid clearance and overall lowering of plasma HDL cholesterol in insulin resistance and hypertriglyceridemia. Interestingly, the increased binding of remnant HDL is hereby not mediated by the LDL receptor nor by SR-BI, because enhanced remnant HDL binding was observed in LDL receptor-deficient cells with or without SR-BI overexpression. Disruption of cell surface heparan sulfate proteoglycans or blockade of apolipoprotein E-mediated lipoprotein binding also did not abolish the enhanced remnant HDL binding.

3.8. Impact of Type 2 Diabetes Mellitus on Phospholipid Transfer Protein

PLTP enhances the transfer of phospholipids from VLDL to HDL particles. In addition, PLTP promotes the generation of small pre-β-HDL particles that are initial acceptors of cell-derived cholesterol [63]. Its activity in plasma is elevated in insulin resistance and type 2 diabetes mellitus in association with high plasma triglycerides and obesity. Despite the stimulatory effect of pre-β-HDL particles on cellular cholesterol efflux, a positive relationship has been documented between pre-β-HDL concentrations and atherosclerosis in type 2 diabetic patients [64]. This suggests that elevated levels of pre-β-HDL indicate an impaired maturation of HDL and that decreased levels would reflect a faster metabolism of such particles into mature α-HDL, contributing to an efficient reverse cholesterol transport [65]. Recently, Dallinga-Thie *et al.* [66] demonstrated a substantial decrease in pre-β-HDL formation by high dose atorvastatin treatment in type 2 diabetic patients. Univariate regression analysis demonstrated that the changes in pre-β-HDL formation were related to the changes in plasma PLTP activity and triglycerides.

In summary, the decrease in HDL cholesterol levels in type 2 diabetes mellitus is the consequence of different simultaneous actions (Fig. 1):

- 1) due to the increase in circulating free fatty acids (FFAs), triglyceride synthesis is increased in the liver, leading to higher VLDL production and subsequently more triglyceride transfer towards HDL in exchange for cholesterol esters *via* CETP. This leads to more triglyceride-enriched HDL and subsequent increased HDL catabolism due to hydrolysis *via* hepatic lipase and endothelial lipase, which are even more expressed under diabetes mellitus;
- 2) due to decreased hepatic ABCA1 expression, and consequently less lipidation and generation of pre-β-HDL, apo A-I, the main protein component of HDL is fast catabolised in the kidney, resulting in less HDL synthesis;
- 3) due to reduced expression of ABCA1 in macrophages, less cholesterol efflux towards pre-β-HDL takes place. In addition, LCAT activity is decreased, resulting in less formation of mature HDL. The efflux of cholesterol from macrophages towards HDL is also impaired *via* reduced ABCG1 expression. Increased expression of SR-BI in macrophages, accompanied with a shift from HDL-mediated cholesterol efflux to cholesterol influx further blocks reverse cholesterol transport.

4. IMPACT OF DIABETES MELLITUS ON THE FUNCTIONALITY OF HDL

The functionality of HDL depends on the shape of HDL (spherical > discoidal [67]), the size and the composition of HDL (fatty acid composition/triglyceride content [68-71], glycated proteins, peroxidized proteins). Whereas the previous paragraph summarized alterations in receptor and protein expression involved in HDL metabolism, influencing HDL remodeling (shape, size and composition (fatty acid composition/triglyceride content)) and consequently HDL functionality, the present paragraph is directed at giving an overview of how the compounds of HDL (apo A-I) or enzymes associated with HDL (paraoxonase (PON)) are structurally, post-translationally influenced, how HDL can be enriched with proteins under diabetes mellitus, and how this affects HDL functionality. Recent studies directed at investigating the HDL proteome by protein chips and mass spectrometry, have brought new insights in the complexity of HDL composition. However, HDL proteomics is still in its infancy and preliminary findings will need to be confirmed with standardized approaches in larger clinical samples. Davidsson *et al.* [72] recently analyzed HDL from controls and patients included in the cohort of the Atherosclerosis and Insulin Resistance Study by protein chips. Patients fulfilled the National Cholesterol Educational Panel definition of the metabolic syndrome and were shown to have peripheral atherosclerosis by ultrasound evaluation of carotid and femoral intima-media thickness [73]. They could demonstrate a significant higher content of serum amyloid A in HDL from patients as from controls. Serum amyloid A is known to decrease the affinity of HDL towards hepatocytes, whereas it increases the affinity for macrophages by 3- to 4-fold. This difference in affinity may account for the clearance of HDL to be redirected from the liver towards macrophages, a process, that has been proposed to shift the anti-atherogenic, anti-inflammatory nature of HDL to a pro-atherogenic one [74,75]. In addition, serum amyloid A has been shown to reduce the capacity of HDL subclasses to promote cholesterol efflux [76]. Perségl *et al.* [70] demonstrated that the ability of HDL from type 2 diabetes mellitus patients to counteract the inhibited endothelium-dependent vasorelaxation by oxidized LDL was impaired and inversely correlated with HDL triglyceride content. The same authors further demonstrated that HDL from type 1 diabetes mellitus patients did not protect against the oxidized LDL-induced inhibition of endothelium-dependent vasorelaxation. This defect could not be explained by abnormalities in HDL composition, size, or para-

