Type 2 diabetes (T2D) frequently occurs in the context of abnormalities of plasma lipoproteins. However, a role for elevated levels of plasma cholesterol in the pathogenesis of this disease is not well established. Recent evidence suggests that alterations of plasma and islet cholesterol levels may contribute to islet dysfunction and loss of insulin secretion. A number of genes involved in lipid metabolism have been implicated in T2D. Recently an important role for ABCA1, a cellular cholesterol transporter, has emerged in regulating cholesterol homeostasis and insulin secretion in pancreatic β cells. Here we review the impact of cholesterol metabolism on islet function and its potential relationship to T2D.

The role of ABCA1 in β cell function: in vivo evidence from animal studies

The most direct evidence for a role of cholesterol metabolism in β cell function comes from the study of the ATP-binding cassette transporter, subfamily A, member 1 (ABCA1). ABCA1 is a cellular cholesterol transporter, and mutations in ABCA1 cause Tangier disease (6–8), characterized by an inability to eliminate excess cellular cholesterol, low levels of HDL cholesterol, and increased risk for coronary artery disease (9–11). Study of the specific role of ABCA1 in T2D began with the observation that mice lacking 

Nonstandard abbreviations used: GK, glucokinase; LBP, LDL receptor-related protein; LXR, liver X receptor; nNOS, neuronal NO synthase; T2D, type 2 diabetes.

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ABCA1 activity in cardiovascular disease (23) and β cell function and diabetes (24), while minimizing the negative side effects of PPAR-γ activation.

**Genetic evidence for a role of cholesterol metabolism in T2D**

Studies of genetic conditions in which cholesterol metabolism is altered in humans and mice provide an additional opportunity to examine the role of cholesterol metabolism in islet function (Table 1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>Variants have been associated with T2D across multiple ethnic groups</td>
<td>47, 56, 57</td>
</tr>
<tr>
<td>LXR</td>
<td>Transcriptional activator of ABCA1; genetic deletion of LXRα results in impaired glucose tolerance and lipid accumulation in islets</td>
<td>26</td>
</tr>
<tr>
<td>LDLR</td>
<td>Identified in a whole-genome association study of diabetes</td>
<td>52</td>
</tr>
<tr>
<td>LRP5</td>
<td>Mice lacking Lrp5 have elevated plasma cholesterol levels and impaired insulin secretion from islets</td>
<td>31</td>
</tr>
<tr>
<td>LRP6</td>
<td>Carriers of a mutation in LRP6 have elevated plasma LDL cholesterol levels and an increased risk of developing T2D</td>
<td>30</td>
</tr>
<tr>
<td>SCD1</td>
<td>Mice lacking Scd1 have a subpopulation of islets with elevated islet cholesterol levels and attenuated insulin secretion</td>
<td>32</td>
</tr>
</tbody>
</table>

In *in vitro* studies investigating the relationship and mechanism between cholesterol and β cell function

Lipoprotein receptors, in particular the LDL receptor and LRP are expressed in mouse islets (34), suggesting that cholesterol may be taken up by islets via the classic receptor-mediated endocytosis pathway (35). Atherogenic lipoproteins such as LDL and VLDL induce apoptotic death in isolated islets and transformed β cell lines (34, 36, 37). This effect is blocked by HDL (34). These studies suggest that cholesterol loading of islets may be directly cytotoxic. Such a concept is consistent with studies in macrophages showing that free cholesterol induces apoptosis by causing stiffening of the ER membrane, with subsequent induction of the unfolded protein response (38).

Oxidized LDL reduces glucose-stimulated insulin secretion in the clonal hamster β cell line, HIT-T15 (39). In contrast, native or acetylated LDL does not reduce insulin secretion. Similarly, oxidized LDL reduces both insulin content and preproinsulin mRNA expression in β cell lines and isolated islets (40) and also increases β cell apoptosis. Treatment of the cells with inhibitors of the JNK pathway prevent this effect of LDL, suggesting that oxidized LDL reduces insulin expression by activating the JNK pathway. Incubation of cells with HDL blocks the ability of LDL to reduce insulin expression and induce apoptosis.

