Published in final edited form as:
Atherosclerosis. 2013 August ; 229(2): 374–380. doi:10.1016/j.atherosclerosis.2013.05.017.

Inhibition of ileal apical but not basolateral bile acid transport reduces atherosclerosis in apoE$^{-/-}$ mice

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Abstract

Objective—Interruption of the enterohepatic circulation of bile acids induces hepatic bile acid synthesis, increases hepatic cholesterol demand, and increases clearance of apoB-containing lipoproteins in plasma. Based on these effects, bile acid sequestrants have been used for many years to treat hypercholesterolemia and the associated atherosclerosis. The objective of this study was to determine the effect of blocking ileal apical versus basolateral membrane bile acid transport on the development of hypercholesterolemia and atherosclerosis in mouse models.

Methods and Results—ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice deficient in the apical sodium-dependent bile acid transporter (Asbt) or apoE$^{-/-}$ mice deficient in the basolateral bile acid transporter (Ostα) were fed an atherogenic diet for 16 weeks. Bile acid metabolism, cholesterol metabolism, gene expression, and development of atherosclerosis were examined. Mice deficient in Asbt exhibited the classic response to interruption of the enterohepatic circulation of bile acids, including significant reductions in hepatic and plasma cholesterol levels, and reduced aortic cholesteryl ester content. Ileal Fibroblast Growth Factor-15 (FGF15) expression was significantly reduced in Asbt$^{-/-}$apoE$^{-/-}$ mice and was inversely correlated with expression of hepatic cholesterol 7α-hydroxylase (Cyp7a1). Ileal FGF15 expression was directly correlated with plasma cholesterol levels and aortic cholesterol content. In contrast, plasma and hepatic cholesterol levels and atherosclerosis development were not reduced in apoE$^{-/-}$ mice deficient in Ostα.

Conclusions—Decreases in ileal FGF15, with subsequent increases in hepatic Cyp7a1 expression and bile acid synthesis appear to be necessary for the plasma cholesterol-lowering and atheroprotective effects associated with blocking intestinal bile acid absorption.

Keywords

Bile acids; cholesterol; apoE knockout mouse; atherosclerosis; Fibroblast growth factor 15; ileum; transporters

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Disclosures
P.A.D. has consulted for Isis Pharmaceuticals, and GlaxoSmithKline, and receive funding from Lumena Pharmaceuticals. Other authors report no conflicts of interest.

1. Introduction

Bile acids are synthesized in the liver, secreted into bile, and delivered to the small intestine, where they act to facilitate absorption of fats, cholesterol, and fat-soluble vitamins. Bile acids are almost quantitatively reclaimed from the intestine, and carried in the portal venous circulation back to the hepatocyte, where they are reabsorbed and resecreted into bile [1]. About 5% of the bile acids entering the small intestine escape reabsorption and are eliminated in the feces. Hepatic conversion of cholesterol to bile acid balances fecal excretion, and this process is the major route for cholesterol catabolism, accounting for almost half of the cholesterol eliminated from the body per day [2]. Disruption of the enterohepatic circulation (EHC) of bile acids stimulates *de novo* hepatic bile acid synthesis, thereby increasing hepatic cholesterol demand, cholesterol synthesis, and LDL receptor expression. This is the basis for the decreased plasma LDL cholesterol levels following ingestion of polymeric bile acid sequestrants [3, 4], administration of ileal bile acid transporter inhibitors [5, 6], or ileal bypass surgery such as in the POSCH study (Program on the Surgical Control of Hyperlipidemias) [7].