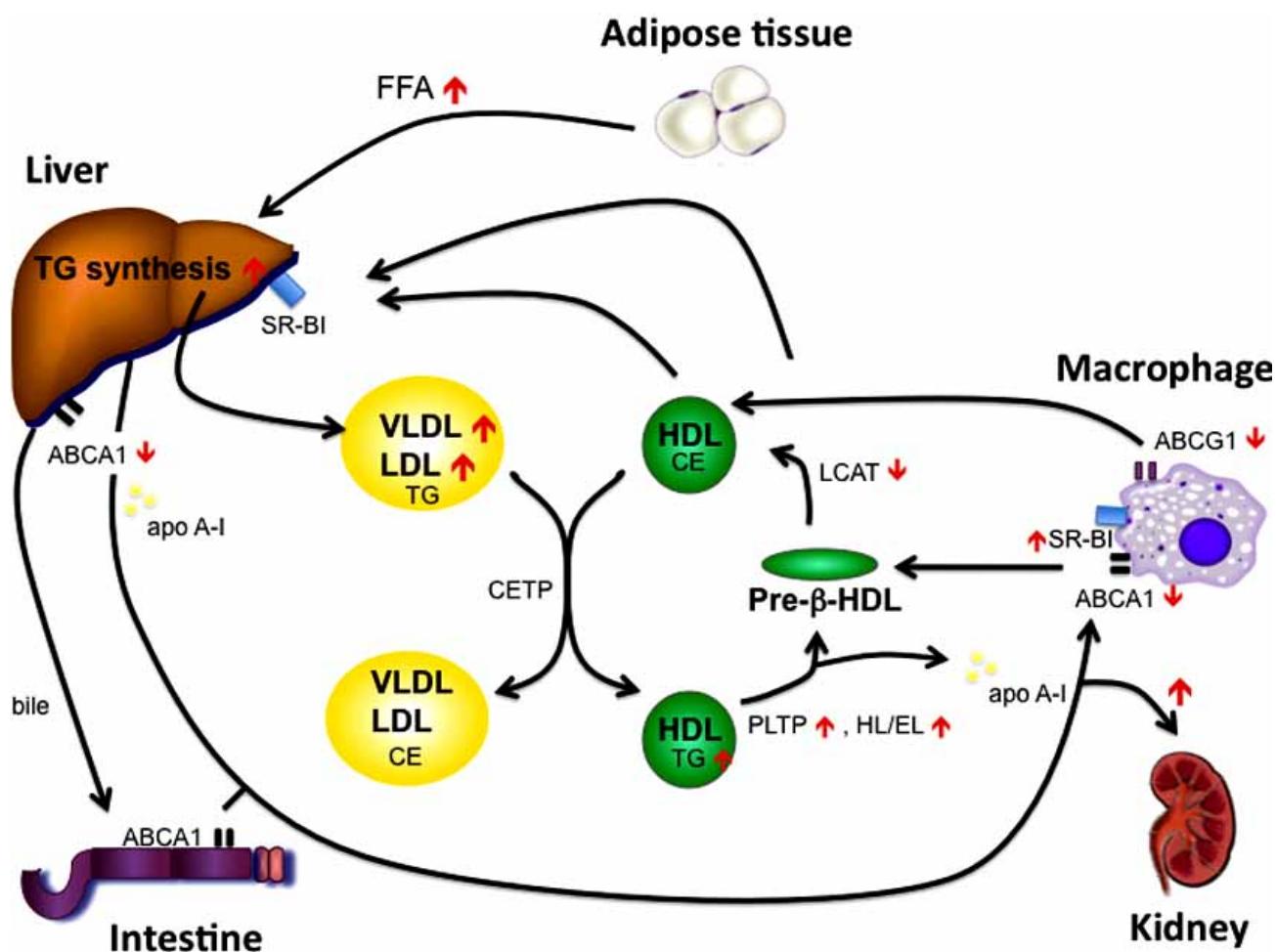


Fig. (1). Altered HDL metabolism under type 2 diabetes mellitus. The major routes of HDL-mediated reverse cholesterol transport from macrophages towards the liver are represented (drawings are not in scale). Changes in regulation or expression are marked by red arrows.

