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Hepatic Overexpression of Abcb11 Promotes Hypercholesterolemia and Obesity in Mice

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Abstract

BACKGROUND & AIMS—ABCB11 is a canalicular transport protein that controls the rate-limiting step in hepatic bile acid secretion. Its expression levels vary in humans—it is not clear how these variations affect lipid metabolism. We investigated whether overexpression of *Abcb11* in mice increases lipid absorption in the intestine and affects the development of obesity or hypercholesterolemia.

METHODS—Transgenic mice that overexpress *Abcb11* in liver (TTR-*Abcb11*) and FVB/NJ mice (controls) were fed a high-cholesterol or high-fat diet for 12 weeks. Intestinal lipid absorption was measured by the dual fecal isotope method. Energy expenditure was measured by indirect calorimetry. The bile acid pool was analyzed by high-performance liquid chromatography.

RESULTS—TTR-*Abcb11* mice had a nearly 2-fold increase in intestinal cholesterol absorption, compared with controls. TTR-*Abcb11* mice fed a high-cholesterol diet had greater increases in plasma and hepatic levels of cholesterol and became more obese than controls; they also had increased intestinal absorption of fatty acids and decreased energy expenditure. In the TTR-*Abcb11* mice, the sizes of plasma and total bile acid pools were reduced; the bile acid pool contained more species of hydrophobic bile acids, compared with controls.

CONCLUSIONS—Hepatic overexpression of *Abcb11* in mice promotes diet-induced obesity and hypercholesterolemia; increased intestinal cholesterol absorption by hydrophobic bile acids might cause these features. Increased absorption fatty acids in the intestine and reduced expenditure of energy could increase weight gain in TTR-*Abcb11* mice. In humans, variations in expression of ABCB11 might confer genetic susceptibility to diet-induced hyperlipidemia and obesity.

Keywords

mouse model; micelle; energy expenditure; bile salt export pump

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Introduction

Bile acids are amphipathic molecules which are essential for intestinal lipid absorption. Bile acids are synthesized in the liver and are subsequently transferred in bile to the lumen of the small intestine where they form mixed micelles to aid in the digestion of dietary fat, cholesterol, and fat-soluble vitamins[1]. Mixed micelles serve as a transport vehicle for lipids across the unstirred water layer of the small intestine to facilitate uptake by enterocytes.

In addition to their function in intestinal lipid absorption, bile acids are also important for hepatic metabolism of cholesterol. Cholesterol is converted to bile acids in the liver, and hepatic secretion of bile acids across the canalicular membrane is coupled to the biliary secretion of cholesterol. Cholesterol secretion into bile, both as bile acids and unesterified cholesterol, serves as a primary mechanism of cholesterol elimination from the human body[2, 3].

ABCB11 encodes for the bile salt export pump located on the canalicular membrane of hepatocytes[4]. Transport across the canalicular membrane by ABCB11 is the rate-limiting step in the hepatocellular transport of bile acids[4]. Mutations resulting in complete loss of function of ABCB11 have been identified in humans. This is the genetic defect underlying progressive familial intrahepatic cholestasis (PFIC) type 2, which results in cirrhosis and death at a young age if liver transplantation is not performed[5]. ABCB11 has been shown to be highly polymorphic in humans, and its level of expression is widely variable [6–10]. Studies have linked such polymorphisms to dyslipidemia and obesity,[11, 12] yet, the impact of variable ABCB11 expression on the development of metabolic diseases is not well established.

We have generated a transgenic mouse which overexpresses *Abcb11* on a liver-specific transthyretin promoter (TTR-*Abcb11*). Initial characterization of the phenotype demonstrates that these mice hypersecrete bile acids, cholesterol, and phospholipids and rapidly develop gallstones on a lithogenic diet[13, 14]. Additionally TTR-*Abcb11* mice demonstrate suppression of hepatic cholesterol 7α-hydroxlyase (CYP7A1) expression, the enzyme controlling the rate-limiting step in bile acid synthesis from cholesterol.

We now aim to explore the effects of a high-cholesterol and high-fat diet on metabolic parameters in mice overexpressing hepatic *Abcb11*. We hypothesize that overexpression of hepatic *Abcb11* will lead to more efficient intestinal lipid absorption predisposing to hypercholesterolemia and obesity.