The transporters that function to maintain the EHC of bile acids have been identified [8]. In the ileum, the apical sodium-dependent bile acid transporter (Asbt or ibat; gene symbol *Slc10a2*) mediates the high capacity uptake of bile acids from the gut lumen across the brush border membrane of the enterocyte, whereas the heteromeric Organic Solute Transporter Ostα-Ostβ (gene symbols: *Osta*, *Slc51a1; Ostb*, *Slc51a1bp*) is responsible for basolateral membrane export of bile acids. Inactivation of either Asbt or Ostα impairs intestinal bile acid transport. However, *Osta* null mice fail to induce hepatic expression of cholesterol 7α-hydroxylase (Cyp7a1) and bile acid synthesis [9, 10], in striking contrast to a block in ileal apical brush border membrane uptake of bile acids [11, 12]. This is thought to be due to an inability to down-regulate expression of the ileal-derived polypeptide hormone fibroblast growth factor (FGF) 15 (human ortholog: FGF19) in Ostα$^{-/-}$ mice [13]. In addition to the flux of bile acids from intestine to liver, a critical role for gut-liver signaling via FGF15 has been recognized in the regulation of hepatic bile acid synthesis [14]. In this process, bile acids signal through the nuclear receptor FXR in ileal enterocytes to induce production of FGF15, which is secreted and carried to the liver, where it signals through the Fibroblast Growth Factor Receptor-4 (FGFR4)/β-klotho complex to repress Cyp7a1 expression and bile acid synthesis [15].

In light of the observed differences in bile acid metabolism between Asbt$^{-/-}$ and Ostα$^{-/-}$ mice, the aim of this study was to determine the effect of blocking ileal apical versus basolateral membrane bile acid transport on the development of hypercholesterolemia and atherosclerosis in mouse models. A second goal was to explore the relationship between ileal FGF15 expression and the atheroprotective effects associated with interruption of the EHC circulation of bile acids.

2. Materials and methods

For detailed methodology, please refer to the data supplement.

2.1. Mice and diets

The Institutional Animal Care and Use Committee approved all experiments. The Asbt$^{-/-}$ (129S6/SvEv) and Ostα$^{-/-}$ (C57BL/6) mice were generated as described previously [9, 11] and crossbred with apoE$^{-/-}$ (C57BL/6) or Ldlr$^{-/-}$ (C57BL/6) to generate the Asbt$^{-/-}$apoE$^{-/-}$, Asbt$^{-/-}$Ldlr$^{-/-}$, and Ostα$^{-/-}$apoE$^{-/-}$ mice. At 6 weeks of age the mice were switched from rodent chow to an atherogenic diet for 16 weeks. The atherogenic diet

contained 11% fat, 18% protein, 71% carbohydrate (% of Calories) and 0.280 mg/Calorie of cholesterol (0.1% w/w) [16].

2.2. Plasma, liver, biliary, and fecal lipid analysis

A fasting blood sample (4 h fast) was collected at the indicated time points to measure total plasma cholesterol (TPC) and triglyceride (TG). Plasma lipoprotein cholesterol distribution was quantified by FPLC size fractionation analysis. Hepatic levels of total cholesterol, free cholesterol, and TG were determined by enzymatic assay (Roche Applied Science) [11]. Gallbladder bile was used to measure phospholipid and bile acid by enzymatic assay; cholesterol was measured by gas-liquid chromatography. Individual bile acid species were quantified by HPLC. Feces were collected to measure total BA content by enzymatic assay and neutral sterol content by gas-liquid chromatography [11].

2.3. Quantification of aortic lipid content and lesion area

Whole aorta (from the sinotubular junction to iliac bifurcate) was removed, fixed in 10% formalin, and the adventitia was cleaned. For Ostα$^{-/-}$apoE$^{-/-}$ and matched apoE$^{-/-}$ mice, aortas were opened along the longitudinal axis and analyzed to quantify the percentage of total aortic surface covered with lesion. After surface lesion quantification, aortic total and free cholesterol concentrations were quantified by gas-liquid chromatography and normalized to aortic protein content. For Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice, aortic cholesterol content but not aortic lesion surface area was measured.

2.4. RNA and protein analyses

Total RNA was extracted from frozen tissue using TRIzol Reagent (Invitrogen). Real time PCR analysis was performed as described [13]. Tissue extracts were prepared and subjected to immunoblotting analysis using an affinity-purified rabbit anti-mouse FGF15 antibody [13]. Blots were also probed with anti-β-actin antibody. Protein expression was quantified by densitometry, and expression data were normalized to levels of the β-actin loading control.