xonase activity [77]. In addition, Sorrentino *et al.* [78] recently demonstrated that the endothelial-vasoprotective effects HDL are impaired in type 2 diabetic patients. The loss in beneficial effect of HDL on vasorelaxation and endothelial NO production could be explained by the observed lipid peroxidation and increased myeloperoxidase (MPO) activity of HDL from diabetic patients. MPO has been shown to modify HDL and its capacity to promote cholesterol efflux from macrophages [79]. Moreover, MPO levels are positively associated with endothelial dysfunction [80] and predict the risk of coronary disease [81].

4.1. Impact of Diabetes Mellitus on Paraoxonase Activity

HDL modulates LDL and cell membrane oxidation through the action of PON1, which is one of the major mechanisms by which HDL is anti-atherogenic. Hedrick *et al.* [82] could demonstrate that incubation of HDL under hyperglycaemic conditions resulted in glycated HDL and was associated with a 65% reduction in PON enzymatic activity. Glycated HDL lost the ability to inhibit monocyte adhesion to human aortic endothelial cells in response to oxidized LDL *in vitro*. HL-mediated non-esterified fatty acid release from HDL lipids was enhanced in glycated HDL compared with control HDL. The same authors also documented a 40% reduction in PON activity in patients with type 2 diabetes mellitus and documented coronary artery disease compared with non-diabetic subjects. Recently, Mastorikou *et al.* [83] found that HDL from type 2 diabetic patients without coronary heart disease had decreased ability to metabolize membrane lipid hydroperoxides. They further showed that *in vitro* glycation of PON1 reduced its

ability to metabolize membrane hydroperoxides by 50%, whereas glycation reduced it by 80%. Ferretti *et al.* [84] demonstrated that HDL from type 1 diabetic patients showed higher levels of lipid hydroperoxides and a lower activity of HDL-PON than healthy subjects. Moreover, HDL of type 1 diabetic patients protected erythrocyte membranes less efficiently against oxidative damage compared with HDL from healthy subjects. A negative correlation was found between HDL-PON activity and the levels of hydroperoxides of HDL, confirming the finding that PON not only reduces LDL and membrane oxidation, but also decreases HDL oxidation [85], suggesting that subjects with low PON activity are more exposed to oxidative damage than subjects with high PON activity.

In summary, it might be hypothesized that the decrease of PON activity in diabetic patients and the lower HDL protective action against membrane peroxidation and LDL oxidation could contribute to acceleration of atherosclerosis in diabetes mellitus.

4.2. Impact of Diabetes Mellitus on Apolipoprotein A-I and Lecithin: Cholesterol Acyltransferase

Hyperglycaemia results in non-enzymatic glycation of plasma proteins, including apo A-I, the most abundant apo in HDL. Glycation of apo A-I alters the conformation of apo A-I in regions critical for LCAT activation and lipid binding and also decreases apo A-I discoidal reconstituted HDL size and surface charge. The rate of LCAT-mediated cholesterol esterification in apo A-I discoidal reconstituted HDL progressively decreases as the extent of apo A-I glycation increases [49]. Importantly, glycation

inhibitors aminoguanidine and pyridoxamine, as well as the insulin sensitiser metformin and the cross-link breaker alagebrium can all decrease the methylglyoxal-mediated glycation of apo A-I in discoidal reconstituted HDL and conserve the ability of the particles to act as substrates for LCAT. However, neither aminoguanidine, pyridoxamine, nor alagebrium could reverse the glycation of apo A-I or restore its ability to activate LCAT [86]. Type 2 diabetes mellitus is associated with increased MPO activity in HDL [78]. Zheng *et al.* [79] demonstrated that apo A-I is a selective target for MPO-catalyzed nitration and chlorination and that MPO-catalyzed oxidation of HDL and apo A-I results in selective inhibition of ABCA1-dependent cholesterol efflux. Moreover, minimally oxidized LDL, which is increased in type 2 diabetes mellitus [87] has been shown to be a potent inhibitor of LCAT activity [88]. Besides its importance in reverse cholesterol transport, LCAT has been shown to hydrolyze platelet-activated factor (PAF) [89] and truncated phosphatidylcholines generated during lipoprotein oxidation, resulting in the abolishment of their pro-inflammatory and cytotoxic effects. In addition to its own anti-oxidative properties, LCAT has an impact on the concentration of the anti-oxidative enzymes PON1 and PAF acetylhydrolase [90]. This suggests that reduction of LCAT activity under diabetes mellitus may not only affect reverse cholesterol transport, but also lead to a decrease in the anti-oxidative capacity of HDL.