Methods

Animals and Diets

Male TTR-*Abcb11* mice (FVB background) and wild-type FVB/NJ mice (Jackson Laboratories, Bar Harbor, ME) age 8–10 weeks were fed a high-cholesterol diet (1.25% cholesterol w/w, 10% kcal%fat), high-fat diet (45% kcal%fat, 0.02% cholesterol w/w), or control diet (10% kcal%fat, 0.02% cholesterol w/w) for 12 weeks (Research Diets, New Brunswick, NJ). Mice were housed in colony cages with a 12-hour light/dark cycle, and were given free access to food and water. Body weight was measured weekly during the 12 week feeding protocol and food consumption was recorded. Mice were fasted for 4 hours prior to sacrifice and were euthanized by CO_2 inhalation. In the animals used for bile salt analysis, the liver, gallbladder, and small intestine were removed and minced in 100% ethanol. In the remaining animals, the livers were rapidly excised and flushed with ice-cold saline. The liver was sectioned and snap-frozen in liquid nitrogen. The gallbladder was

removed and examined for the presence of gallstones. The small intestine was removed, flushed with ice-cold saline, sectioned into 5 cm segments, and snap frozen in liquid nitrogen. Ileal tissue was defined as the distal 5cm of the small intestine. Jejunal tissue was defined as a segment 15 to 20cm distal to the stomach. The livers and small intestine were stored at −80°C until analysis. All animal protocols were approved by the Northwestern University Animal Care and Use Committee (ACUC).

Plasma and Liver Chemistries

Liver samples were homogenized in Dulbecco's phosphate buffered saline for hepatic lipid analysis (100mg liver tissue/mL). Cholesterol levels were measured in liver homogenate and plasma using an Infinity spectrophotometric assay per the manufacturer's protocol (Thermo Electron Corporation, Melbourne, Australia). Plasma bile acid concentrations were measured by a spectrophotometric assay (Bioquant, San Diego, CA).

Analysis of Gene Expression by Quantitative PCR

Total RNA from frozen liver and intestinal samples was isolated using TRIzol reagent. Two micrograms of total RNA was used for reverse transcription PCR using a qScript cDNA Synthesis Kit (Quanta BioSciences, Gaithersburg, MD). Real-time quantitative PCR was performed using 2 μL of cDNA from each sample in a 25 μL reaction mixture containing Quantitect SYBR Green PCR Mastermix (Qiagen, Valencia, CA) along with primers specific for the gene of interest. GAPDH was employed as a housekeeping gene. The primer sequences are shown in Supplemental Table 1. Amplification was performed on an ABI 7300 sequence detector (Applied Biosystems, Foster City, CA). Gene expression was calculated relative to controls using the comparative threshold cycle method as described in the Applied Biosystems Sequence Detection Systems instruction guide.

Intestinal Lipid Absorption

Intestinal cholesterol absorption was measured using the dual fecal isotope method of Wang et al[15]. TTR-*Abcb11* and FVB/NJ mice were fed a control diet or control diet supplemented with 2% cholestyramine for 4 weeks prior to measuring intestinal cholesterol absorption. Briefly, mice were given by oral gavage an intragastric bolus of 150μL medium-chain triglycerides containing 1μCi [^{14}C] cholesterol and 2μCi [^{3}H] sitostanol. Stool was collected for 4 days during which time mice were maintained on a control or cholestyramine-supplemented diet. The ratios of [^{14}C] cholesterol and [^{3}H] sitostanol were measured in fecal extracts and the dosing mixture.

Intestinal oleic acid absorption was measured using a modified dual-fecal isotope method. TTR-*Abcb11* and FVB/NJ mice fed a high-fat diet or high-fat diet supplemented with 2% cholestyramine for 4 weeks and were then given by oral gavage an intragastric bolus of 150μL olive oil containing 1μCi [^{14}C] oleic acid and 1μCi [^{3}H] sitostanol. Stool was collected for 4 days during which time the mice were maintained on a high-fat diet or high-fat diet supplemented with cholestyramine. The ratios of [^{14}C] oleic acid and [^{3}H] sitostanol were measured in fecal extracts and the dosing mixture.

HPLC assay

Total bile salt pool size and composition were measured by high-performance liquid chromatography (HPLC) as previously described [13]. Samples were spiked with glycocholic acid as an internal standard to control for extraction efficiency. Individual bile salt species were identified by their characteristic retention times as well as spiking with standards. The bile salt pool size and composition was calculated from a standard curve. Bile salt levels are reported as μmol/100g body weight (BW).

