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HDLs and beta cells

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Abstract
High-density lipoproteins (HDLs) exert a series of potentially beneficial effects on many cell types including anti-atherogenic actions on the endothelium and macrophage foam cells. HDLs may also exert anti-diabetogenic functions on the beta cells of the endocrine pancreas, notably by potently inhibiting stress-induced cell death and enhancing glucose-stimulated insulin secretion. HDLs have also been found to stimulate insulin-dependent and insulin-independent glucose uptake into skeletal muscle, adipose tissue, and liver. These experimental findings and the inverse association of HDL-cholesterol levels with the risk of diabetes development have generated the notion that appropriate HDL levels and functionality must be maintained in humans to diminish the risks of developing diabetes. In this article, we review our knowledge on the beneficial effects of HDLs in pancreatic beta cells and how these effects are mediated. We discuss the capacity of HDLs to modulate ER stress and how this affects beta cell survival. We also point out the gaps in our understanding on the signaling properties of HDLs in beta cells. Hopefully, this review will foster the interest of scientists in working on beta cells and diabetes to better define the cellular pathways activated by HDLs in beta cells. Such knowledge will be of importance to design therapeutic tools to preserve the proper functioning of the insulin-secreting cells in our body.

Introduction

Diabetes mellitus is defined by the finding of a fasting and postprandial plasma glucose level higher than 7.0 mM and 11.1 mM, respectively, or a glycated hemoglobin A1c level above 6.5%. Diabetes mellitus is differentiated into diabetes mellitus type 1 (T1DM), diabetes mellitus type 2 (T2DM) and several rarer forms of diabetes such as gestational diabetes or inherited forms (1). The rarer T1DM predominantly develops in children and adolescents and is caused by a primary loss of insulin production due to autoimmune beta cell destruction. The much more frequent T2DM is manifested mostly in adults after many years of insulin resistance. In this case, diabetes develops when the pancreatic beta cells can no longer produce the increased insulin secretion required to compensate insulin resistance. Usually, the pre-diabetic insulin resistant state is clinically silent but revealed upon finding of impaired fasting plasma glucose (5.6 to 7.0 mM), glucose intolerance (2-hours plasma glucose level after exposure to 75 g glucose ranging between 7.0 and 11.1 mM), or glycated hemoglobin level ranging between 5.4 and 6.5% (1). This pre-diabetic state is frequently accompanied by abdominal obesity (waist circumference > 102 cm in men or > 88 cm in women), arterial hypertension, and a dyslipidemia characterized by fasting plasma triglycerides above 2.3 mM as well as high density lipoprotein (HDL) cholesterol levels below 1.05 mM in men or below 1.25 mM in women. These confounding cardiovascular risk factors, which are defining criteria of the metabolic syndrome, put pre-diabetic patients at substantially increased risk for cardiovascular disease (1).

By its increasing prevalence, T2DM has become a major public health problem. From 1990 to 2010, diabetes-related mortality doubled and since has become the worldwide ninth most prevalent cause of death. With respect to life years lost, diabetes ranks 19 worldwide and 13 in Western Europe (2). The major reasons for premature mortality of diabetic patients are cardiovascular diseases, chronic kidney disease and cancer. Glycemic control is a mainstay to prevent acute metabolic decompensation and microvascular complications, notably nephropathy and retinopathy, as well as peripheral neuropathy. Lowering of low density lipoprotein cholesterol (LDL-C) by statins has emerged as the most effective means of reducing the risk of myocardial infarction, even in diabetic subjects. However, statins were also found to dose-dependently increase the risk of manifesting diabetes mellitus, especially in patients who are already affected by one or several components of the metabolic syndrome and hence have increased risks of both cardiovascular events and manifestation of diabetes mellitus (3-5). It has been argued that the cardiovascular benefit of statins measured by the reduction of clinical endpoints outweighs the risk of diabetes mellitus, the latter being revealed by hyperglycemia rather than by a hard clinical endpoint. It is however also important to note that the impact of statins for microvascular and neurological complications of diabetes is not well established.

Low HDL levels as a risk factor for diabetes (and cardiovascular disease) development

A low level of HDL-cholesterol (HDL-C) is a well-defined risk factor for the development of cardiovascular diseases (6) and overall survival in general (7), even when LDL-cholesterol (LDL-C) levels are optimally controlled (8, 9). More recently, evidence has accumulated that a low HDL-C levels also is an independent risk factor for the development of diabetes (10-13).

While a low HDL-C level (< 1mM HDL-cholesterol) is a bad prognosis for both diabetes and cardiovascular disease development, very high HDL-C levels do not appear to be associated with less risk than intermediate HDL-C levels, at least for cardiovascular diseases (14). In two recent meta-analyses of population studies and statin trials published in JAMA (15) and Circulation (16), the 20% individuals with the highest HDL-cholesterol levels (HDL-C concentrations above 1.62 mM and 1.49 mM, respectively) were not better protected from coronary heart diseases than the individuals in the 60^{th} to 80^{th} percentiles with HDL-cholesterol concentrations ranging from 1.35 to 1.62 mM and 1.23 to 1.43 mM, respectively.