5. IMPACT OF HDL ON GLUCOSE HOMEOSTASIS

Insulin resistance turns out into type 2 diabetes mellitus when pancreatic β -cells do not produce enough insulin to overcome insulin resistance. Among others, glucose, free fatty acids and inflammatory cytokines derived from adipose tissue (e.g. tumor necrosis factor-alpha (TNF- α) or the innate immune system (e.g. interleukin-1beta (IL-1 β)) contribute to β -cell dysfunction and apoptosis [91]. Accumulating evidence indicates that also low HDL cholesterol, high LDL cholesterol and hypertriglyceridemia, previously only seen as early symptoms of insulin resistance, which may later progress to diabetes mellitus, can actively contribute to β -cell failure and thus to the manifestation of diabetes mellitus [92,93]. Roehrich *et al.* [92] were the first to demonstrate that β -cells express lipoprotein receptors, including SR-BI, LDLR, VLDLR and apoER2 and can bind and internalize VLDL, LDL, and HDL. In addition, they showed that human VLDL and LDL particles reduced *insulin* mRNA levels and β -cell proliferation and induced a dose-dependent increase in the rate of apoptosis. Islets from mice lacking the LDL receptor showed a dramatic decrease in LDL uptake and were partially resistant to apoptosis caused by LDL. The pro-apoptotic signaling of LDL and VLDL was antagonized by HDL particles (Fig. 2), which was mediated, in part, by inhibition of caspase-3 cleavage and activation of Akt/protein kinase B. Rutti *et al.* [93] recently further demonstrated that both the delipidated protein or the deproteinated lipid moieties of HDL, apo A-I, or S1P, could inhibit IL-1 β -induced β -cell apoptosis. ABCA1 has been shown to modulate insulin secretion [94] and HDL can reverse the deleterious effects of oxidized LDL on insulin secretion (Fig. 2) [95]. Besides direct insulin-dependent mechanisms in the pancreas, HDL can also modulate plasma glucose through insulin-independent mechanisms, i.e. HDL may increase glucose disposal through direct effects in skeletal muscle, the major site of glucose disposal in the body. Han *et al.* [96] demonstrated that apo A-I was able to stimulate the phosphorylation of the key metabolic regulatory enzyme AMPK and elevated glucose uptake in C2C12 myocytes (Fig. 2). AMPK is regulated by nutritional status, exercise, and adipokines and plays a pivotal role in skeletal muscle glucose disposal. In apo A-I $^{-/-}$ mice, AMPK phosphorylation was reduced in skeletal muscle and liver, and expression of gluconeogenic enzymes was increased in liver. In addition, the apo A-I $^{-/-}$ mice had increased fat content and compromised glucose tolerance, indicating that apo A-I has a protective effect against diabetes *via* activation of AMPK. Recently, Drew *et al.* [97]