Indirect Calorimetry

Indirect calorimetry experiments were performed at the University of Cincinnati Mouse Metabolic Phenotyping Center using an Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH). Data was measured at 30 minute intervals for 24 hours. Mice had free access to standard rodent diet and water during this time period.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Comparisons between groups were performed using Student's *t*-test analysis.

RESULTS

TTR-*Abcb11* mice develop hypercholesterolemia and increased hepatic cholesterol accumulation when fed a high-cholesterol diet

As shown in Figure 1, TTR-*Abcb11* mice were found to have decreased baseline plasma cholesterol levels compared to wild-type mice (138 ± 20 vs 189 ± 36 mg/dL in WT, $p<0.01$). However, when fed a high-cholesterol diet for 12 weeks, TTR-*Abcb11* mice developed increased plasma cholesterol levels compared to wild-type mice (207 ± 37 vs 178 ± 22 mg/dL in WT, $p<0.05$).

When fed a control diet, TTR-*Abcb11* and wild-type mice had similar levels of cholesterol in liver homogenate (51.4 ± 9.1 vs 47.5 ± 10.2 mg chol/g prot in controls, NS) (Figure 1). However, when fed a high-cholesterol diet, TTR-*Abcb11* mice demonstrated enhanced hepatic cholesterol accumulation compared to wild-type mice (133.9 ± 29.2 vs 98.6 ± 31.4 mg chol/g prot in WT, $p<0.01$).

We have previously shown that TTR-*Abcb11* mice fed a lithogenic diet rapidly develop gallstones[14]. In the present experiments, TTR-*Abcb11* mice did not develop gallstones when fed a high-cholesterol diet.

Given the increased plasma and hepatic cholesterol content in TTR-*Abcb11* mice fed a high cholesterol diet, we next measured the expression of hepatic genes involved in cholesterol synthesis and transport (Table 1). TTR-*Abcb11* mice demonstrated lower baseline expression of hepatic HMG-CoA reductase (HMG-CoAR), the rate-limiting enzyme for cholesterol synthesis (0.58 ± 0.23 vs 1.00 ± 0.09 in WT, $p<0.01$). As expected, HMG-CoAR mRNA expression was suppressed in response to cholesterol feeding in wild-type mice (0.32 ± 0.07 vs 1.00 ± 0.09, $p<0.01$). However, cholesterol-fed TTR-*Abcb11* mice did not demonstrate significant suppression of HMG-CoAR compared to TTR-*Abcb11* mice fed a control diet (0.40 ± 0.18 vs 0.58 ± 0.23, NS). ABCG5 and ABCG8 constitute the heterodimeric transporter complex responsible for biliary cholesterol excretion. Expression of hepatic *Abcg5* and *Abcg8* mRNA was not significantly different between TTR-*Abcb11* and wild-type mice on a control diet and was equally upregulated by high-cholesterol feeding. There was no significant change in mRNA expression of LDL receptor (LDL-R) among any of the cohorts. Hepatic Niemann Pick C1-like 1 (NPC1L1) is a canalicular transmembrane protein that has been shown to reduce biliary cholesterol excretion, and is thought to counterbalance cholesterol efflux by ABCG5/8[16]. Hepatic NPC1L1 mRNA expression was not significantly different in TTR-*Abcb11* and wild-type mice on a control diet, however, when challenged with a high cholesterol diet, TTR-*Abcb11* showed increased expression of NPC1L1 (1.62 ± 0.10 vs 0.79 ± 0.34 in WT, $p<0.01$).

As we have previously shown, TTR-*Abcb11* mice demonstrated suppression of hepatic CYP7A1 mRNA[13]. However, when fed a high cholesterol diet, TTR-*Abcb11* mice

upregulated CYP7A1 expression relative to TTR-*Abcb11* mice fed a control diet (0.94 ± 0.43 vs 0.44 ± 0.38, p<0.05). Fibroblast growth factor (FGF) 15, an ileal derived peptide, is the primary mediator of bile acid feedback inhibition of CYP7A1[17]. At baseline, expression of ileal FGF15 mRNA was increased >2-fold in TTR-*Abcb11* mice compared to wild-type mice. High-cholesterol feeding increased FGF15 expression to a similar degree in TTR-*Abcb11* and wild-type mice (Table 1).

