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Impact of Inhibiting Ileal Apical Versus Basolateral Bile acid Transport on Cholesterol Metabolism and Atherosclerosis in Mice

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Abstract

Background—Bile acid sequestrants have been used for many years to treat hypercholesterolemia by increasing hepatic conversion of cholesterol to bile acids, thereby inducing hepatic LDL receptor expression and clearance of apoB-containing particles. In order to further understand the underlying molecular mechanisms linking gut-liver signaling and cholesterol homeostasis, mouse models defective in ileal apical membrane bile acid transport (*Asbt* null) and ileal basolateral membrane bile acid transport (*Ostα* null) were studied under basal and hypercholesterolemic conditions.

Key Messages—Hepatic conversion of cholesterol to bile acids is the major pathway for cholesterol catabolism and a major mechanism for cholesterol elimination. Blocking ileal apical membrane bile acid transport (*Asbt* null mice) increases fecal bile acid excretion, hepatic Cyp7a1 expression and the relative proportion of taurocholate in the bile acid pool, but decreases ileal FGF15 expression, bile acid pool size, and hepatic cholesterol content. In contrast, blocking ileal basolateral membrane bile acid transport (*Ostα* null mice) increases ileal FGF15 expression, reduces hepatic Cyp7a1 expression, and increases the proportion of tauro-β-muricholic acid in the bile acid pool. In the hypercholesterolemic *apoE* null background, plasma cholesterol levels and measurements of atherosclerosis were reduced in *Asbt/apoE* null mice but not in *Ostα/apoE* null mice.

Conclusions—Blocking intestinal absorption of bile acids at the apical versus basolateral membrane differentially affects bile acid and cholesterol metabolism, including the development of hypercholesterolemia-associated atherosclerosis. The molecular mechanism likely involves altered regulation of ileal FGF15 expression.

Keywords

Enterohepatic circulation; Intestine; liver; cholesterol; atherosclerosis

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Conflicts of Interest

Dr. Dawson has served as a consultant for Lumena Pharmaceuticals in the past.

Introduction

Bile acids are synthesized from cholesterol via 2 major pathways, the “classical” neutral pathway (Cholesterol 7α-hydroxylase, Cyp7a1, pathway) that favors cholic acid biosynthesis, and an “alternative” acidic pathway (Sterol 27-hydroxylase pathway) that favors biosynthesis of chenodeoxycholic acid in humans and biosynthesis of muricholic acid in mice. After their synthesis, bile acids are secreted and stored in the gall bladder [1]. Following a lipid-rich meal, the gall bladder empties its contents into the small intestine, where bile acids facilitate the digestion and absorption of dietary fat and cholesterol [2]. In the distal small intestine, bile acids are almost quantitatively reabsorbed and carried in the portal venous circulation back to the liver, where they are taken up, transported across the hepatocyte, and resecreted into bile [1]. The major transporters that function to maintain the EHC of bile acids have been identified [1]. In ileum, the apical sodium-dependent bile acid transporter (Asbt or ibat; *Slc10a2*) mediates uptake of bile acids from the gut lumen across the apical brush border membrane of the enterocyte [3], whereas the heteromeric transporter Ostα-Ostβ (Ost = Organic solute transporter) mediates intestinal basolateral bile acid export [4]. Ostα-Ostβ is comprised of two subunits, a 352 amino acid polytopic membrane protein (Ostα, *Slc51a*), and a 154 amino acid type I membrane protein (Ostβ, *Slc51b*) [5].

Intestinal Bile Acid Transport and Cholesterol Metabolism

In order to maintain the bile acid pool size under normal physiological conditions [6], hepatic conversion of cholesterol to bile acids balances the fecal excretion. This process is the major route for cholesterol catabolism and accounts for almost half of the cholesterol eliminated from the body each day [7]. Disruption of the enterohepatic circulation (EHC) of bile acids stimulates their *de novo* synthesis from cholesterol. The hepatic demand for cholesterol is met by increasing hepatic cholesterol synthesis and plasma clearance of lipoproteins such as LDL. Indeed, this mechanism is thought to be responsible for the lower plasma cholesterol levels and reduced cardiovascular disease associated with partial ileal bypass surgery in the Program on the Surgical Control of Hyperlipidemias (POSCH) study [8] or with the use of bile acid sequestrants in the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) [9]. The POSCH study is notable for being one of the first lipid-lowering clinical trials to show a statistically significant decrease in cardiovascular disease mortality, underscoring the beneficial effect of aggressively lowering plasma cholesterol levels [10]. Similar to treatment with bile acid sequestrants or ileal bypass, patients with ASBT mutations malabsorb bile acids and maintain decreased plasma LDL cholesterol levels [11]. In *Asbt* null mice, intestinal return of bile acids to the liver is significantly impaired, and hepatic Cyp7a1 mRNA expression and cholesterol conversion to bile acids is significantly increased [12,13]. Careful analysis of the cholesterol balance in wild type versus *Asbt* null mice revealed that cholesterol excretion as neutral and acidic sterols is increased almost 10-fold [12]. Cholesterol turnover in *Asbt* null mice is shown schematically in Fig. 1. The increase in cholesterol excretion is due to several mechanisms including increased fecal neutral sterol excretion secondary to decreased intestinal cholesterol absorption or possibly increased direct intestinal cholesterol excretion, and increased fecal bile acid excretion.