demonstrated that intravenous reconstituted HDL reduced plasma glucose in patients with type 2 diabetes mellitus by increasing plasma insulin and activating AMPK in skeletal muscle (Fig. 2). Furthermore, both HDL and apo A-I increased glucose uptake in primary human skeletal muscle cell cultures established from patients with type 2 diabetes mellitus. Recently, Rapizzi *et al.* [98] demonstrated that the HDL-associated sphingolipid S1P can increase glucose uptake in skeletal muscle through transactivation of the insulin receptor. As shortly outlined above, inflammatory cytokines, including TNF- α , IL-6, and leptin, derived from adipose tissue contribute to β -cell dysfunction. Previous studies have shown that infiltration of macrophages, the major source of TNF- α , is of critical importance in adipose tissue inflammation and the development of insulin resistance [99]. Besides the pro-inflammatory adipokines TNF- α and leptin, adipose tissue also expresses an adipocyte-derived cytokine with anti-inflammatory, anti-diabetic features, namely adiponectin. Adiponectin stimulates glucose utilization and fatty acid oxidation by activating AMPK and enhances hepatic insulin action (Fig. 2) [100-102]. Decreased serum levels of adiponectin are an independent risk factor for the progression of type 2 diabetes in a Japanese population [103]. Adiponectin, but not inflammatory markers such as C-reactive protein and IL-6, has been shown to be significantly related to the development of type 2 diabetes in Pima Indians [104] and to be associated with the metabolic syndrome stronger than any other inflammatory marker [105]. Prospective and longitudinal studies [106,107] have demonstrated that lower adiponectin levels are associated with a higher incidence of diabetes. Intriguingly, accumulating evidence from epidemiological studies [108-114] and intervention studies [115,116] indicates a positive correlation between adiponectin and HDL cholesterol. The impact of adipose tissue metabolism on HDL cholesterol levels is well established [108-111,113,115,117,118]. Recently, a direct role of adiponectin on HDL catabolism has been suggested by the negative correlation between adiponectin and the fractional catabolic rate of apo A-I, which occurred independently of obesity, insulin resistance, and the content of triglycerides in HDL particles [112]. Van Linthout *et al.* [119] recently demonstrated that HDL can reciprocally increase adiponectin expression in a PI3K-dependent way. This suggests that HDL might indirectly - *via* increasing adiponectin expression - regulate glucose homeostasis. Peterson *et al.* [120] demonstrated that treatment with the apo A-I mimetic peptide L-4F increased serum adiponectin levels and decreased IL-1 β and IL-6 levels in obese mice, which was paralleled with improved insulin sensitivity and improved glucose tolerance. Moreover, in a further study they showed that L-4F decreased both subcutaneous and visceral adipose tissue, reduced hepatic lipid content, and increased the presence of insulin-sensitive adipocytes (Fig. 2) [121]. Finally, we could demonstrate that apo A-I gene transfer resulted in a decrease in cardiac glycogen content in an experimental model of diabetic cardiopathy, potentially *via* an Akt-glycogen synthase kinase (GSK)-3 β dependent pathway (Fig. 2) [122]. Whether HDL can influence cardiac glucose metabolism, involving AMPK, is under current investigation in an experimental model of the metabolic syndrome.

6. DIABETES MELLITUS AND CARDIOVASCULAR COMPLICATIONS

Diabetes mellitus has been shown to be an independent risk factor for cardiovascular disease by a large body of epidemiological and pathological data. Endothelial dysfunction plays a critical role in the pathogenesis of diabetic macro/micro-angiopathy [123], which is the most common cause of mortality and morbidity in diabetic patients. Dysfunction of the endothelium is an early phase of atherosclerosis, without significant morphological changes of the vessel wall, mainly associated with an abnormality of the physiology of nitric oxide (NO) [124] and subsequent alteration in endothelium-dependent relaxation [124,125]. Both diabetes