TTR-*Abcb11* mice demonstrate increased intestinal cholesterol absorption

Given the findings of enhanced hepatic cholesterol accumulation and increased plasma cholesterol levels in TTR-*Abcb11* mice in response to dietary cholesterol, we next explored the hypothesis that TTR-*Abcb11* mice more efficiently absorb dietary cholesterol. TTR-*Abcb11* mice demonstrated a nearly twofold increase in intestinal cholesterol absorption by the dual fecal isotope measurement (76.1 ± 7.0% vs 40.9 ± 13.4% in WT, p<0.01) (Table 2). We hypothesize that the enhanced intestinal cholesterol absorption in TTR-*Abcb11* mice is due to more efficient micelle formation by bile acids. Therefore we next explored the effect of cholestyramine feeding on cholesterol absorption in TTR-*Abcb11* mice. The enhanced intestinal cholesterol absorption was attenuated in TTR-*Abcb11* mice fed a diet supplemented with cholestyramine (57.4 ± 17.1%, p<0.05 vs control-fed TTR-*Abcb11*). There was no significant effect of diet or strain on jejunal expression of NPC1L1, *Abca1*, *Abcg5* or *Abcg8* expression, which are key regulators of intestinal cholesterol absorption[18–21] (Table 1).

TTR-*Abcb11* mice demonstrate increased weight gain when fed a high-fat diet

We next explored the effects of a high-fat diet on body weight gain in TTR-*Abcb11* mice. TTR-*Abcb11* mice demonstrated a greater increase in body weight compared to wild-type mice beginning at 5 weeks of high-fat feeding (Figure 2). There was no significant change in weight gain between TTR-*Abcb11* and wild-type mice fed a control diet. There was no difference in food consumption between TTR-*Abcb11* and wild-type mice to explain the differences in weight gain in response to high-fat feeding (3.1 ± 0.3 vs 3.1 ± 0.5 g/mouse/day, NS).

TTR-*Abcb11* mice demonstrate increased intestinal fat absorption

TTR-*Abcb11* and wild-type mice were fed a high-fat diet for 4 weeks prior to measurement of intestinal oleic acid absorption. TTR-*Abcb11* mice showed an increase in intestinal oleic acid absorption compared to wild-type mice by the dual fecal isotope method (98.5 ± 0.4% vs 97.8 ± 0.6%, p<0.05) (Table 3). We next measured intestinal oleic acid absorption in mice fed a high-fat diet supplemented with 2% cholestyramine for 4 weeks. Both TTR-*Abcb11* and FVB/NJ controls showed a marked reduction in oleic acid absorption when cholestytramine was added to the high-fat diet (87.7 ± 15.6% and 84.3 ± 15.4% in TTR-*Abcb11* and FVB/NJ mice respectively, NS).

We next examined the expression of intestinal genes involved in fatty acid absorption (Table 4). Fatty acid transport protein 4 (*Fatp4*) is localized to the apical brush border of intestinal epithelial cells and is thought to regulate absorption of dietary lipids[22]. Jejunal *Fatp4* expression was upregulated by high-fat feeding in wild-type but not TTR-*Abcb11* mice. Diacylglycerol O-acyltransferase 2 (*Dgat2*) is involved in intestinal intracellular formation of triglyceride in intestinal epithelial cells. Jejunal *Dgat2* expression was suppressed in TTR-*Abcb11* mice on a control diet and further suppressed by high-fat feeding. The scavenger receptor, CD36, located on the apical membrane of enterocytes is believed to mediate facilitated fatty acid uptake[23]. CD36 expression was similar in TTR-*Abcb11* and wild-type mice and was unaffected by diet.

As outlined above, high-fat diet fed TTR-*Abcb11* mice gained an average of 0.97g/wk compared to 0.74g/wk in wild-type mice, which equates to a difference of 0.23g/wk. Assuming that 100% of the weight gain was due to an increase in adipose tissue (9kcal/g fat), there is an average energy differential of 2.1 kcal/wk between TTR-*Abcb11* and wild-type mice. The observed increase in intestinal fat absorption in TTR-*Abcb11* mice accounts for an additional 0.32 kcal absorbed per week. As such, the weight gain observed in TTR-*Abcb11* mice cannot be fully explained by increased intestinal fat absorption.