Like the association with coronary heart disease, the association of low HDL-C with increased risk of T2DM is independent of other risk factors and confounders such as glucose, HbA1c, body mass index, triglycerides, and blood pressure. This statistical independence does not imply causality. In fact the association of low HDL-C with increased risk of T2DM was traditionally interpreted to be a bystander of insulin resistance rather than an indication of pathogenic causality. Indeed, there are indications for reverse causality, meaning that low HDL

cholesterol levels are the consequence of the pre-diabetic and diabetic state rather than a cause of diabetes. In particular, indirect mechanisms involving triglyceride-rich lipoproteins, free fatty acids, microRNA 33 (miR33), insulin resistance and the resulting hyperinsulinemia may promote a decrease in HDL cholesterol (**Figure 1**). This may explain why research on the anti-diabetic potential of HDL has been relatively neglected until recently (i.e. the middle of the last decade). This contrasts with the research on the anti-atherogenic role of HDL that has been stimulated for more than 50 years by the inverse association of low HDL-C and cardiovascular risks. In this dispute of causality, one must also consider the option that both relationships may be true: in a vicious cycle, increased insulin resistance and hyperinsulinemia compromise HDL metabolism and lead to quantitative and qualitative alterations of HDL, which in turn interfere with the production and action of insulin (17-20). The insulin-secreting pancreatic beta cells and the cells that respond to insulin such as skeletal muscle cells are two cell categories that could benefit from a beneficial effect of HDLs in the context of glucose homeostasis and diabetes (21). Positive effects of HDL on insulin-independent glucose uptake and utilization by adipocytes, myocytes and hepatocyte as well as HDL-mediated control of inflammation could also participate in the protection against diabetes (22-26). These latter effects have been the focus of recent excellent review articles (21, 27). Therefore and because it is beta cell failure that determines the conversion of insulin-resistant pre-diabetes into manifest T2DM we here focus on the role played by HDLs in beta cells and on the mechanisms that can be activated by HDLs in these cells to mediate their beneficial effects. But before we move to the main topic of this review, let us raise two points that are sometimes overlooked when working with HDLs.

Signaling molecules carried by HDLs: the problem of heterogeneity and scarcity

HDL particles are the most complex and heterogeneous lipoproteins. More than 80 different proteins and hundreds of different lipid species have been found associated with HDL particles (28). In addition, HDLs can carry various miRNAs and deliver them to different tissues (29, 30). HDL particles are mainly produced by the liver, but also, to a lower extent, by the intestine. These tissues produce the major HDL protein component, apolipoprotein A-I (ApoA-I), as well as its major lipids (phosphatidylcholine and cholesterol). Many minor HDL components are not only produced by hepatocytes (e.g. paraoxonase) but also by other organs (e.g. ApoM both in the liver and kidney; clusterin and sphingosine-1-phosphate (S1P) almost ubiquitously). HDLs acquire these bioactive molecules by interacting with other cell types. For example, both native and reconstituted HDLs were found to induce S1P efflux from cardiomyocytes and erythrocytes [(31) and Suter and von Eckardstein (unpublished results)]. Hence, HDLs can locally induce the release of bioactive molecules (e.g. S1P, phosphatidylserine, clusterin or ApoE) and incorporate them in their structure. In this scenario even artificial HDL consisting only of ApoA-I and phosphatidylcholine may acquire additional bioactive molecules when exposed to target cells (e.g. pancreatic beta cells). They can then present these bioactive molecules to cells in an autocrine manner or transport them to other cells to elicit paracrine or endocrine effects.

A given HDL particle may not carry all the molecules identified by isolation of HDLs through ultracentrifugation, gel filtration or affinity chromatography. Indeed, some bioactive components are only carried by a minority of HDL particles (28). For example, sphingosine-1-phosphate (S1P), which has been reported to mediate the capacity of HDLs to induce NO release and relaxation of the vascular endothelium (32), is found on ~2% of circulating HDLs(28), potentially even less if there is a sub-group of HDL particles that is “specialized” in carrying S1P and therefore carries more than one S1P molecule per HDL particle. The scarcity of some bioactive molecules may greatly complicate the interpretation of studies assessing the signaling capacities of HDLs because the concentration of HDL particles carrying a given bioactive molecule is rarely determined. It should however not be concluded that if a bioactive molecule is rarely encountered on HDL particles it should not play a physiological role. If we take S1P for example, the concentration of HDLs carrying this lipid is about 500 nM. This should be amply sufficient to induce vasorelaxation of vessels as the half-maximal response induced by purified S1P is around 100 nM (32).

The HDL concentration conundrum

A rather wide range of concentrations of native or reconstituted HDL as well as lipid-free apolipoproteins has been used by various laboratories to induce specific cellular responses such as cholesterol efflux, cell protection, and the stimulation of various signal transduction pathways. Yet, it is often difficult to compare the

experimental settings from various publications because the amounts of HDLs reported in the literature are calibrated in multiple ways based on either total protein, ApoA-I, cholesterol, or phosphatidylcholine using mass or molar concentrations. As HDLs are quite heterogeneous in composition it is difficult to give exact conversion numbers but roughly one can calculate, based on the content of cholesterol vs protein in HDLs [18% vs 52%; (33)], that 1 mM (~0.4 mg/ml) HDL-cholesterol corresponds to ~1.2 mg/ml HDL-protein, about 80% of which corresponds to ApoA-I. Efficacious doses range from around 50 μg/ml HDL-protein (~0.06 mM HDL-cholesterol) to 1 mg/ml HDL-protein (~ 1.2 mM HDL-cholesterol). The maximal HDL concentrations used experimentally are within the range of plasmatic concentrations (1-2 mM HDL-cholesterol). Are non-endothelial cells exposed to such plasmatic concentrations *in vivo*? HDL concentrations have been determined in a few extravascular compartments. For example, there is 0.4, 0.7, and 0.3-0.6 mM HDL-cholesterol in the lymph, the synovial fluid, and the ovarian follicular fluid, respectively (34-37). Hence, these concentrations amount to only 20% to 50% of the intravascular ones. If one considers that the islets of Langerhans are vascularized by fenestrated capillaries, HDL concentrations in the vicinity of beta cells may however not be far from 1 mg/ml HDL-protein or 1 mM HDL-cholesterol (38).