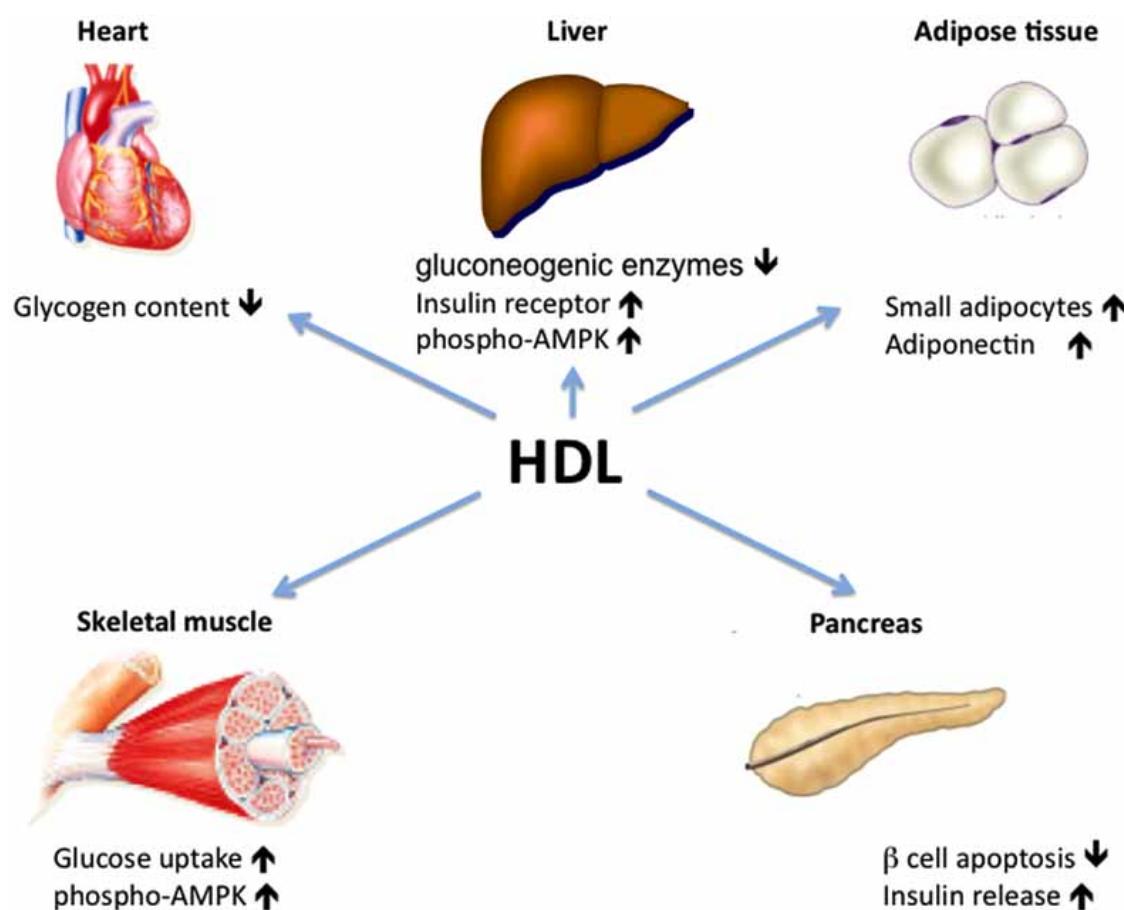


Fig. (2). Effect of HDL on glucose homeostasis. HDL decreases glucose levels *via* insulin-dependent and -independent mechanisms. In the liver, HDL decreases the expression of gluconeogenic enzymes increases insulin receptor phosphorylation and AMPK phosphorylation. In adipose tissue, HDL increases the amount of small, i.e. insulin-sensitive adipocytes and the expression of the insulin-sensitizing agent: adiponectin. In the pancreas, HDL decreases β -cell apoptosis induced by (oxidized) LDL, IL-1 β , TNF- α . In the skeletal muscle, HDL increases glucose uptake involving increased phosphorylation of AMPK. In the heart, HDL decreases glycogen content involving an Akt-GSK3 β -axis.

mellitus-associated hyperglycemia and increased angiotensin (Ang) II levels [126] induce reactive oxygen species (ROS), which contribute to endothelial dysfunction, partly *via* oxidative degradation of NO. NO has important anti-inflammatory, anti-apoptotic and anti-thrombotic properties leading to the assumption that impaired endothelium-dependent vasodilation also reflects the alteration of other important functions of the endothelium. Numerous studies have shown that activation of the Ang II type 1 receptor (AT1R) contributes to the induction of oxidative stress and apoptosis of vascular cells, thus promoting the initiation and progression of endothelial dysfunction [127]. The expression levels of the AT1R define the biological efficacy of Ang II and have been shown to be regulated by several agonists such as Ang II, glucose, insulin, ROS, LDL and many others [128,129] including diabetes mellitus [125,130]. The importance of the enhanced vascular AT1R expression and Ang II-mediated signaling in diabetes mellitus-associated endothelial dysfunction follows from the finding that AT1R antagonism in diabetes mellitus improves endothelial function [131,132]. Not only neighboring mature endothelial cells are responsible for the repair of denuded vessel walls, but also circulating endothelial progenitor cells (EPCs) take part in the re-endothelialization at sites of endothelial injury [133-136]. Consequently, the reduction in number [137] and function [137,138] of EPCs under diabetes mellitus might be involved in the diabetes mellitus-associated endothelial dysfunction [139].

Diabetes mellitus is associated with diabetic cardiopathy, a primary myocardial injury, which occurs in the absence of hyper-

tension and coronary macroangiopathy. Diabetic cardiopathy is associated with impaired cardiac ventricle function due to cardiomyocyte hypertrophy, a change of myocardial extracellular matrix (interstitial and perivascular fibrosis), intramyocardial microangiopathy, interstitial inflammation, abnormal intracellular Ca^{2+} -handling, cardiac glycogen accumulation, endothelial dysfunction, and cardiomyocyte apoptosis [122,140-144]. The incidence of apoptosis increases in the heart of diabetic patients [145] and streptozotocin (STZ)-induced diabetic animals [146] and is directly linked to hyperglycemia-induced oxidative stress [146]. Hyperglycemia induces oxidative stress by inducing the generation of ROS on the one hand and reducing the production of anti-oxidant enzymes on the other hand [147]. Besides inducing lipid peroxidation, ROS can alter cellular proteins and initiate diverse stress-signaling pathways like Erk, JNK, and p38 MAPK. Activation of cardiac p38 MAPK is of pathological importance in the diabetic heart indicated by the finding that p38 MAPK inhibition improves left ventricular dysfunction in STZ-induced diabetic mice [148]. The importance of the anti-oxidant enzymes superoxide dismutase (SOD), SOD-1, SOD-2, and extracellular (ec)-SOD for the heart, which convert O_2^- anions into molecular oxygen and hydrogen peroxide, has been outlined in transgenic and knock-out animal models [149], and recently for SOD-2 in a diabetic setting [150]. In the heart, overexpression of ec-SOD decreases macrophage infiltration and fibrosis, and improves left ventricular dysfunction [149], whereas overexpression of SOD-2 protects mitochondrial respiratory function and blocks apoptosis induction [151].