TTR-*Abcb11* mice demonstrate reduced energy expenditure

As an alternative explanation for the enhanced weight gain observed in TTR-*Abcb11* mice on a high-fat diet, we next assessed energy expenditure using indirect calorimetry. As shown in Figure 3, TTR-*Abcb11* mice demonstrated reduced oxygen consumption (Figure 3A) and carbon dioxide production (Figure 3B), translating to a decrease in respiratory quotient (Figure 3C) and energy expenditure (Figure 3D).

The bile acid pool of TTR-*Abcb11* mice has an increased content of hydrophobic bile acid species

It has been shown that intestinal lipid absorption is facilitated by hydrophobic bile acids and inhibited by hydrophilic bile acids[24]. Additionally, bile acids have been shown to modulate energy expenditure[25]. We next analyzed the plasma bile acid levels and total bile acid pool size and composition in TTR-*Abcb11* and wild-type mice. Consistent with the finding of suppressed CYP7A1 expression, TTR-*Abcb11* mice showed a decreased total bile acid pool size compared to wild-type mice (23.4 ± 1.8 vs 38.1 ± 3.1 μmol/100g BW, $p<0.01$) (Supplemental Table 2). Likewise, the plasma bile acid concentration was reduced in TTR-*Abcb11* mice compared to controls (24.0 ± 7.6 vs 34.5 ± 7.6 μmole/L, $p=0.02$). High-cholesterol feeding resulted in expansion of the total bile acid pool in TTR-*Abcb11* mice (33.9 ± 1.8 vs 23.4 ± 1.8 μmol/100g BW in high-cholesterol and control-fed, respectively, $p<0.01$) but not in wild-type mice (41.0 ± 3.2 vs 38.1 ± 3.1 μmol/100g BW in high cholesterol and control-fed, respectively, NS). TTR-*Abcb11* mice demonstrated an increased content of the hydrophobic bile acid, taurodeoxycholic acid (TDCA) (0.56 ± 0.06 vs 0.16 ± 0.13 μmol/100g BW in WT, $p<0.01$) and a decreased content of the hydrophilic bile acid, tauromuricholic acid (TMCA) (12.7 ± 1.5 vs 25.9 ± 1.8 μmol/100g BW in WT, $p<0.01$). High cholesterol feeding increased the content of hydrophobic bile acid species, taurochenodeoxycholic acid (TCDCA) and TDCA, to a greater degree in TTR-*Abcb11* mice than in controls. The content of TMCA was decreased in TTR-*Abcb11* mice compared to controls on a high cholesterol diet (17.5 ± 2.4 vs 27.6 ± 2.5 μmol/100g BW, $p<0.01$).

High-fat feeding expanded the bile acid pool size in TTR-*Abcb11* (34.6 ± 1.7 vs 23.4 ± 1.8 μmol/100g BW, $p<0.05$) but not in wild-type mice (Supplemental Table 2). TTR-*Abcb11* mice fed a high-fat diet demonstrated a reduction in content of TMCA (19.1 ± 1.1 vs 25.2 ± 3.4 μmol/100g BW, $p<0.01$) and an increase in the content of TDCA (0.8 ± 0.5 vs 0.1 ± 0.1 μmol/100g BW, $p<0.05$).

DISCUSSION

Our findings indicate that hepatic overexpression of *Abcb11* enhances intestinal cholesterol and fatty acid absorption and reduces energy expenditure in mice. Wang et al have recently shown that hepatic overexpression of *Abcb11* in an AKR background results in enhanced intestinal cholesterol absorption, however, the physiologic relevance of this finding with respect to dyslipidemia and hepatic cholesterol accumulation was not explored.[26] Additionally, whether hepatic *Abcb11* overexpression results in enhanced intestinal fat absorption or causes obesity was previously unknown. We have now shown that Abcb11

overexpression causes increased plasma total cholesterol levels in mice fed a high-cholesterol diet and increased weight gain in mice fed a high-fat diet. We propose that the enhanced cholesterol absorption causes the hypercholesterolemia observed in TTR-*Abcb11* mice and both enhanced fatty acid absorption and decreased energy expenditure contribute to the increased weight gain. There were no increases in plasma cholesterol or weight gain in TTR-*Abcb11* mice fed a control (low-fat, low-cholesterol) diet, indicating that *Abcb11* overexpression results in a metabolic phenotype only when stressed by high intestinal lipid loads. This high-fat diet-induced obesity seen with murine *Abcb11* overexpression is similar to the obesity that occurs in humans when they consume a high-fat, high-cholesterol "Western" diet. This may implicate ABCB11 in the pathogenesis of diet-induced dyslipidemia and obesity in humans who consume a "Western" diet.