Gut-Liver Signaling, and Metabolism of Bile Acids and Cholesterol

Although generally assumed that bile acid sequestrants act by reducing delivery of bile acids to the liver [14], recent data suggests that bile acid sequestrant effects on gut-liver signaling may also be important. The regulation of hepatic Cyp7a1 expression and bile acid synthesis is complex [6]. Whereas a role for hepatic FXR in regulating Cyp7a1 expression has been appreciated for more than a decade [15,16], intestinal FXR's contribution was recognized only more recently [17,18]. In that FXR-FGF15 gut-liver signaling pathway, bile acids are taken up by ileal enterocytes where they activate FXR to induce expression of fibroblast growth factor (FGF) 15 (the mouse ortholog of human FGF19) [17]. FGF15 is then released into the portal circulation, and carried to the liver where it binds its receptor, the FGFR4/β-klotho complex on the surface of hepatocytes. This activates a signaling cascade that down-regulates expression of Cyp7a1 and other genes critical for hepatic bile acid biosynthesis [19,20].

In *Asbt* null mice, the contributions of altered hepatic versus altered ileal bile acid signaling could not be discerned. However, the *Ostα* null mouse, generated previously to test the hypothesis that Ostα-Ostβ is the major intestinal basolateral membrane bile acid transporter, provided such an opportunity. Both *Asbt* and *Ostα* null mice exhibit impaired ileal bile acid absorption, decreased return of bile acids to the liver and a decreased bile acid pool size [21,22]. However hepatic Cyp7a1 expression and bile acid synthesis is paradoxically suppressed in *Ostα* null mice, secondary to elevated ileal FGF15 production [23]. These results are significant with regard to the mechanism of action of bile acid sequestrants and also the potential side effect of dietary constituents, supplements, or drugs. Blocking intestinal absorption of bile acids at the enterocyte apical brush border membrane (e.g., bile acid sequestrants, dietary fiber, Asbt inhibitors) suppresses ileal FGF15 expression, induces hepatic bile acid synthesis, and reduces plasma cholesterol levels. Conversely, blocking basolateral membrane export may induce ileal FGF15 expression, inhibit hepatic bile acid synthesis, and potentially raise plasma cholesterol levels. Ostα-Ostβ's broad substrate specificity raises the concern that dietary constituents, dietary supplements, or drugs could act as Ostα-Ostβ inhibitors to slow bile acid export, activate FXR to induce intestinal FGF15/19 expression, and raise plasma cholesterol levels. Precedence already exists for part of this pathway. Cafestol, a component in unfiltered coffee that has been described as one of the most potent cholesterol-elevating compounds known in the human diet, is believed to operate by directly activating intestinal FXR to induce FGF19 expression and reduce hepatic Cyp7a1 expression [24]. Increased plasma LDL cholesterol levels have also been observed in a clinical trial using the synthetic FXR agonist, obeticholic acid [25].

Intestinal Bile Acid Transport and Atherosclerosis

The differences in sterol metabolism observed with blocking ileal apical membrane versus basolateral membrane bile acid transport may translate to altered susceptibility to development of hypercholesterolemia and atherosclerosis. Although wild type mice are relatively resistant to atherosclerosis, the genetically modified *LDLr* null and *apoE* null mice reproducibly develop hypercholesterolemia and atherosclerotic lesions, and are commonly used as experimental models to study the underlying mechanisms [26,27]. Since bile acid

sequestrants have been used for many years to treat hypercholesterolemia [28], it is surprising that there are relatively few published studies using *LDLr* or *apoE* null mice to examine the molecular mechanisms underlying their effects on the development of hypercholesterolemia and atherosclerosis.

