Hepatic ABCA1 Expression Improves β-Cell Function and Glucose Tolerance

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Low HDL is a risk factor for the development of type 2 diabetes. Hepatic ABCA1 is the rate-limiting protein in HDL biogenesis, and mice lacking hepatic ABCA1 (ABCA1−/−) have very low plasma HDL concentrations. To investigate the role of hepatic ABCA1 in glucose tolerance and β-cell function, we used ABCA1−/− mice, which showed impaired glucose tolerance without changes in insulin sensitivity. Insulin secretion was reduced following glucose gavage. Ex vivo, glucose stimulated insulin secretion from β-cells from wild-type (WT) and ABCA1−/− mice was similar. Insulin secretion was, however, reduced upon addition of ABCA1−/− serum to the medium compared with WT serum, whereas islets lacking β-cell ABCA1 were not affected differently by ABCA1−/− or WT serum. After high-fat feeding, WT and ABCA1−/− mice showed no difference in glucose tolerance or insulin secretion, and serum from ABCA1−/− and WT mice fed a high-fat diet did not affect insulin secretion differently. We conclude that hepatic ABCA1 improves glucose tolerance by improving β-cell function through both HDL production and interaction with β-cell ABCA1. The beneficial effect of hepatic ABCA1 is decreased under metabolic stress. Increasing hepatic ABCA1 may represent a novel therapeutic strategy for improving glucose homeostasis in diabetes.

Low HDL is an independent risk factor for the development of cardiovascular disease and type 2 diabetes (1). Type 2 diabetes is characterized by elevated plasma glucose concentrations, insulin resistance, and β-cell dysfunction (2). There is mounting evidence that HDL and apolipoprotein A-I (apoA-I), its most abundant apolipoprotein, improve glucose homeostasis (3–6). Reconstituted HDL and apoA-I have been shown to increase insulin secretion in vitro (3), and apoA-I injections have been shown to improve insulin secretion, insulin sensitivity, and glucose tolerance in mice (4,5). In diabetic patients, infusions with reconstituted HDL increase plasma insulin concentrations and reduce plasma glucose concentrations (6). Furthermore, the cholesteryl ester transfer protein (CETP) inhibitor torcetrapib increases HDL and lowers blood glucose in hamsters (7), and CETP inhibition is associated with an increase in plasma insulin concentrations in humans (8). Taken together, these indicate a beneficial role of HDL-increasing therapies in glucose homeostasis. The failure of torcetrapib, the first CETP inhibitor, to improve cardiovascular outcome (9) raised the question of whether other targets increasing HDL concentrations may also have beneficial effects on glucose tolerance. The ATP binding cassette transporter (ABC) A1 is the major determinant of plasma HDL concentrations in mammals (10,11). Therefore, ABCA1 may be a promising target for novel HDL-increasing strategies that could reduce cardiovascular disease and improve glucose homeostasis in type 2 diabetes.

ABCA1 is a cholesterol transporter that interacts with lipid-free apoA-I and small HDL to mediate the efflux of cholesterol from cells and to generate HDL (10,11). ABCA1 deficiency in humans and ABCA1 knockdown in mice leads to extremely low plasma HDL-cholesterol concentrations, indicating that ABCA1 is the rate-limiting protein in HDL production (10,11). Mice with hepatocyte-specific ABCA1 deletion (ABCA1−/−) have over an 80% reduction in plasma HDL-cholesterol and plasma apoA-I concentrations (12,13), demonstrating that the liver is the most important site for HDL biogenesis. ABCA1, however, is widely expressed (14). Our group has
demonstrated a critical role for ABCA1 in the pancreatic β-cell. In mice lacking ABCA1 specifically in the β-cell (ABCA1⁻/⁻), glucose tolerance is impaired because of a defect in exocytosis of insulin granules, resulting in impaired insulin secretion (15,16). Therefore, hepatic ABCA1 may improve β-cell function through its role in HDL biogenesis and potentially via a subsequent impact on β-cell ABCA1. The aim of this study, therefore, was to evaluate whether hepatic ABCA1 affects glucose tolerance in vivo and to elucidate the mechanisms by which hepatic ABCA1 may affect glucose homeostasis.