The level of expression of ABCB11 in humans is highly variable. Schuetz et al reported a 7-fold variation in ABCB11 expression in normal human liver samples[9]. The TTR-*Abcb11* mouse overexpresses *Abcb11* only 2-fold which is well within the range of ABCB11 variation in the general population. Therefore, we feel that the observed results may have important physiologic relevance to human disease.

We have previously shown that *Abcb11* overexpression in mice results in bile acid hypersecretion[13]. The enhanced intestinal lipid absorption in TTR-*Abcb11* mice could be explained by the increased biliary bile acid secretion leading to increased availability of bile acids in the intestine to promote micellar formation. Additionally, we have previously shown that *Abcb11* overexpression shifts the bile acid pool composition to an increased content of hydrophobic species and decreased content of hydrophilic species. In the present study we confirm this shift in bile acid composition persists when TTR-*Abcb11* mice are challenged with a high-cholesterol or high-fat diet. Hydrophobic bile acids increase micellar lipid solubility, thereby facilitating intestinal cholesterol and fat absorption[24, 27, 28]. The altered bile acid pool composition in TTR-*Abcb11* mice favors lipid solubilization in mixed micelles and may contribute to the enhanced intestinal lipid absorption. There was no significant change in the expression of intestinal lipid transport proteins to alternatively explain the enhanced intestinal lipid absorption.

The efficiency of intestinal cholesterol absorption is highly variable among and within species. In humans, intestinal cholesterol absorption ranges from 30 to 80%[29]. Among inbred strains of mice, intestinal cholesterol absorption ranges from 20 to 90%[15]. Modulating intestinal cholesterol absorption in humans has been shown to impact plasma cholesterol levels[30, 31]. Ezetimibe, for example, reduces intestinal cholesterol absorption by inhibiting NPC1L1 and is now widely used to treat hypercholesterolemia[31, 32]. The underlying etiology of the wide variation in cholesterol absorption in humans is poorly understood. Although this is likely to have a polygenic basis, our studies support the hypothesis that variable expression of ABCB11 may be a contributing factor. Supporting this hypothesis is the recent finding that polymorphisms in human ABCB11 are linked to alterations in total serum cholesterol level[11]. Pharmacologic therapies that target ABCB11 may prove to be beneficial in the management of disorders of lipid metabolism.

A potential role of ABCB11 in the development of obesity is supported by the recent observation that polymorphisms in ABCB11 may be linked to obesity in humans[12]. Whether this association is causative is highly speculative. Therefore, our studies aimed to directly address the physiologic consequences of *Abcb11* overexpression on the development of obesity in a murine model. We found a small but statistically significant increase in intestinal fat absorption in TTR-*Abcb11* mice. Our calculations of the energy differential demonstrate that this small increase in intestinal fat absorption does not entirely account for the increased weight gain in TTR-*Abcb11* mice relative to wild-type mice. As such, we

measured energy expenditure which was found to be significantly reduced in TTR-*Abcb11* mice. Bile acids have been shown to increase energy expenditure in brown adipose tissue via a TGR5-cAMP-D2 signaling pathway.[25] We speculate that the reduced plasma bile acid levels in TTR-*Abcb11* mice may contribute to the observed reduction in energy expenditure in this model. Despite the reduced energy expenditure at baseline, TTR-*Abcb11* mice did not gain significantly more weight than wild-type mice when maintained on a control diet indicating that other compensatory mechanisms are contributing. Food consumption was not statistically different between the strains. However, as previously proposed[33], it is possible that small differences in food intake, below the limits of detection, could account for small changes in body weight over prolonged periods of time. For example, the difference in energy expenditure between TTR-*Abcb11* and FVB/NJ mice equates to 6.0kcal/wk. To compensate for this degree of reduction in energy expenditure, TTR-*Abcb11* mice would have to decrease their food consumption by 0.2g/day (control diet = 3.85 kcal/g), which is within one standard deviation for this measurement, and therefore within the margin of error. Based on these calculations, we can conclude that a statistically insignificant reduction in food consumption could feasibly compensate for the reduced energy expenditure in TTR-*Abcb11* mice resulting in no significant weight gain when fed a control diet. Furthermore, this indiscernible change in food consumption may be lost in response to a high-fat diet, leading to weight gain in TTR-*Abcb11* mice. Additionally, the fact that TTR-*Abcb11* demonstrated phenotypic changes only when challenged with a high-fat diet may highlight the important contribution of enhanced intestinal fat absorption in this model.