Importantly, the different HDL concentrations eliciting given cellular responses in specific cell types may point to different mechanism of action. Responses that are elicited at low concentrations may be mediated by interactions of HDL with specific receptors (e.g. SR-BI, S1P receptors), while responses to high HDL concentrations could be receptor-independent, as a consequence for example of alterations in cellular cholesterol homeostasis resulting from aqueous diffusion of cholesterol from the plasma membrane to HDL particles (28). Discrepant responses to HDLs in different beta cell types, e.g. primary cells from human or murine islets, rat (INS1E) or mouse (MIN6) insulinomas, in the context of different stress stimuli (e.g. thapsigargin, tunicamycin, cytokines, LDLs) may point to different modes of action and mechanisms elicited by HDLs.

In experiments using lipid-free ApoA-I, a wide range of concentrations has also been used: 10 μg/ml (2 μM) to 900 mg/l (32 μM). However, in this case only the lower concentration appears to reflect physiological situations. Higher concentrations correspond to total plasmatic ApoA-I that is both lipid-free and lipid-bound. However, only 5 to 10 percent of total plasma ApoA-I occurs in a lipid-free form which, by its electrophoretic preβ1-mobility, can be differentiated from the lipidated ApoA-I of the α- or even pre-α-migrating HDL particles (39, 40). In extravascular and interstitial fluids, the proportion of preβ1-HDL (i.e. lipid-free ApoA-I) is higher than in plasma but does not amount to 100% of total ApoA-I (41) and is diluted as compared to plasma (because the HDL concentration in the extravascular space is lower than in plasma; see above). Moreover, due to its much smaller structural heterogeneity, cellular responses to lipid-free ApoA-I appear to be initiated by less diverse mechanisms than responses to the highly heterogeneous HDL particles: as yet only ATP-binding cassette sub-family A member 1 (ABCA1) and the ectopic β-chain of the F_0F_1 ATPase have been identified as cell proteins interacting with lipid-free ApoA-I (42-44). In these cases, these interactions could be detected at rather low ApoA-I concentrations (10 μg/ml to 30 μg/ml) in all cell types but beta cells where 10 fold higher concentrations were used to enhance glucose-stimulated insulin secretion (45).

HDLs are potent beta cell protectors

The majority of data reporting a beneficial effect of HDLs in beta cells concern their survival. Numerous studies have indeed shown that HDLs are very efficient in inhibiting apoptosis of beta cells. **Figure 2** reports the capacity of HDLs to counteract beta cell death induced by a variety of stimuli. These include inflammatory cytokines, free fatty acids (e.g. palmitate), thapsigargin, tunicamycin, protein over-expression, etc. Many of these stimuli induce endoplasmic reticulum (ER) stress. As ER stress has been proposed to be a driving parameter in beta cell dysfunction and death in the course of diabetes development (46-48), the capacity of HDLs to protect beta cells from ER stressors could be one mechanism underlying their potential ability to prevent type 2 diabetes (see below).

As can be seen in **Figure 2**, the beta cell protective concentrations of HDLs are around 1 mM HDL-cholesterol in most cases. In Min6 cells, the protection against thapsigargin and tunicamycin was decreased at lower HDL concentrations (49), indicating that 1 mM HDL-cholesterol was the optimal protective in these particular conditions. Interestingly however, about 10 times lower amounts of HDLs were necessary to inhibit thapsigargin-induced apoptosis in the rat INS1e insulinoma cell line. Maximal anti-apoptotic effects were seen at HDL protein concentrations as low as 100 mg/l (corresponding to 0.12 mM HDL-cholesterol) (Annema and von Eckardstein, unpublished results). Whether this is a reflection of different mechanisms of protection in INS1 cells compared to

other insulinoma cells remains to be determined. Protection of rat beta cells against LDL-mediated death by doses that are 100 fold lower than 1 mM HDL-cholesterol (50) (**Figure 2**) is an odd, and as yet not reproduced, observation.

Little information is available on the molecules carried by HDLs that mediate their anti-apoptotic effect. The inhibitory effect of HDL on IL1β- and high glucose-induced apoptosis of primary beta cells in murine islets was found in both the protein and lipid fractions of HDLs and mimicked by both lipid-free ApoA-I (at a concentration of 20 mg/l) and S1P (at a 1 μM concentration, which is in the upper physiological concentration range) (51). In contrast, the components mediating the protective effect of HDLs against the ER stressors thapsigargin, tunicamycin, palmitic acid, and oxidized LDL have not yet been identified.

In the previous paragraph, we have proposed that responses induced by mM ranges of cholesterol could be HDL receptor-independent. SR-BI, the HDL receptor mediating, for example, eNOS activation in CHO cells and human microvascular endothelial cells (52), may therefore not be involved in HDL-mediated protection of beta cells. Indeed this has been verified experimentally. Beta cells lacking SR-BI, either as a result of gene knock-out or siRNA-mediated silencing, are protected by HDLs as efficiently as control SR-BI-positive cells (51, 53). The way HDL particles engage beta cells remains therefore mysterious.

HDL and anti-apoptotic signaling pathways: still a black box!

How do HDLs induce their anti-apoptotic activity in beta cells? What is the signaling mode employed? Anti-apoptotic signaling pathways include NFkB signaling (54), Akt signaling (55), and the unfolded protein response (UPR).

The NFkB transcription factors can inhibit cell death responses by inducing the expression of anti-apoptotic genes, such as those coding for inhibitor of apoptosis (IAP) family members, but they can also induce pro-apoptotic genes, such as death receptors (56). Hence depending on the cell type and how the cells are stimulated, NFkB can either promote or inhibit apoptosis. Pancreatic beta cells appear not very tolerant to NFkB stimulation, especially when this signaling route is sustained or strongly activated (57-59). NFkB activation has indeed been shown to induce beta cell apoptosis in a number of situations (60, 61). One could argue that these responses require a strong NFkB stimulation and that NFkB could be protective when stimulated to low extent. However, even when mildly activated, NFkB does not contribute to cell survival (62). Therefore, NFkB is an unlikely target of HDLs in beta cells and indeed HDLs do not appear to stimulate this transcription factor in beta cells (63) [nor do they do in other cell types (64)].