RESEARCH DESIGN AND METHODS

Mice

Mice lacking hepatic ABCA1 (ABCA1⁻⁻⁻⁻) or β-cell ABCA1 (ABCA1⁻⁻⁻⁻⁻⁻⁻) were generated by crossbreeding floxed ABCA1 mice with Alb-cre or Ins2-cre mice, as described previously (12,15). Unless otherwise indicated, 2–month-old female mice were used. Mice had ad libitum access to water and chow or a high-fat, high-cholesterol (HFHC) diet (21% milk fat, 0.21% cholesterol; D12079B, Research Diets). Experiments were approved by the University of British Columbia animal ethics committee.

Plasma Analysis

Blood was drawn from mice fasted for 4 h and analyzed for glucose (One Touch glucose meter), insulin (Insulin ELISA; Merodia, Uppsala, Sweden), C-peptide (C-peptide ELISA; Alpco, Salem, NH), triglycerides (triglyceride reagent; Roche, Penzberg, Germany), and cholesterol (Infinity Reagent; Fisher, Waltham, MA), according to manufacturers’ protocols. HDL-cholesterol was measured after precipitation of the lipoproteins containing apolipoprotein B with heparin and manganese chloride (17).

Glucose Tolerance, Insulin Sensitivity, and Insulin Secretion Testing

Mice were fasted for 4 h and received 2 g/kg glucose (Sigma) in PBS orally or 1U/kg insulin (Novo Nordisk Bagsværd, Denmark) in PBS intraperitoneally. Blood was drawn at the indicated time points and was assayed for glucose, insulin, or C-peptide (17). Islets were isolated after perfusing the pancreas with collagenase (Sigma). Insulin secretion in response to low (1.67 mmol/L) and high (16.7 mmol/L) glucose was assessed. Insulin secretion was calculated as the percentage of islet insulin content and was normalized to basal secretion (15). Murine HDL was isolated by ultracentrifugation and dialyzed against PBS. ApoA-I was from BioVision (San Francisco, CA).

Statistical Analysis

Data were analyzed with two-way ANOVA or the Student t test using Prism 5 (GraphPad, La Jolla, CA). P < 0.05 was considered significant.

Figure 1—Impaired glucose tolerance in hepatocyte specific ABCA1 knockout mice. A and B: Blood was drawn from female WT mice (white bars) and ABCA1⁻⁻⁻⁻ mice (black bars) after 4 h of fasting. Blood glucose concentrations and plasma insulin concentrations were measured (n = 14–16). C and D: ABCA1⁻⁻⁻⁻ mice (black bar/circles) and control littermates (white bar/circles) received an oral gavage of 2 g/kg glucose in PBS. Blood was drawn at the indicated time points and assayed for glucose (n = 6–7 in C). Values are mean ± SEM. *P < 0.05; **P < 0.01. AUC, area under the curve.
RESULTS

Impaired Glucose Tolerance in Mice Lacking Hepatic ABCA1

Previous studies showed a crucial role for hepatic ABCA1 in HDL formation (12,13). We used hepatocyte-specific ABCA1 knockout mice (ABCA1-/-) to assess glucose homeostasis in mice with low HDL concentrations but with functional ABCA1 in other tissues, including β-cells. We observed a ~90% decrease in hepatic ABCA1 protein levels (Supplementary Fig. 1A). Total plasma cholesterol and HDL-cholesterol concentrations were greatly reduced in ABCA1-/- mice (Supplementary Fig. 1B and C). Plasma triglyceride concentrations and body weight were not affected in ABCA1-/- mice (Supplementary Fig. 1D and E). This confirms the previously described phenotype of ABCA1-/- mice (12).

To assess whether low HDL in ABCA1-/- mice is associated with alterations in glucose homeostasis, we measured plasma glucose and insulin. We observed an increase in glucose concentrations (Fig. 1A), as well as a reduction in plasma insulin concentrations (Fig. 1B), in ABCA1-/- mice. To evaluate whether hepatic ABCA1 affects glucose tolerance, we gave the mice an oral gavage of glucose and measured their glucose response. We observed significantly impaired glucose tolerance in female ABCA1-/- mice (+23%; P < 0.01; Fig. 1C and D) and in male mice (+17%; P < 0.05; Supplementary Fig. 2A and B).