TTR-*Abcb11* mice demonstrated reduced plasma cholesterol levels at baseline. We attribute this finding to the fact that TTR-*Abcb11* mice hypersecrete biliary cholesterol and eliminate it in stool[13]. We propose that when a low cholesterol diet is fed (e.g. rodent chow containing <0.02% cholesterol), the cholesterol lowering effect of enhanced biliary cholesterol secretion outweighs the effect of enhanced intestinal cholesterol absorption. Conversely, when TTR-*Abcb11* mice are fed a diet rich in cholesterol, the enhanced intestinal cholesterol absorption is the major determinant of cholesterol balance. In this way, *Abcb11* overexpression may be implicated specifically in diet-induced hypercholesterolemia.

We attribute the increased hepatic cholesterol content in cholesterol-fed TTR-*Abcb11* mice primarily to enhanced intestinal cholesterol absorption resulting in increased delivery of cholesterol to the liver. However, other contributing factors must be considered. Although HMG-CoAR expression in TTR-*Abcb11* mice was equal to wild-type mice on a high-cholesterol diet, the expression was not suppressed relative to TTR-*Abcb11* mice fed a control diet. This may indicate that TTR-*Abcb11* mice do not appropriately suppress HMG-CoAR in response to dietary cholesterol, which could contribute to the enhanced hepatic cholesterol accumulation. Additionally, the expression of hepatic NPC1L1 was increased in TTR-*Abcb11* in response to a high-cholesterol diet, which may promote accumulation of hepatic cholesterol via influx across the canalicular membrane. The mechanism by which *Abcb11* overexpression results in inadequate suppression of HMG-CoAR and NPC1L1 is unclear. We speculate that hypersecretion of cholesterol across the canalicular membrane in TTR-*Abcb11* mice may result in dysregulation of compensatory feedback inhibition pathways.

In the present study, we have implicated *Abcb11* overexpression in diet-induced metabolic disease via enhancement of intestinal lipid absorption and reduction of energy expenditure. Given the wide variability of ABCB11 expression among humans, the results of this work implicate ABCB11 as a novel gene target for the management of human disorders of lipid metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and terminology

TTR	transthyretin
CYP7A1	cholesterol 7α-hydroxylase
LDL-R	low density lipoprotein receptor
HMG-CoAR	HMG-CoA reductase
NPC1L1	Niemann-Pick C1 like 1
TMCA	tauromuricholic acid
TCA	taurocholic acid
TCDCA	taurochenodeoxycholic acid
TDCA	taurodeoxycholic acid, FGF, fibroblast growth factor
FGF	fibroblast growth factor

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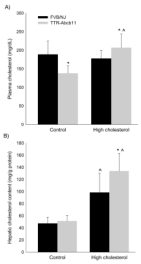

Figure 1.

TTR-*Abcb11* mice develop increased plasma and hepatic cholesterol levels relative to FVB/NJ control mice when fed a high-cholesterol diet. A) Plasma and B) Hepatic cholesterol levels in TTR-*Abcb11* and FVB/NJ control mice fed a control or 1.25% cholesterol diet for 12 weeks. Mean ± SD, *p<0.05 vs FVB/NJ controls on the same diet, ^p<0.05 vs control-fed mice of the same strain.

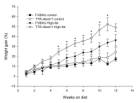

Figure 2.
TTR-*Abcb11* mice exhibit enhanced weight gain compared to FVB/NJ control mice when fed a high-fat diet. Weight gain (%) in TTR-*Abcb11* and FVB/NJ control mice fed a control or 45% high-fat diet for 12 weeks. Mean ± SEM, **p*<0.05 vs FVB/NJ on high-fat diet

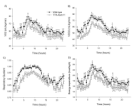

Figure 3.