Cholesterol may directly impair β cell function and glucose-stimulated insulin secretion (40). Elevating islet cholesterol levels, either in mice lacking Apoe on the diabeticogenic obese ob/ob background or by directly overloading transformed β cell lines with cholesterol, reduces glucose-stimulated insulin secretion. This is consistent with the reduction in insulin secretion and elevation in islet cholesterol in mice lacking β cell Abca1 (12), suggesting a direct effect of cholesterol in reducing β cell function. Conversely, lowering β cell cholesterol levels with methyl-β-cyclodextrin (40, 41), which depletes membrane cholesterol, or with a statin (40) increases insulin secretion.

Collectively, these studies indicate that cholesterol is taken up by β cells via lipoprotein receptors and that increasing islet cholesterol leads to reduced β cell function and, in some studies, increased β cell apoptosis. The degree to which these results can be extrapolated to the physiological situation in vivo remains to be determined.

It is essential to determine the mechanisms by which ABCA1 and islet cholesterol impact β cell function in order to therapeutically modify these pathways to potentially treat T2D. Some of the potential pathways involved are illustrated in Figure 1. Based on the *in vitro* studies described above, one could predict that the islet cholesterol accumulation that occurs in the absence of β cell ABCA1 would induce apoptosis. However, mice lacking...
β cell Abca1 do not appear to have a reduction in β cell mass (12). While this finding does not rule out an increased rate of apoptosis, it does suggest that the predominant effect of Abca1 deficiency and islet cholesterol accumulation in vivo is on β cell function rather than mass.

Elevated β cell cholesterol may also impair insulin secretion by affecting glucose metabolism upstream (40). Depleting cholesterol from transformed rat (INS-1) β cells has been reported to increase glucokinase (GK) activity, whereas culturing cells in cholesterol in the presence of high (20 mM) glucose reduces GK activity. Therefore, GK, which controls the rate-limiting step in β cell glucose metabolism, appears to be impacted by β cell cholesterol levels. Islet cholesterol levels may also modulate the dimerization state of β cell neuronal NO synthase (nNOS) (40), suggesting that excess cholesterol may impair β cell glucose metabolism and sensing by increasing the proportion of GK bound to nNOS dimers on the insulin secretory granule membrane, where it is inactive (40).

Cholesterol is known to be a component of both secretory granules and plasma membranes, and cholesterol-rich microdomains in the plasma membrane are thought to be essential for normal exocytosis (42, 43). Cholesterol is also enriched in the trans-Golgi network and plays an important role in normal granule formation and trafficking. Lipid rafts rich in cholesterol and sphingolipids are sites of accumulation of soluble N-ethylmaleimide–sensitive fusion protein attachment protein receptors (SNAREs) and other key proteins in exocytosis. Depletion of membrane cholesterol from β cells enhances insulin exocytosis by redistributing potassium channels out of lipid rafts (41). It is therefore possible that accumulation of cholesterol in the plasma membrane of β cells results in retention of potassium channels in lipid rafts where they are maintained in the open configuration, thus resulting in a reduction in stimulus-coupled insulin secretion.

Role of ABCA1 in glucose metabolism in humans
ABC1A clearly plays an important role in islet function in the mouse, but what data exist for a role of ABCA1 in glucose metabolism in humans? A number of polymorphisms and mutations in ABCA1 have been associated with T2D across multiple ethnic groups (Table 2). In the Mexican population, the R230C polymorphism is associated with low HDL cholesterol and increased body mass index (BMI) (47). This polymorphism is also significantly associated with the metabolic syndrome, and in a limited sample, individuals with the C230 allele had a 4.5-fold increased risk for T2D, a risk that remained significant even after adjusting for HDL cholesterol, BMI, apoA-I levels, and ethnic admixture. In 2 additional larger cohorts of Mexican individuals, the R230C variant was found to be significantly associated with T2D, particularly early-onset T2D (48). This variant was also associated with higher hemoglobin A1C levels and reduced fasting insulin. Interestingly, of the 10 individuals homozygous for the C230 allele, all 10 had T2D. While functional studies of the
R230C variant have not been reported, this variant is predicted to impair the function of the ABCA1 protein based on the evolutionary conservation at this site in related proteins (49).