Akt (also known as PKB) is a kinase family of proteins with powerful anti-apoptotic activities (65, 66). For example, the anti-apoptotic RasGAP fragment generated by the caspase-3/RasGAP stress sensing module (66) protects cells, including beta cells, in an Akt-dependent manner (61, 67-69). Other protective responses in beta cells that require Akt include those activated by Cyclin-dependent kinase 5 (70), Glucose metabolism-related protein 1 (71), Erythropoietin (72), Adiponectin (73), Glucose-dependent insulinotropic polypeptide (74), Cxcl12 (75), FGF-21 (76), and even glucose itself (77). It has to be noted that Akt activation can, in some situations, lead to beta cells death. For example, palmitate appears to require Akt to induce apoptosis in the rat insulinoma INS-1 cell line (78). Additionally, forced expression of a constitutive active Akt mutant in INS-1 cells causes their death, unless NFkB is inhibited (61). Hence, Akt modulates beta cell apoptosis in a context-dependent manner. Nevertheless, as Akt is involved in beta cell survival in many instances, it is conceivable that HDLs use Akt to protect beta cells, especially since it is known that this kinase is activated by HDLs in other cell types (79-82) and this has, at least on one instance, shown to be required for the HDL-mediated anti-apoptotic response (83). To our knowledge, there is only one published study that assessed Akt activation by HDLs in beta cells (50). This was done by immuno-precipitating Akt and assessing the ability of the pulled down material to phosphorylate glycogen synthase kinase, one of the Akt substrates. Using this methodology the authors reported an augmentation of Akt activity induced by HDLs but the data were neither quantitated nor was the number of replicated experiments mentioned. Moreover, whether Akt was involved in the protective activity of HDLs was not investigated in this study. The implication of Akt in the anti-apoptotic response mediated by HDLs remains therefore to be demonstrated (**Figure 3**).

Oxidized LDLs were reported to reduce the expression of the anti-apoptotic Bcl2 protein and induce apoptosis of MIN6 cells. This was reported to be mediated by an up-regulation of JNK (c-Jun N-terminal kinase; also known as mitogen-activated protein kinase 8 [MAPK8]) as a consequence of a decrease in the expression of islet-

brain 1 (IB1; also known as MAPK8 interacting protein 1). HDL counteracted the pro-apoptotic effects of oxidized LDLs on Bcl2, JNK and IB1. However, it has not been clarified if these anti-apoptotic effects of HDLs on the JNK pathway are exerted directly on the beta cells by interference with the apoptotic signaling or indirectly by inactivating oxidized LDLs (84).

In murine islets, HDLs were found to down-regulate the extrinsic death receptor FAS and to upregulate its inhibitor FLIP (51). Additionally, IL1β-induced up-regulation of iNOS (inducible nitric oxide synthase) was suppressed by HDLs. However, no interference experiments were reported to show that HDLs inhibit IL1β-induced apoptosis through these pathways. Interestingly, the expression of Bcl-2 and SOCS-3, which are also known to inhibit apoptosis in beta cells in response to cytokines (68, 85, 86), were not altered (51).

HDL and the ER connection

As indicated above, many apoptotic inducers in beta cells induce ER stress which leads to the activation of a conserved physiological stress response called the unfolded protein response (UPR). The UPR is activated when the functionality of the ER is perturbed such as when too much protein misfolding in the ER takes place. The UPR consists in the activation of three main arms depending on three key proteins, inositol-requiring protein 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum (ER) kinase (PERK) and activating transcription factor 6 (ATF6) (87, 88). These proteins are kept inactive when binding to the Bip (GRP78) chaperone. In the presence of an ER stress, the ER accumulates misfolded protein. Bip binds these misfolded proteins and this frees and activates IRE1a, PERK, and ATF6. The initial consequence of this activation is to induce the expression of genes that will help restoring the folding capacity of the ER and the elimination of terminally misfolded proteins on the one hand and decrease the loading of the ER with newly synthesized proteins by inhibiting global translation on the other hand (87, 88). If this "repair" phase fails, the UPR eventually activates an apoptotic response leading to the elimination of the non-functioning cell (87, 88). The initial phase of the UPR may also be involved in physiological adaptation to metabolic changes and in several differentiation and cellular activation processes (87).

As indicated above, many of the apoptotic stimuli that can be counteracted by HDLs (see **Figure 2**) are able to induce ER stress. This raises the possibility that HDL modulate such ER stress responses to exert their protective function in beta cells. Recent data show this to be true in certain cases but not in others (49, 53).

Thapsigargin is an irreversible inhibitor of SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase), an ER-associated calcium pump (89). SERCA inhibition leads to disruption of the calcium gradient between the ER and the cytoplasm and this causes profound ER stress as assessed by increased activation of IRE1, PERK, and ATF6 (53, 90-93). HDLs were found to fully block thapsigargin-induced apoptosis of beta cells, with a concomitant inhibition of UPR activation and restoration of the capacity of the ER to fold proteins and to export them further down the secretory pathway (53). Blocking trafficking between the ER and the Golgi with Brefeldin A prevented HDLs from inhibiting thapsigargin-induced beta cell death (53). This is an indication that HDLs need to preserve the functionality of the ER to counteract the apoptotic response induced by thapsigargin. However, since Brefeldin A can affect cell activity and viability on its own (94-97), this interpretation should be taken with caution. Palmitate, a patho-physiological relevant pro-diabetogenic compound, induced, similarly to thapsigargin, the activation of the UPR (53, 98). In this case too, HDLs fully prevented stimulation of ER stressors and apoptosis induced by palmitate and they maintained the functionality of the ER in terms of protein folding and protein trafficking (53). These results suggest that HDLs protect beta cells by allowing them to retain a functional ER despite the presence of stressful conditions, an obviously clear benefit for professional secretory cells that beta cells are (**Figure 3**).