7. IMPACT OF HDL ON DIABETES MELLITUS-ASSOCIATED CARDIOVASCULAR COMPLICATIONS

7.1. Impact of HDL on Diabetes-Associated Endothelial Dysfunction

Low plasma HDL is an independent predictor of endothelial dysfunction in healthy individuals and diabetic patients [152-154]. Recently, it has been shown that the endothelial-protective effects of HDL also include the potential to increase the number of circulating EPCs [155] and to accelerate endothelial regeneration [156]. A role for the HDL-associated sphingolipid S1P in the stimulation of the functional capacity of EPCs has hereby also been demonstrated [157]. In addition, studies have shown that infusions of recombinant and reconstituted HDL have modest effects on coronary plaque morphology and volume [158,159] and improve endothelial function in type 2 diabetes mellitus [160]. Kruger *et al.* [161] demonstrated that the apo A-I mimetic peptide D-4F induces decreased endothelial cell sloughing and improves vascular reactivity in a rat model of diabetes mellitus involving an induction in heme oxygenase-1 and extracellular superoxide dismutase expression. Whetzel *et al.* [162] demonstrated that S1P could prevent monocyte/endothelial interactions in experimental type 1 diabetic vascular endothelium involving activation of the S1P1 receptor. We could define the down-regulation of the AT1R as a novel additional vascular-protective effect of HDL. Apo A-I gene transfer in an experimental model of diabetes mellitus improved vascular relaxation, along with a down-regulation of aortic AT1R and NADPH oxidase activity and decreased eNOS uncoupling [163]. These findings were further corroborated *in vitro*, where we showed that the HDL-mediated down-regulation of the AT1R in human aortic endothelial cells was associated with a decrease in hyperglycemia-induced oxidative stress, and with a decrease in the hyperglycemia-induced responsiveness to Ang II. Recently, it has been demonstrated that HDL from diabetic patients loses its capacity to induce early EPC-mediated endothelial repair. Treatment with the HDL-raising drug extended-release niacin in type 2 diabetes mellitus patients increased not only HDL plasma levels but markedly improved endothelial-protective functions of HDL in these patients, including the promotion of EPC-mediated endothelial repair [78].

7.2. Impact of HDL on Diabetic Cardiopathy

Besides the well-known vasculoprotective effects [5,6,163-165], also cardioprotective effects [166-169] of HDL have been consistently demonstrated, be it to a lesser extent. A role for S1P in the HDL-mediated cardioprotective effects has been shown in models of ischemia/reperfusion [147] and myocardial infarction [170] and is extensively reviewed by Means and Brown [171]. In

the context of diabetes mellitus, we could demonstrate that apo A-I gene transfer reduced the development of experimental diabetic cardiopathy *via* reduction of cardiac oxidative stress, inflammation, fibrosis, apoptosis, and glycogen accumulation, leading to improved left ventricular function despite severe hyperglycemia and unaltered levels of LDL cholesterol [122]. The reduction in cardiac expression of the inflammation marker vascular cell adhesion molecule-1 (VCAM-1) in diabetic rats treated with apo A-I gene transfer compared to diabetic control rats is demonstrated by immunohistochemistry in Fig. 3. We could show that the cardioprotective effects of HDL were associated with a normalization of the diabetes-reduced phosphorylation/activation state of the protein kinase B Akt [172] and of its effector endothelial nitric oxide synthase (eNOS) to levels found in non-diabetic hearts. Immunofluorescence staining illustrated the presence of activated Akt in cardiomyocytes as well as in cardiac endothelial cells. Since Akt is a critical regulator of cell survival [173], these findings might have contributed to the reduction in cardiomyocyte apoptosis as well as in the improvement of endothelial integrity found in the hearts of the diabetic rats treated with apo A-I gene transfer. On ultrastructural level demonstrated by electron microscopy, the anti-apoptotic effects of apo A-I gene transfer were translated in a reduced number of cardiomyocytes with swollen mitochondria and apoptotic bodies. In parallel, apo A-I gene transfer led to an improved connected structure of the sarcomere (actin-myosin filaments), sharper intercalated discs, more intact endothelium and basement membrane, and less cardiac fibrosis and glycogen accumulation. These findings suggest that the combination of these effects on the different compartments of the heart (cardiomyocytes, cardiac endothelium, and extracellular matrix) contributed to the improved cardiac function found after apo A-I gene transfer compared to STZ-diabetic rats. HDL has previously been shown to affect matrix regulation [174]. How exactly HDL reduces cardiac fibrosis in the setting of diabetes mellitus needs further investigation.