TTR-*Abcb11* mice demonstrate reduced energy expenditure relative FVB/NJ control mice. A) Oxygen consumption (mL/kg/min), B) Carbon dioxide production (mL/kg/min), C) Respiratory quotient (RQ), and D) Energy expenditure (kcal/kg/hr) in TTR-*Abcb11* and FVB/NJ control mice over 24 hours. *$p<0.05$

Table 1

Hepatic and intestinal gene expression in TTR-*Abcb11* and FVB/NJ mice fed a control or high cholesterol diet for 12 weeks

	Control diet		High cholesterol diet	
Hepatic	**FVB/NJ**	**TTR-*Abcb11***	**FVB/NJ**	**TTR-*Abcb11***
HMG-CoAR	1.00 ± 0.09	0.58 ± 0.23*	0.32 ± 0.07^	0.40 ± 0.18
Abcg5	1.01 ± 0.12	0.86 ± 0.42	2.03 ± 0.49^	2.21 ± 0.46^
Abcg8	1.01 ± 0.14	0.88 ± 0.37	1.62 ± 0.30^	2.15 ± 0.31^
Abca1	1.04 ± 0.32	1.71 ± 1.20	1.78 ± 0.31^	2.54 ± 0.61
LDL-R	1.02 ± 0.22	1.18 ± 0.86	0.72 ± 0.31	1.06 ± 0.32
NPC1L1	1.00 ± 0.11	0.77 ± 0.23	0.79 ± 0.34	1.62 ± 0.10*^
CYP7A1	1.11 ± 0.51	0.44 ± 0.38*	0.53 ± 0.29^	0.94 ± 0.43^
Jejunal				
NPC1L1	1.12 ± 0.51	1.73 ± 0.57	0.69 ± 0.32	1.02 ± 0.33
Abca1	1.04 ± 0.30	1.22 ± 0.84	1.06 ± 0.31	1.20 ± 0.36
Abcg5	1.10 ± 0.52	1.52 ± 0.58	1.12 ± 0.10	1.41 ± 0.17
Abcg8	1.13 ± 0.63	1.80 ± 1.01	1.24 ± 0.12	1.20 ± 0.23
Ileal				
FGF15	1.11 ± 0.46	2.68 ± 1.53*	5.57 ± 4.04^	4.66 ± 1.92^

Values expressed as mean (n=6) ± SD.

* $p<0.05$ vs wild-type on matched diet,

^ $p<0.05$ vs matched strain on control diet.

Table 2

Intestinal cholesterol absorption (%) in TTR-*Abcb11* mice fed a control diet +/− cholestyramine (CSM) for 4 weeks

	Wild-type	TTR-*Abcb11*
Control diet	40.9 ± 13.3	76.1 ± 25.4*
Control diet + CSM	48.7 ± 9.4	57.4 ± 17.1^

Values expressed as mean (n=10) ± SD.

* $p<0.05$ vs wild-type on the same diet,

^ $p<0.05$ vs same strain on control diet

Table 3

Intestinal oleic acid absorption (%) in TTR-*Abcb11* mice fed a high-fat diet +/− cholestyramine (CSM) for 4 weeks

	Wild-type	TTR-*Abcb11*
High-fat diet	97.8 ± 0.6	98.5 ± 0.4*
High-fat diet + CSM	87.7 ± 15.6^	84.3 ± 15.4^

Values expressed as mean (n=10) ± SD.

* $p<0.05$ vs wild-type on the same diet,

^ $p<0.05$ vs same strain on control diet

Table 4

Intestinal gene expression in TTR-*Abcb11* and FVB/NJ mice fed a control or high-fat diet for 12 weeks

	Control diet		High fat diet	
Jejunal	**FVB/NJ**	**TTR-*Abcb11***	**FVB/NJ**	**TTR-*Abcb11***
CD36	1.02 ± 0.22	1.02 ± 0.36	0.87 ± 0.39	0.72 ± 0.21
Fatp4	1.08 ± 0.49	1.57 ± 0.96	2.26 ± 0.37^	1.68 ± 1.00
Dgat2	1.16 ± 0.67	0.51 ± 0.07*	0.70 ± 0.16	0.38 ± 0.16*

Values expressed as mean (n=6) ± SD.

* $p<0.05$ vs wild-type on matched diet,

^ $p<0.05$ vs matched strain on control diet.