The R230C variant is also seen in the Oji-Cree population and is linked in these subjects to familial low HDL levels (50). Interestingly, the incidence of T2D in the Oji-Cree population is exceptionally high (51), raising the question of whether the R230C variant may predispose to diabetes in this population. Although the R230C allele is very frequent in the Mexican population, it is not found in Caucasians (50). This might explain why ABCA1 was not identified as a risk locus for T2D in 4 recently published genome-wide association studies in primarily Caucasian populations (52–55).

In a Japanese population of T2D patients and normoglycemic controls, an ABCA1 haplotype block in intron 2 was associated with T2D with an odds ratio of 2.5 (56). This association remained significant after adjustment for BMI and triglyceride levels (57). In the Copenhagen Heart Study, male carriers of the K776N variant had significantly lower HDL cholesterol and an increased risk of cardiovascular disease. The frequency of T2D was nearly double in male carriers (11%) compared with non-carriers (6%) of this variant (61). Although this result did not reach statistical significance, it suggests that men with the K776N variant may be at increased risk of developing T2D.

In the Copenhagen Heart Study, male carriers of the K776N ABCA1 variant had significantly lower HDL cholesterol and an increased risk of cardiovascular disease. The frequency of T2D was nearly double in male carriers (11%) compared with non-carriers (6%) of this variant (61). Although this result did not reach statistical significance, it suggests that men with the K776N variant may be at increased risk of developing T2D.

Patients with T2D have lower levels of ABCA1 mRNA in monocytes compared with nondiabetic control subjects (62). This raises the question of whether a reduction in ABCA1 levels could contribute to the pathogenesis of T2D in the general population. Alternatively, hyperglycemia may downregulate ABCA1, a concept supported by the finding that leukocyte ABCA1 mRNA levels are inversely correlated with fasting plasma glucose levels in normoglycemic individuals (63). ABCA1 protein levels are reduced by unsaturated FFAs (64, 65) and advanced glycosylation end products (AGEs) (66), suggesting that the imbalances associated with the metabolic syndrome, such as elevated fatty acid levels and hyperglycemia, may themselves reduce ABCA1 activity. In adipocytes, ABCA1 levels are downregulated by insulin (67). This suggests that levels of ABCA1 in cells are associated with circulating levels of both glucose and insulin. It is not known whether the reduction in ABCA1 levels in diabetic subjects is cause or consequence of their disease. However, these findings raise the question of whether reduced ABCA1 activity could predispose to T2D in the general population. It would be useful to determine whether the changes in ABCA1 mRNA levels reflect genetic variation in ABCA1 and whether they have an impact on cholesterol efflux from β cells, which could explain the alterations in fasting glucose levels with which they are associated.

Collectively these studies suggest that impaired ABCA1 activity is associated with an increased risk of diabetes. However, the important question of whether Tangier disease — a rare inherited disorder caused by homozygous mutations in ABCA1 and characterized by a severe reduction in HDL levels (68) — itself predisposes to diabetes remains unanswered. Despite four decades of intensive research into Tangier disease, there is a paucity of published data on glucose homeostasis in these patients. However, the fact that diabetes is not commonly reported as a clinical feature of this disorder would suggest that the patients do not frequently develop T2D. One issue in addressing this question is that humans with Tangier disease have significantly reduced total plasma cholesterol, and in particular, LDL cholesterol is reduced by 40%–70% (69). These beneficial reductions in plasma lipids may limit the extent of β cell cholesterol accumulation that occurs in the complete absence of ABCA1 and could partially mask a disturbance in glucose homeostasis.

An analogous situation exists in Abca1 total knockout mice, which have very low levels of total plasma cholesterol (70) and may be partially protected from islet cholesterol accumulation. Indeed, the glucose intolerance phenotype is much more severe in Abca1 β cell–specific knockout mice, which have higher levels of circulating cholesterol (12). As a consequence, it may be necessary to study humans heterozygous for ABCA1 mutations, with higher levels of total plasma cholesterol, to detect a phenotype. It should be possible with techniques currently available to determine whether patients heterozygous for an ABCA1 mutation have impaired β cell function.