2.5. Statistical analyses

Mean values±SEM are shown unless otherwise indicated. The data were evaluated for statistically significant differences using the two-tailed Student's t test. Plasma cholesterol and TG levels were evaluated for statistically significant differences using a 2-way repeated measures ANOVA with genotype and time as factors and post hoc analyses using the Tukey-Kramer honestly significant difference test. The correlations were calculated using a Spearman Test. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Effect of Asbt deficiency on plasma lipids, hepatic lipids, and atherosclerosis

The Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice were indistinguishable from their apoE$^{-/-}$ and Ldlr$^{-/-}$ littermates in terms of survival, gross appearance, and behaviors. As previously noted for the Asbt$^{-/-}$ mice [17], there was a small but significant decrease in body weight and liver weight in Asbt$^{-/-}$apoE$^{-/-}$ versus and apoE$^{-/-}$ mice after 16 weeks on diet; a similar decrease in liver but not body weight was also observed in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice (Supplemental Table 2). Plasma lipids were measured at baseline and during the 16-week diet study. As analyzed by repeated measures ANOVA, there was a significant decrease in TPC in the Asbt$^{-/-}$apoE$^{-/-}$ mice versus apoE$^{-/-}$ mice over the 16-week atherogenic diet challenge (Fig. 1A). There was also statistically significant reduction in the calculated TPC area under the curve (AUC) as evaluated using the two-tailed Student's t test

(18067±1448 versus 23222±1898 Arbitrary units; n=13–16, $p<0.05$). Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice also had higher plasma TG levels (Fig. 1B). After 16 weeks on diet, plasma was collected and lipoproteins were fractionated by FPLC to determine the cholesterol distribution. The cholesterol concentration was significantly lower for the apoB-containing lipoprotein fraction (VLDL and LDL) but not for the HDL fraction in Asbt$^{-/-}$apoE$^{-/-}$ mice (Fig. 1C and D). Hepatic lipids were measured after 16 weeks on diet; cholesteryl ester content was decreased by 68% in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice, but hepatic TG content remained elevated in both genotypes (302±21 versus 347±26 μg TG/mg liver wet weight in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice) (Fig. 1E). Aortic total cholesterol (Fig. 1F) and cholesteryl ester (2.66±0.77 versus 16.57±2.52 μg/mg protein; n=13–16, $p<0.05$) content was significantly reduced by approximately 54% and 84%, respectively, in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. As shown in Supplemental Fig. 1, reductions were also observed for TPC, apoB-containing lipoprotein (VLDL plus LDL) cholesterol, hepatic cholesteryl ester content, and aortic total cholesterol and cholesteryl ester content (5.49±1.01 versus 9.08±1.26 μg/mg protein; n=15–19, $p<0.05$) in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice. In addition, hepatic TG levels were also reduced in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice (Supplemental Fig. 1E). These findings are in general agreement with previous reports of apoE$^{-/-}$ and Ldlr$^{-/-}$ mice treated with a bile acid sequestrant [18, 19], and apoE$^{-/-}$, Ldlr$^{-/-}$/apoE$^{-/-}$, and SR-BI$^{-/-}$/apoE$^{-/-}$ mice treated with Asbt inhibitors [20–22]. As the findings were similar in the Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice, subsequent studies focused on examining the effects of Ostα deficiency in the apoE$^{-/-}$ mice.

3.2. Effect of Ostα deficiency on plasma lipids, hepatic lipids, and atherosclerosis

The Ostα$^{-/-}$apoE$^{-/-}$ mice were indistinguishable from their apoE$^{-/-}$ littermates in terms of survival, gross appearance, and behaviors. As previously noted for Ostα$^{-/-}$ mice [17], the small intestine was significantly longer and heavier, with ileum being approximately 60% heavier per unit length in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (Supplemental Table 3). Plasma lipids were measured at baseline and during the 16-week diet study. As analyzed by repeated measures ANOVA, there was no difference between the Ostα$^{-/-}$apoE$^{-/-}$ mice versus apoE$^{-/-}$ mice over the 16-week atherogenic diet challenge (Fig. 2A). Similarly, there was no statistically significant difference in the calculated TPC area under the curve. Plasma TG levels were similar in Ostα$^{-/-}$apoE$^{-/-}$ and apoE$^{-/-}$ mice throughout the study. After 16 weeks on diet, the TPC was increased approximately 10% in Ostα$^{-/-}$apoE$^{-/-}$ mice (886±52 versus 997±105 mg/dl; n=10) and cholesterol in the LDL fraction was significantly increased by 30% (Fig. 2C and D). Hepatic free cholesterol, cholesteryl ester, and TG content were similar in Ostα$^{-/-}$apoE$^{-/-}$ and apoE$^{-/-}$ mice (Fig. 2E). Atherosclerosis, measured as aortic total cholesterol content (Fig. 2F) or surface lesion area (Fig. 2G), was similar in Ostα$^{-/-}$apoE$^{-/-}$ and apoE$^{-/-}$ mice. There was no significant difference in the aortic cholesteryl ester content between the 2 genotypes (33.3±3.71 versus 34.9±5.1 μg/mg protein in Ostα$^{-/-}$apoE$^{-/-}$ and apoE$^{-/-}$ mice, respectively; n= 9–13 mice per group).