Decreased Insulin Secretion in Mice Lacking Hepatic ABCA1

Changes in insulin resistance or insulin availability may underlie altered glucose tolerance (2). First, we evaluated whether changed insulin sensitivity could explain impaired glucose tolerance. An intraperitoneal bolus of insulin reduced plasma glucose concentrations to a similar extent in wild-type (WT) and ABCA1-/- mice (Fig. 2A and B and Supplementary Fig. 2C and D). This suggests that glucose intolerance in ABCA1-/- mice is not altered because of insulin resistance but may be a result of β-cell dysfunction. Indeed, compared with WT mice, ABCA1-/- mice demonstrated significantly lower insulin secretion in

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**Figure 2**—Impaired insulin secretion in hepatocyte-specific ABCA1 knockout mice. A and B: ABCA1-/- mice (black bar/circles) and control littersmates (white bar/circles) received a bolus of insulin to assess insulin sensitivity, and glucose was measured at the indicated time points. C and D: ABCA1-/- mice (black bar/circles) and control littersmates (white bar/circles) received an oral gavage of 2 g/kg glucose in PBS. Blood was drawn at the indicated time points and assayed for insulin. Values are mean ± SEM (n = 6–7). *P < 0.05; **P < 0.01. AUC, area under the curve; NS, not significant.
response to an oral glucose load (−33%; P < 0.05) (Fig. 2C and D). C-peptide is released after cleavage from pro-insulin. ABCA1<sup>−/−</sup> mice showed lower plasma C-peptide concentrations after glucose gavage (Supplementary Fig. 3). This suggests that hepatic ABCA1 improves glucose tolerance mainly by affecting β-cell function.

To evaluate how β-cell function is affected, we isolated pancreatic islets and measured their insulin secretion capacity. Glucose-stimulated insulin secretion from islets of ABCA1<sup>−/−</sup> mice was unaltered (Fig. 3A). Because ABCA1<sup>−/−</sup> mice have low plasma HDL concentrations, and HDL has been shown to affect insulin secretion directly (3), we hypothesized that insulin secretion may be affected by the interaction with HDL in plasma. To confirm that HDL directly affects insulin secretion, we incubated pancreatic islets in the absence or presence of murine HDL or apoA-I. Indeed, HDL and apoA-I stimulated insulin secretion (Supplementary Fig. 4A). Next, we assessed how insulin secretion is affected by serum from WT and ABCA1<sup>−/−</sup> mice. Interestingly, glucose-stimulated insulin secretion was significantly reduced in the presence of 10% serum from ABCA1<sup>−/−</sup> mice compared with WT serum (−56%; P < 0.05; Fig. 3B), suggesting a direct effect of plasma HDL on insulin secretion. We observed a similar difference in insulin secretion in the presence of WT or ABCA1<sup>−/−</sup> plasma when stimulated with potassium chloride (KCl) instead of glucose (Fig. 3C). KCl directly depolarizes the membrane. Therefore, these data suggest that plasma HDL affects insulin granule exocytosis.

We previously showed that β-cell ABCA1 stimulates insulin secretion by stimulating the exocytosis of insulin granules (15,16). Furthermore, HDL and apoA-I were unable to stimulate insulin secretion from ABCA1-deficient β-cells (3) (Supplementary Fig. 4B). Therefore, we evaluated whether β-cell ABCA1 is involved in our observations. Indeed, no differences in the insulin secretion from ABCA1-deficient β-cells in the presence of serum from WT or ABCA1<sup>−/−</sup> mice were observed (Fig. 3D). Taken together, these observations suggest that there are no major defects in the β-cells from ABCA1<sup>−/−</sup> mice, but that the presence of HDL in the plasma stimulates insulin secretion through interaction with ABCA1.

**Hepatocyte-Specific ABCA1 Knockout Mice Show No Impairment of Glucose Tolerance When Fed an HFHC Diet**

It has been suggested that HDL becomes dysfunctional under conditions of metabolic stress (18–21). To induce metabolic stress in our mice, we fed them an HFHC diet.