Moreover, we confirmed the anti-apoptotic effect of HDL on cardiomyocytes under hyperglycemia *ex vivo*, showing that HDL supplementation on cardiomyocytes in hyperglycemia reduces apoptosis. In agreement with the increased phosphorylation state of Akt and eNOS in hearts of diabetic rats, which underwent apo A-I gene transfer, supporting a HDL-Akt-eNOS pathway, the anti-apoptotic effect of HDL was found to be PI3K- and NO-dependent. In addition, although HDL had no effect on the contractility of isolated cardiomyocytes under control conditions, HDL improved their function under hyperglycemia-induced stress in a PI3K- and NO-dependent manner. This suggests that besides beneficial vascular/cardiac-protective long-term effects, including an improvement of cardiac, vascular, and matrix remodeling, also direct

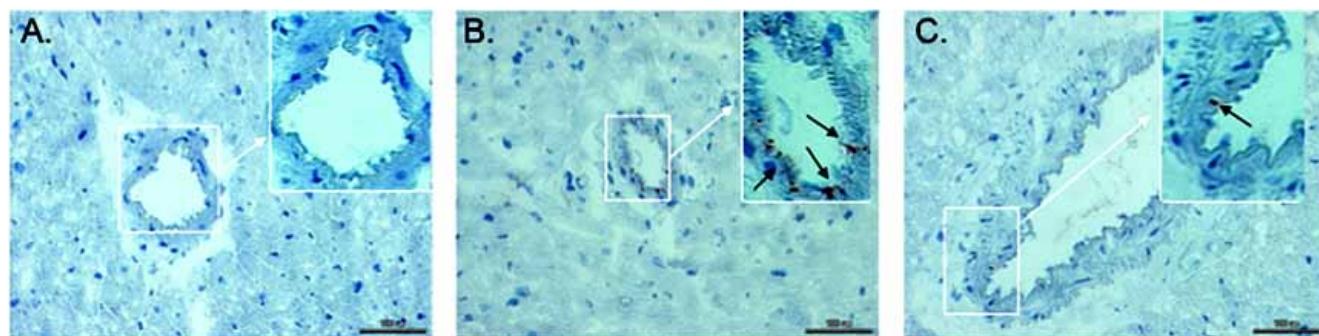


Fig. (3). Apolipoprotein A-I gene transfer reduces cardiac vascular cellular adhesion molecule-1 expression in an experimental model of diabetic cardiopathy. Panel A, B, and C: representative immunohistochemistry pictures of cardiac VCAM-1 stainings of control, streptozotocin-induced diabetic rats, and streptozotocin-induced diabetic rats, which underwent apo A-I gene transfer, respectively. Magnification 200x, insets magnification 400x.

myocardial effects of HDL may have contributed to the improvement of cardiac function under severe STZ-induced stress.

This study [122] performed in an animal model characterized by severe hyperglycemia, oxidative stress, and an HDL cholesterol to LDL cholesterol ratio of 1, strongly suggests that HDL has direct cardioprotective effects. However, the relevance of the use of HDL-raising therapies for the co-treatment of diabetic cardiomyopathy urges for future studies investigating also the effect of increasing HDL on established diabetic cardiomyopathy as well as studying the effect in type 2 diabetes mellitus.

CONCLUSION

Type 2 diabetes mellitus has got a prone impact on HDL metabolism leading to low HDL cholesterol levels. In addition, diabetes mellitus leads to changes in HDL size, shape, and composition, the latter partly due to glycation and peroxidation, resulting in impaired HDL functionality. Among others, reverse cholesterol transport is impaired and HDL loses its anti-oxidative and endothelial-vasoprotective capacity. The emerging notion that HDL quality has more predictive power than HDL quantity stimulates for further exploration of the HDL proteome. Moreover, it underscores that in the development of new therapeutic agents targeting HDL, not only the levels of HDL cholesterol levels but especially HDL functionality should be evaluated.

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