Interestingly, even for the transcription factor-7–like 2 (TCF7L2) gene, one of the genes with the strongest genetic evidence for involvement in the pathogenesis of T2D (71, 72), the effect on insulin secretion in humans is quite modest (73). This appears to be the case for other genes strongly linked with T2D (74). If a similar situation holds true for ABCA1, it will be necessary to perform detailed phenotypic studies of β cell function to accurately characterize the role of ABCA1 in islet function in humans.

Another exception seems to be familial hypercholesterolemia, in which patients have extremely high levels of LDL cholesterol in plasma but are not reported to develop T2D more frequently or at an earlier age than controls. It is interesting to note, however,

### Table 2

**ABCA1 variants associated with T2D**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R230C</td>
<td>Increased risk (4.5-fold) of T2D development in Mexican population</td>
<td>47</td>
</tr>
<tr>
<td>K776N</td>
<td>Increased risk (1.8-fold) of T2D development in males in Copenhagen Heart Study</td>
<td>61</td>
</tr>
<tr>
<td>R1615P</td>
<td>Associated with T2D in a heterozygous patient</td>
<td>59</td>
</tr>
<tr>
<td>Haplotype block in intron 2</td>
<td>Increased (2.5-fold) risk of T2D development in Japanese population</td>
<td>56</td>
</tr>
<tr>
<td>Exon 4 mutation</td>
<td>Associated with T2D development in a Japanese patient</td>
<td>60</td>
</tr>
</tbody>
</table>
that all 5 genetic conditions in humans associated with raised LDL cholesterol levels involve a reduction in the number or activity of LDL receptors (75, 76). Cholesterol uptake into islets appears to require the presence of LDL receptors, as it is reduced in islets isolated from mice lacking the LDL receptor (34). This could indicate that familial hypercholesterolemia and related disorders result in very high levels of cholesterol in plasma but a reduced uptake of cholesterol into islets, potentially explaining the finding that islet function does not appear to be impaired under these conditions.

Plasma cholesterol levels are associated with islet cholesterol content in mice (40). A corollary to this is that lowering levels of plasma cholesterol should reduce islet cholesterol levels and thereby reduce the risk of T2D. Indeed this seems to be the case, at least in certain circumstances. In the West of Scotland Coronary Prevention Study (WOSCOPS) trial, plasma LDL cholesterol was lowered by 26% with pravastatin, and over the following 6 years the risk of developing T2D was reduced by 30% (77, 78). Lipid lowering is also reported to decrease the risk of T2D after a myocardial infarction (79). However, not all statins have been reported to reduce the risk of T2D (80, 81). The reasons for this difference are not known but could relate to the finding that certain statins appear to negatively impact insulin secretion in vitro (45, 82), independently from their lipid-lowering effect.

Conclusion

T2D is amongst the major health crises of the present age. Data from several sources suggest that alterations of plasma and islet cholesterol metabolism may contribute to the pathogenesis of this disease. ABCA1, which regulates islet cholesterol efflux, is essential for normal β cell function, and absence of ABCA1 results in islet cholesterol overload and impaired insulin release. Several genetic alterations of cholesterol metabolism are associated with T2D. In vitro studies suggest that increasing islet cholesterol can induce β cell death and impairment of insulin secretion, while reducing islet cholesterol enhances insulin secretion. Individuals with T2D frequently have elevated plasma cholesterol levels, and lowering plasma cholesterol may reduce the risk of developing this disease.

Several important questions remain. Do humans with mutations in ABCA1 have impaired β cell function? What are the precise mechanisms by which ABCA1 and cholesterol impact β cell function? Do certain statins that reduce the incidence of T2D do so via cholesterol lowering? Many of these questions can now be addressed with currently available tools, and their answers may lead us closer to the tantalizing prospect of targeting cholesterol metabolism in order to simultaneously treat diabetes and the number one cause of death of persons with diabetes, namely cardiovascular disease.

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