Effect of Blocking Ileal Apical Brush Border Membrane Bile Acid Transport on the Development of Hypercholesterolemia and Atherosclerosis

The effect of inactivation of the Asbt on development of hypercholesterolemia and atherosclerosis in female *LDLr* and *apoE* null mice maintained for 16 weeks on an atherogenic diet has been reported recently [29]. In that study, introducing the *Asbt* null allele into the *LDLr* null or *apoE* null backgrounds resulted in significant reductions in total plasma cholesterol, apoB-containing lipoprotein (VLDL plus LDL) cholesterol, and the plasma cholesterol exposure (area under the curve) over the 16-week dietary challenge. In agreement with reductions in the apoB-containing lipoprotein fraction (VLDL and LDL), it was not surprising to find that aortic total cholesterol and cholesteryl ester content, markers of atherosclerosis [30], were also significantly reduced in *LDLr* and *apoE* null mice lacking the Asbt. The reduction in plasma cholesterol levels in the *Asbt/LDLr* and *Asbt/apoE* null mice appears to be secondary to the increased hepatic cholesterol demand for bile acid synthesis. Numerous other mouse studies have demonstrated a central role for hepatic Cyp7a1 and bile acid synthesis in cholesterol homeostasis, including the findings that the apoB-containing lipoprotein cholesterol and atherosclerosis are reduced in Cyp7a1 transgenic mice [31] and in models that induce hepatic Cyp7a1 expression, such as the Retnla transgenic mice [32]. The findings for the *Asbt/LDLr* and *Asbt/apoE* null mice also agree with previous reports of *LDLr* null mice or *apoE* null mice treated with a bile acid sequestrant [33,34], and *apoE* null, *LDLr/apoE* null, and *SR-BI/apoE* null mice treated with small molecule inhibitors of the Asbt [35–37]. Notably, the finding that blocking intestinal apical membrane bile acid absorption reduces plasma cholesterol levels even in the absence of LDL receptors and apoE [35] suggests that the atheroprotective effects are related to induction of a negative cholesterol balance and operates via mechanisms in addition to classical hepatic lipoprotein clearance [33]. The question remains to be answered whether this involves non-biliary mechanisms for cholesterol elimination such as trans-intestinal cholesterol excretion [38,39]. The recent study of the *Asbt/apoE* null mice also provided insight to the role of gut-liver signaling in this process. In agreement with the proposed role of the FXR-FGF15 pathway as a negative regulator of hepatic bile acid synthesis, there was a strong inverse correlation between the levels of mRNA expression for ileal FGF15 and hepatic Cyp7a1 in these models. However even more intriguing, there was a strong direct correlation between ileal FGF15 mRNA expression and total plasma cholesterol levels, and between ileal FGF15 mRNA expression and aortic cholesterol content, a measure of atherosclerosis [29]. The question of whether a similar relationship exists between plasma levels of FGF19 (human ortholog of mouse FGF15) and markers of atherosclerosis exists in humans has not yet been examined.

Effect of Blocking Ileal Basolateral Membrane Bile Acid Transport on the Development of Hypercholesterolemia and Atherosclerosis

It is generally assumed that different interventions that block intestinal return of bile acids to the liver should have similar effects on cholesterol homeostasis [7]. However the interventions examined to date, such as ileal bypass, bile acid sequestrants, and Asbt inhibitors, only blocked enterohepatic cycling of bile acids at the intestinal apical brush border membrane. As such, it was not possible to isolate individual contributions of ileal enterocyte and hepatocyte signaling to the phenotype. To further understand these underlying mechanisms, the effects of *Ostα* deficiency were studied in the *apoE* null mice [29]. In contrast to the findings in the *Asbt/apoE* null mice, blocking intestinal bile acid absorption by inactivating *Ostα* failed had no effect on hepatic cholesterol content, and the expression of genes important for cholesterol and bile acid synthesis, such as HMG-CoA reductase, HMG-CoA synthase, Cyp7a1 and Cyp8b1, were unaffected or reduced in *Ostα/apoE* null mice. Finally in *Ostα/apoE* null mice, ileal expression of FGF15 also tended to be higher or similar to the levels in *apoE* null mice, and significantly higher than *Asbt/apoE* null mice. In aggregate, this evidence supports a model (Figure 2) where repression of ileal FGF15 expression and induction of hepatic bile acid synthesis is required for the atheroprotective effects associated with the interruption of the enterohepatic circulation of bile acids.