Is the capacity of HDLs to maintain ER functionality its only mode of protection in beta cells? The answer to this question is certainly no. First, some apoptotic stimuli, such as starvation or even basal apoptosis, which are counteracted by HDLs, have not been reported to induce an ER stress response. Second, HDLs, while blocking beta cell death induced by the ER stressor tunicamycin, does not modulate the UPR response activated by tunicamycin. In other words, HDLs inhibit tunicamycin-induced beta cell apoptosis without a significant reduction in the induction of stress markers (such as Xbp1 splicing, Bip expression, PERK activation, and CHOP induction) (49). In this case it can be concluded that the protection conferred by HDLs occurs distally to the UPR (**Figure 3**). HDLs therefore use multiple routes of protection in beta cells, including one that maintains the functionality of the ER.

Other beneficial effects of HDLs in beta cells

Besides protecting beta cells from death, HDLs may also favor their survival and function by augmenting their proliferation or their insulin secretory capacity (21). Whereas a stimulatory effect of HDL on beta-cell proliferation was ruled out (51), *in vivo* and *in vitro* evidence has been provided for stimulation of insulin production and secretion. Infusion of reconstituted HDLs in type 2 diabetes patients increased their HOMA-B index, an indirect measurement of pancreatic beta cell function (99). Also, the treatment of healthy volunteers for two weeks with an inhibitor of cholesteryl ester transfer protein (CETP) was found to increase postprandial insulin and C-peptide levels (100). Treatment with a CETP inhibitor (CETPi) increased plasma HDL cholesterol by 46% and HDL-associated ApoA-I by 22% as well as insulin levels by 30% as compared to placebo. The plasma of CETPi-treated volunteers had an increased capacity to stimulate cholesterol efflux and glucose-stimulated insulin secretion from MIN6 cell. The CETPi itself did not stimulate insulin secretion leading the authors to conclude that the improved secretory potential was caused by an increase in HDL particle size and/or concentration (100). This small short term study in normolipidemic and euglycemic volunteers mirrors the results of a post-hoc analysis of the large ILLUMINATE trial (101). In this randomized controlled endpoint study, the CETPi torcetrapib was found to increase cardiovascular mortality and morbidity of statin-treated patients despite substantial increase in HDL cholesterol levels, possibly due to off-target effects on aldosterone production and blood pressure. In a post-hoc analysis of a subgroup of diabetic ILLUMINATE participants, atorvastatin + placebo treatment led to an increase in glucose and HbA1c levels which was not observed in the atorvastatin + torcetrapib treated probands (101).

In vitro data on the effects of HDL or ApoA-I on insulin secretion are however controversial. HDL-treated βTC3 cells and MIN6 cells were found to express more insulin mRNA than untreated control cells (50, 84). Stimulation of the Min6 insulinoma cell line for one hour with 4 to 32 μM lipid-free recombinant ApoA-I, ApoA-II or discoidal reconstituted HDLs dose-dependently increased both basal and glucose-stimulated insulin secretion (45). The supraphysiological dosages of 32 μM increased basal and glucose-stimulated insulin secretion by a factor of 4 and 3, respectively. A concentration of 1 mg/ml HDL-protein (~1.2 mM HDL-cholesterol) led to a doubling of insulin secretion but this particular experiment was only performed once (45). By RNA interference the authors showed that the stimulatory effect of lipid-free ApoA-I and reconstituted HDLs on insulin secretion depended on ATP-binding cassette (ABC) transporters A1 and G1, respectively (45).

Min6 cells incubated 3 days with 50 μg/ml HDL-protein (~60 μM HDL-cholesterol) boosted acute glucose-induced insulin secretion by ~6-fold (99). However, it is not clear how specific this HDL effect on insulin secretion is. Bovine serum albumin was able to increase glucose-stimulated insulin secretion in Min6 cells (50-60% more than glucose alone) (45) indicating that the mere presence of proteins can positively affect insulin secretion by Min6 cells. Taking into consideration an earlier study reporting no effect of HDLs (0.8-1 mM HDL-cholesterol) on basal or glucose-stimulated insulin secretion by mouse and human islet cells (51), it appears premature to conclude that HDLs have a direct effect on the insulin secretory capacity of beta cells. In this context, a hyperinsulinemic euglycemic clamp in human individuals injected or not with reconstituted HDLs would be particularly informative.

Although the stimulatory effect of HDL on insulin secretion is still controversial, there is good evidence that the ABC transporters ABCA1 and ABCG1 modulate insulin secretion from pancreatic beta cells. These transporters mediate cholesterol efflux, in cells such as macrophages for example, in the presence of lipid-free apolipoproteins (ApoA-I in particular) and HDLs, respectively (102, 103). However, we will see that in beta cells ABCA1 favors insulin secretion by promoting cholesterol efflux while ABCG1 does so by inducing cholesterol transfer to insulin granules. The evidence for these notions has been generated both *in vivo* and *in vitro*. Mice with a targeted knock-out of ABCA1 in pancreatic beta cells and cross-bred with LDL-receptor knock-out mice to induce hypercholesterolemia, were found to be less glucose tolerant than LDL receptor knock-out only mice (104). The beta-cell specific ABCA1 knock-out mice also showed reduced insulin secretion in response to glucose administration. Islets isolated from these mice showed altered cholesterol homeostasis and impaired insulin secretion *in vitro* (104). Later studies by the same group showed that the lack of β-cell ABCA1 results in impaired depolarization-induced exocytotic fusion of insulin granules, disturbances in membrane micro-domain organization, and alteration in Golgi and insulin granule morphology. Acute cholesterol depletion rescued the exocytotic defect in β-cells lacking ABCA1, suggesting that elevated islet cholesterol accumulation directly impairs granule fusion and insulin secretion (105). *In vitro*, adenoviral overexpression in beta cells of microRNAs 33a and 145 (miR-33a and miR145), which target ABCA1, led to increased cholesterol levels and to decreased glucose-stimulated insulin