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**Figure 3**—Reduced insulin secretion in the presence of serum from hepatocyte-specific ABCA1 knockout mice. A: Islets were isolated from ABCA1<sup>−/−</sup> mice (black bar) and control mice (white bar) and insulin secretion upon incubation in Krebs-Ringer bicarbonate buffer with high glucose was measured. B: Islets were isolated and insulin secretion in response to high glucose was assessed in the presence of serum from WT (white bar) or ABCA1<sup>−/−</sup> mice (gray bar). C: Insulin secretion in response to potassium chloride (KCl) was assessed in the presence of serum from WT (white bar) or ABCA1<sup>−/−</sup> mice (gray bar). D: WT (hatched bar) and ABCA1<sup>p/p</sup> islets (white/gray bars) were isolated and glucose-stimulated insulin secretion was assessed in the presence of serum from WT (white bar) or ABCA1<sup>−/−</sup> mice (gray bar). Values are mean ± SEM (n = 6–7). Data are pooled from two independent experiments. *P < 0.05; **P < 0.01.
This diet increased body weight by ~27% without differences between the genotypes. Similar to mice fed chow, total and HDL cholesterol were reduced in ABCA1−/− mice, without changes in VLDL cholesterol or triglycerides (Supplementary Fig. 5). We observed no difference in glucose tolerance (Fig. 4A and B) or insulin secretion (Fig. 4C and D) in ABCA1−/− mice compared with WT mice on an HFHC diet. Furthermore, insulin secretion was reduced upon addition of serum from mice fed an HFHC diet, but we did not observe a further decrease in insulin secretion in the presence of ABCA1−/− serum (Fig. 4E). Also, HDL isolated from mice fed an HFHC diet was not able to stimulate insulin secretion (Fig. 4F). This may support the paradigm that HDL becomes dysfunctional under metabolic stress.

**DISCUSSION**

We previously demonstrated a crucial role for ABCA1 in β-cells in the regulation of insulin secretion through the modulation of insulin granule exocytosis (15,16). Furthermore, we and others showed that hepatic ABCA1 plays a critical role in the HDL biogenesis (12,13). We now show that hepatic ABCA1 affects glucose tolerance and insulin secretion in vivo, most likely through its role in the release of HDL in plasma and its interaction with β-cell ABCA1.
Low HDL in ABCA1\(^{-/-}\) mice leads to impaired glucose tolerance and decreased insulin secretion upon glucose gavage. We found no effect of hepatic ABCA1 on insulin sensitivity. This indicates that impaired glucose tolerance is caused by reduced insulin availability in mice with low HDL due to a lack of hepatic ABCA1.

Insulin secretion from islets from ABCA1\(^{-/-}\) mice ex vivo was not affected. Insulin secretion from pancreatic islets, however, was higher in the presence of serum from WT mice compared with serum from ABCA1\(^{-/-}\) mice. Furthermore, HDL isolated from mouse serum stimulated insulin secretion. This suggests that low HDL does not cause β-cell damage or β-cell death, but that plasma HDL directly affects insulin secretion. KCl directly depolarizes the plasma membrane. The observation that insulin secretion stimulated by KCl was higher in the presence of serum from WT mice compared with serum from ABCA1\(^{-/-}\) therefore suggests that exocytosis is affected. We demonstrated that β-cell ABCA1 stimulates insulin granule exocytosis (16). Insulin secretion in ABCA1 deficient islets was not differentially affected by plasma from ABCA1\(^{-/-}\) and WT mice. This is consistent with the observation that apoA-I and HDL require β-cell ABCA1 to stimulate insulin secretion. Together, these observations suggest that hepatic ABCA1 stimulates insulin secretion through the biogenesis of HDL and that HDL may interact directly with β-cell ABCA1 to stimulate insulin secretion.

It has been suggested that HDL becomes dysfunctional with regard to cholesterol efflux under metabolic stress in obesity (18–21). It was recently shown that apoA-I isolated from human atheroma is oxidized and dysfunctional with regard to ABCA1-dependent cholesterol efflux (21), supporting the hypothesis of HDL dysfunction in atherosclerosis. This observation may also be relevant to diabetes. Indeed, in patients with type 2 diabetes, HDL functionality, as measured by efflux capacity, was correlated with β-cell function (22). We show here that hepatic ABCA1 loses its protective role with regard to glucose tolerance and insulin secretion upon consumption of an HFHC diet. Insulin secretion is reduced in the presence of serum from mice fed an HFHC diet compared with those fed chow. This supports the paradigm that HDL becomes dysfunctional under metabolic stress.

Taken together, our findings confirm that HDL synthesized by hepatic ABCA1 improves β-cell function. HDL-increasing therapies for the treatment of patients with dyslipidemia are currently under development (23–25). ABCA1 is considered an important target for the development of such HDL-increasing strategies. Increased ABCA1 concentrations due to treatment with liver X receptor agonists or microRNA-33 inhibitors have been shown to increase plasma HDL-cholesterol concentrations in mice and monkeys, and these interventions reduce atherosclerosis in hyperlipidemic mice (23–25). Our data suggest that such drugs may also be beneficial and improve β-cell function in diabetes.