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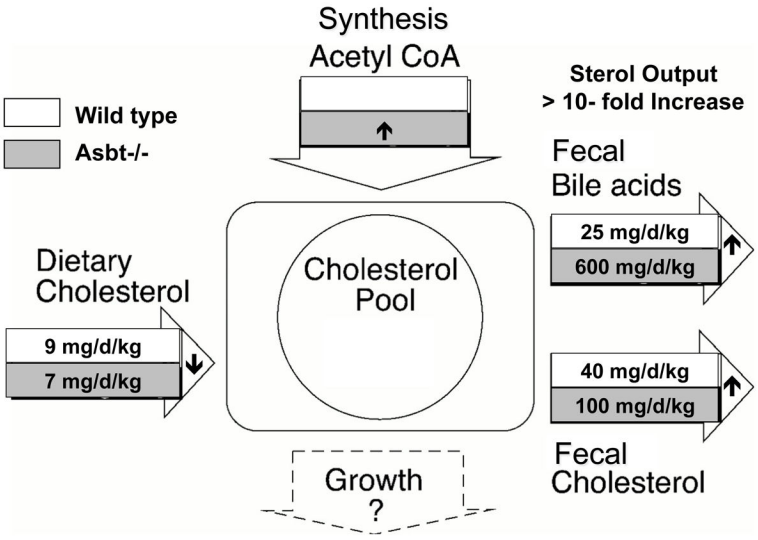
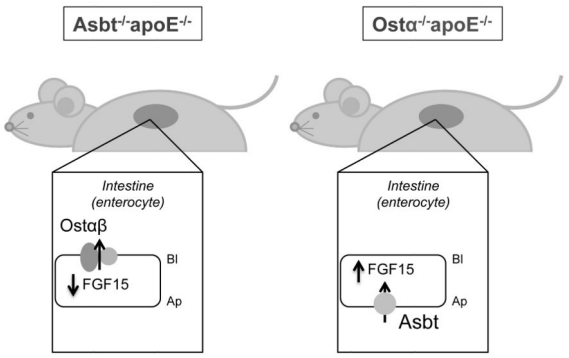


Fig. 1. Cholesterol Balance in Wild Type and Asbt null Mice

Schematic depicting sterol turnover in three-month old male wild type and *Asbt* null mice (129S6/SvEv background). The dietary cholesterol input was calculated by measuring daily food consumption and adjusting for the percent cholesterol absorption, as measured using the fecal dual isotope ratio method. Aliquots of quantitative fecal collections were used to measure bile acids and neutral sterols by colorimetric assay and gas-liquid chromatography, respectively. Cholesterol synthesis from acetyl CoA was not measured directly, but hepatic expression of HMG CoA reductase mRNA was increased in the *Asbt* null mice versus wild type mice. The whole body cholesterol pool (cholesterol content) was not measured, but is predicted to be unchanged and approximately 2200 mg per kg body weight [40]. Growth rates for the three-month old wild type and *Asbt* null mice appeared to be comparable. The small arrows indicate the direction of change in *Asbt* null versus wild type mice. The balance is calculated using the results from reference [12] and reproduced with permission.



Parameter	Asbt-/-apoE-/-	Ostα-/-apoE-/-
Plasma Lipids	↓ TPC, ↓ VLDL-C, ↓ LDL-C	(−) TPC, (−) VLDL-C, ↑ or (−) LDL-C
Hepatic Lipids	↓ TC, ↓ CE	(−) TC, (−) CE
Fecal Sterols	↑ Bile acids, ↑ neutral sterols	↓ Bile acids, ↑ neutral sterols
Hepatic Gene Expression	↑ Cyp7a1, Cyp8b1, Hmgcr, Hmgcs	↓ Cyp7a1, Cyp8b1, Hmgcr, Hmgcr
Ileal Gene Expression	↓ FGF15, Ibabp, Shp	↑ FGF15, Ibabp, Shp

Fig. 2. Phenotypic Differences Between *Asbt/apoE* and *Ostα/apoE* null mice

The arrows indicated the direction of change in *Asbt/apoE* null mice or *Ostα/apoE* null mice versus the matched *apoE* null mice. *Asbt/apoE* null mice exhibit decreased plasma total cholesterol (TPC), VLDL cholesterol (VLDL-C), and LDL cholesterol (LDL-C), and a decreased hepatic total cholesterol (TC) and cholesteryl ester (CE). The increased hepatic demand for cholesterol was secondary to a significant increase in hepatic Cyp7a1 expression, which was inversely correlated with significantly reduced levels of ileal FGF15 expression. Plasma and hepatic cholesterol levels were not significantly changed in *Ostα/apoE* null mice versus *apoE* null mice. In contrast to a block in intestinal bile acid absorption at the apical brush border membrane, disrupting the enterohepatic circulation of bile acids at the basolateral membrane failed to increase hepatic demand for cholesterol by inducing hepatic Cyp7a1 expression and bile acid synthesis.