secretion (106, 107). This compromised insulin secretion was again rescued by cholesterol depletion. Inhibition of miR-33a expression in apolipoprotein E knockout islets and ABCA1 overexpression in β-cell-specific ABCA1 knockout islets rescued normal insulin secretion and reduced islet cholesterol (107). Therefore, cholesterol seems to be one of the bad guys negatively affecting beta cell function. However, the situation is much more complex than this simple interpretation. Indeed, mice lacking another cholesterol transporter, ABCG1, were also found to be glucose-intolerant due to reduced insulin secretion but this resulted from an inefficient transfer of cholesterol to insulin granules, which negatively impacted on their beta cell insulin secretory capacity (108). These defects could be rescued by exogenous addition of cholesterol (108). Hence, when beta cells lack ABCA1, the ensuing insulin secretion dysfunctionality can be alleviated by depleting cells from cholesterol. Yet, when beta cells lack ABCG1, insulin secretion is restored by exogenous addition of cholesterol. These studies show that a delicate balance of cholesterol concentrations between different sub-cellular compartments must be achieved to allow optimal beta cell functionality. Whether HDLs cooperate with ABCA1 or ABCG1 to induce its cellular effects in beta cells is unknown (**Figure 3**).

As expected from the data presented above, the ABCA1 and ABCG1 cholesterol transporters play non-redundant functions in beta cell activity. Combined deficiency of ABCA1 and ABCG1 aggravated the diabetic phenotype found in the single knock-out animals (109). This also resulted in significant cholesterol accumulation in beta cells. Islet inflammation was also increased as indicated by augmented expression of interleukin-1β and macrophage infiltration (109).

Taken together these findings indicate that cholesterol homeostasis and its regulation by ABCA1 and ABCG1 are critical for the secretory β-cell function. The relevance of these findings in humans is unclear however. On the one hand, decreased glucose-induced insulin secretion is reported in ABCA1 deficient patients with Tangier disease or heterozygous carriers of ABCA1 mutations (110, 111). On the other hand, mutations in ABCA1 have not been associated with increased risk of diabetes (112). Nevertheless, data on the role of ABCA1 and ABCG1 may be of special importance for patients treated with statins, which appear to interfere with insulin secretion by inhibiting the production of cholesterol for granules as well as sterol intermediates which are important for insulin secretion, and by enhancing the uptake of LDL (17, 113).

Conclusion

The beneficial effect of HDLs on beta cells is undisputable. Numerous studies have shown that HDLs induce potent anti-apoptotic signals in beta cells stressed by a plethora of stressful stimuli. HDLs may also favor their insulin secretory function. In humans, there is a strong association with low HDL levels and an increase in the risk of developing several diseases, including type 2 diabetes. While it is clear that HDLs induce protective signals in beta cells, our knowledge on the molecular mechanisms underlying the beneficial functions of HDLs in beta cells is at best rudimentary. We need now to invest time and resources to decipher the protective signaling pathways activated by HDLs in beta cells. Not only will this increase our fundamental understanding on how pancreatic beta cells can be spared from stress-induced death, this may also lead to the identification of markers associated with diabetes development, with the hope that some of which can be used as prognostic markers. Finally, knowing exactly how beta cells are protected by HDLs is a pre-requisite for the development of drugs that stimulate or mimic the anti-diabetic effects of HDLs in order to lower the risk, for example in overweight patients, to manifest this disease.

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Figure legends

Figure 1. Disturbed HDL metabolism in insulin resistance
Insulin resistance implies both reduced insulin sensitivity of some organs and increased response of other organs to the compensatory hyperinsulinemia. Hyperinsulinism increases hepatic production of triglycerides and very low density lipoproteins by upregulating the expression of the transcription factor SREBP1c (sterol regulatory element binding protein 1c) and the microRNA miR33b that is encoded by an intron of SREBP1c (18-20). In the adipose tissue, insulin resistance is interfering with lipogenesis and enhancing lipolysis so that the concentration of circulating free fatty acids is increasing, which in turn results in the stimulation of hepatic lipogenesis and hence VLDL production. The increased secretion of VLDL produces hypertriglyceridemia, which is not sufficiently cleared because of reduced lipoprotein lipase (LPL) activity. LPL is released from adipocytes upon insulin stimulation but this is compromised in insulin resistance. Reduced lipolysis of VLDL decreases the production of surface remnants that contribute to the maturation of HDL. Moreover, hypertriglyceridemia increases the activity of cholesteryl ester transfer protein (CETP) that exchanges cholesteryl esters of HDL against triglycerides of VLDL. This leads to a decrease in the concentration of HDL cholesterol (18). Finally, the production of HDL precursors in the liver and intestine is disturbed in insulin resistance states because the ATP binding cassette transporters A1 and G1 are inhibited by free fatty acids at both the transcriptional and post-translational levels, as well as by miR33 acting at the post-transcriptional level (19, 114).

Figure 2. HDLs are potent inhibitors of beta cell death
This figure depicts the effect of HDLs against apoptosis induced by the stimuli in the right-most column in the indicated cell types. When HDLs protect the cells, a green light is shown, while when there is no protection, a red light is depicted. In the left-most column the HDL concentration used is indicated by the cursor. When the cursor is yellow, the HDL concentration used in the studies reporting the effect of HDL on beta cell apoptosis was determined based on the cholesterol content of the particles. When the cursor is blue, this concentration was based on the protein content of the particles but was converted here back to HDL-cholesterol concentrations.
Min6 cells are derived from pancreatic tumors of C57BL/6 mice expressing the SV40 large T antigen under the control of the human insulin promoter (115). The βTC3 cell line is derived from pancreatic tumors of B6D2F1/J mice expressing the SV40 large T antigen under the control of the rat insulin II promoter (116, 117). INS1 cells are derived from X-ray-induced NEDH rat pancreatic tumors (118, 119).
References for the data presented in this figure: Min6 cells (49, 53, 63, 84); βTC3 (50, 63); INS1e (Annema and von Eckardstein, unpublished results); mouse β cells (51); rat β cells (53, 120); human β cells (51, 53).

Figure 3. HDLs and beta cell protection: a series of missing links
HDLs exert potent anti-apoptotic signals in beta cells and they may favor insulin secretion. At present virtually nothing is known on how this is achieved at the molecular level (see the question mark inside a puff of smoke). At the cellular level, HDLs can maintain the ER functionality in response to certain types of stresses (e.g. palmitate) and thereby diminish ER stress and apoptosis. This will obviously also have a positive impact on insulin secretion but other mechanisms (e.g. cooperation with ABC transporters) can conceptually also participate in enhanced insulin secretion. Even in the presence of an ER stress can HDLs inhibit apoptosis (e.g. when cells are stimulated with the tunicamycin ER stressor). HDLs can also protect beta cells against stimuli that do not induce an ER stress response such as some inflammatory cytokines (e.g. IL1β) or starvation. Whether the anti-apoptotic activity of HDLs in beta cells relies on specific receptors, whether there is a signaling cascade that is activated following binding of HDLs to their putative receptors, or whether bioactive molecues (e.g. S1P) are transferred from the HDL particle to the beta cell to mediate the protective response will need to be investigated. Note that in beta cells SR-BI, the classical HDL docking platform/receptor, is dispensable for the HDL-mediated effects.

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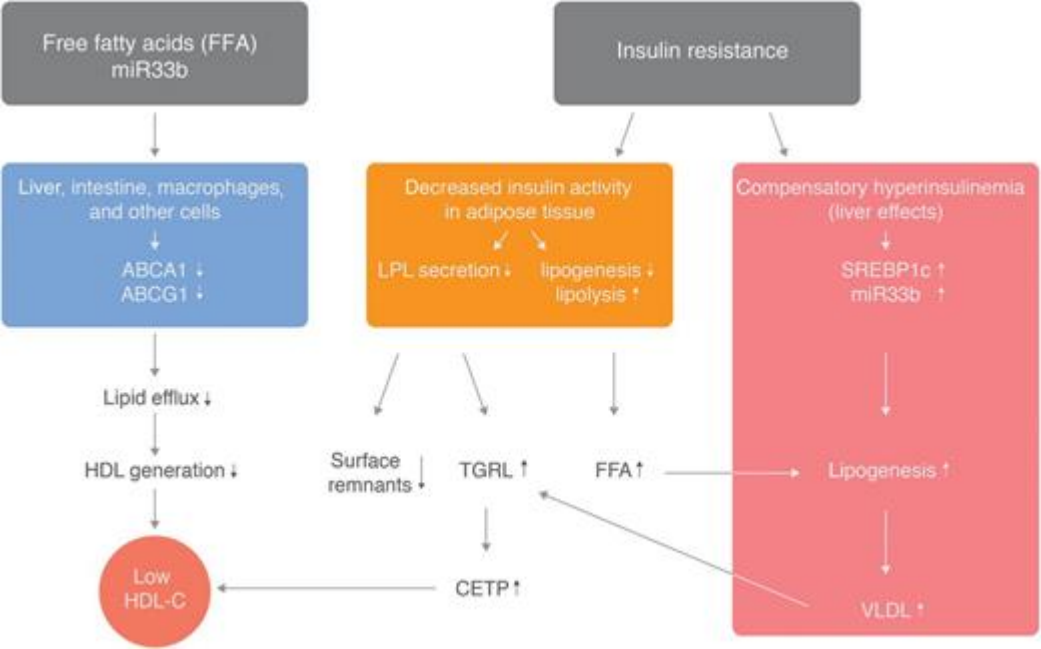
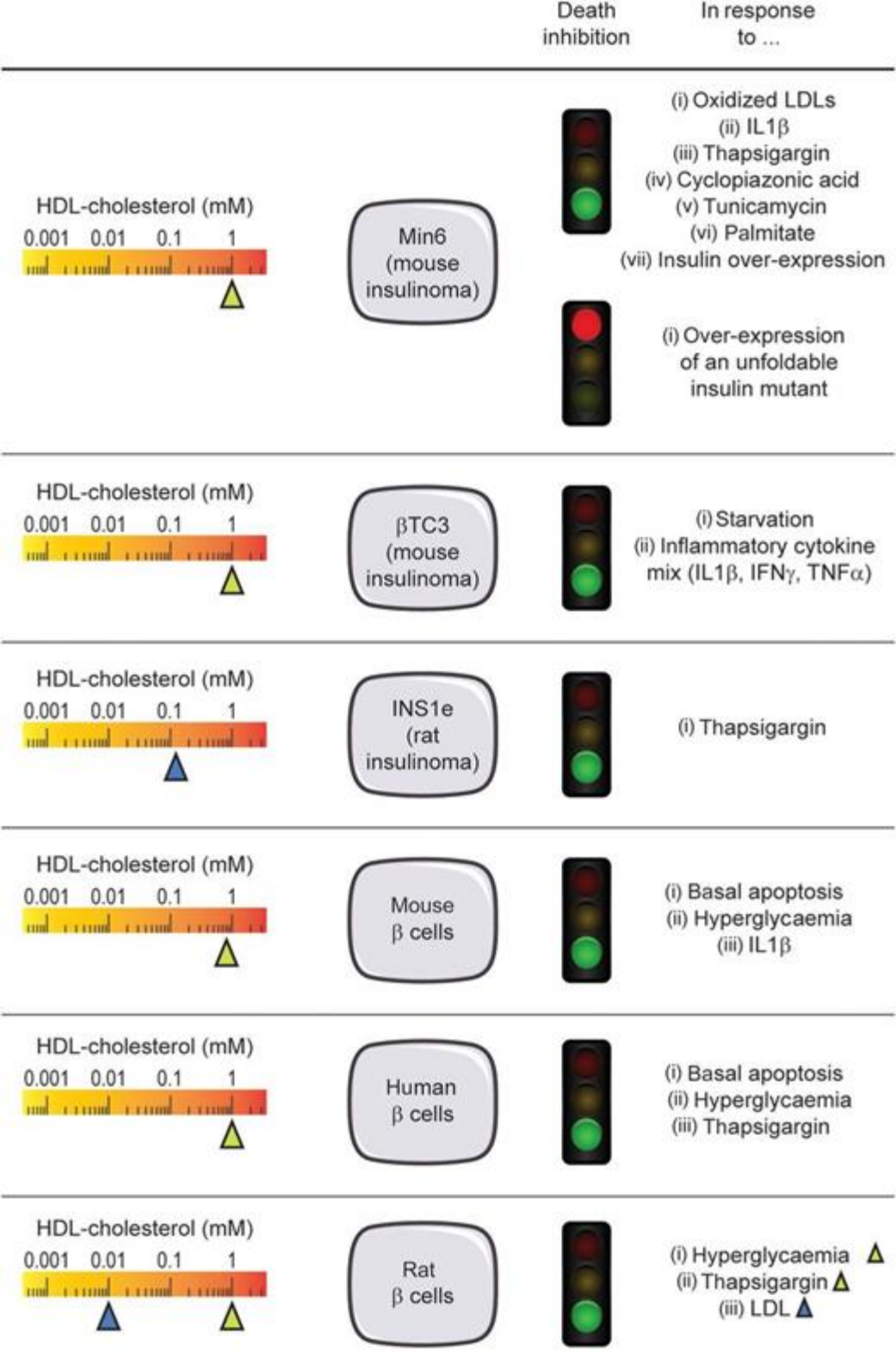
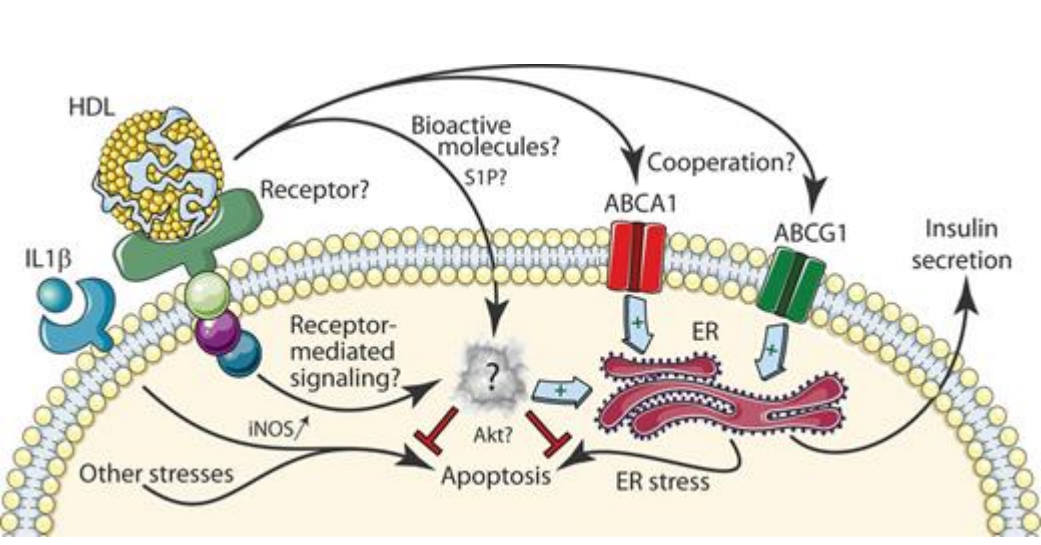

Free fatty acids (FFA)
miR33b
Insulin resistance
Liver, intestine, macrophages, and other cells
ABCA1 ↓
ABCG1 ↓
Decreased insulin activity in adipose tissue
LPL secretion ↓
lipogenesis ↓
lipolysis ↑
Compensatory hyperinsulinemia (liver effects)
SREBP1c ↑
miR33b ↑
Lipid efflux ↓
HDL generation ↓
Surface remnants ↓
TGRL ↑
FFA ↑
Lipogenesis ↑
Low HDL-C
CETP ↑
VLDL ↑


Death inhibition
In response to ...
HDL-cholesterol (mM)
0.001 0.01 0.1 1
Min6 (mouse insulinoma)
(i) Oxidized LDLs
(ii) IL1β
(iii) Thapsigargin
(iv) Cyclopiazonic acid
(v) Tunicamycin
(vi) Palmitate
(vii) Insulin over-expression
(i) Over-expression of an unfoldable insulin mutant
βTC3 (mouse insulinoma)
(i) Starvation
(ii) Inflammatory cytokine mix (IL1β, IFNγ, TNFα)
INS1e (rat insulinoma)
(i) Thapsigargin
Mouse β cells
(i) Basal apoptosis
(ii) Hyperglycaemia
(iii) IL1β
Human β cells
(i) Basal apoptosis
(ii) Hyperglycaemia
(iii) Thapsigargin
Rat β cells
(i) Hyperglycaemia
(ii) Thapsigargin
(iii) LDL


HDL
Bioactive molecules?
S1P?
Cooperation?
Receptor?
ABCA1
ABCG1
Insulin secretion
IL1β
Receptor-mediated signaling?
?
ER
iNOS
Akt?
Other stresses
Apoptosis
ER stress