3.3. Effect of Asbt and Ostα deficiency on hepatic and ileal gene expression

In order to understand the mechanisms responsible for the differential effects on hepatic and plasma cholesterol observed in Asbt$^{-/-}$apoE$^{-/-}$ and Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice, hepatic and intestinal expression of genes important for cholesterol and bile acid synthesis were measured. As previously demonstrated in Asbt$^{-/-}$ mice [11, 12], hepatic expression of Cyp7a1, sterol 12α-hydroxylase (Cyp8b1), HMG CoA reductase (Hmgcr), and HMG CoA synthase (Hmgcs) were elevated in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (Fig. 3A). In agreement with the reduced bile acid absorption by ileum, mRNA expression of the FXR target genes Ostβ, Ibabp, Shp, and FGF15 were all reduced (Fig. 3C). Similar changes in gene expression were observed in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice (Supplemental Table 4). By contrast in Ostα$^{-/-}$apoE$^{-/-}$ mice, the expression of hepatic genes important for

cholesterol and bile acid synthesis tended to be lower or similar to levels in background-matched apoE$^{-/-}$ mice (Fig. 3B). In agreement with the predicted decrease in bile acid synthesis via the Cyp7a1 pathway, the ratio in gallbladder bile of the Cyp7a1-derived taurocholate to the alternative pathway-derived tauro-β-muricholate was reduced by 25% (0.68±0.03 versus 1.08±0.05; n=5; $p<0.05$) in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. In gallbladder bile, the mole percent bile acid was also reduced (71.7±0.8 versus 79.3±1.3%; n=9–10; $p<0.05$), with corresponding increases in the mole percent phospholipid (26.6±0.9 versus 17.0±1.4%) and cholesterol (5.7±0.1 versus 3.7±0.5%; $p<0.05$). As previous reported for Ostα$^{-/-}$ mice, expression of the Asbt was lower in the Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. In ileum, the expression of the FXR target genes Ibabp, Ostβ, Shp, and FGF15 tended to be higher or similar to the levels in apoE$^{-/-}$ mice (Fig 3D).

As FGF15 is thought to be a major physiological regulator of Cyp7a1 expression in the mouse, ileal FGF15 mRNA and protein expression was measured in a larger set of animals. Ileal FGF15 protein correlated with ileal FGF15 mRNA expression (Spearman $r_s = 0.83$; $p<0.0001$) (Supplemental Fig. 2B) and their expression was reduced approximately 80% in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (Fig. 3E). In those same mice, ileal FGF15 expression was negatively correlated with hepatic Cyp7a1 mRNA expression (Spearman $r_s = -0.57$; $p=0.0126$; Supplemental Fig. 2C), and positively correlated with TPC (Spearman $r_s = 0.60$; $p=0.0079$; Supplemental Fig. 2D) and aortic total cholesterol content (Spearman $r_s = 0.84$; $p<0.0001$; Supplemental Fig 2E). Ileal FGF15 mRNA and protein expression were higher in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (Fig. 3F). Ileal total FGF15 mRNA and protein expression (normalized for the increased ileal mRNA and protein content secondary to the increased ileal cell number) was increased approximately 3.6-fold in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (data not shown). An inverse correlation was also observed between ileal total FGF15 expression and hepatic Cyp7a1 mRNA expression in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (Supplemental Fig. 3C). However, little difference in TPC and aortic cholesterol content was observed between the Ostα$^{-/-}$apoE$^{-/-}$ and apoE$^{-/-}$ mice (Fig. 2), and there was no significant correlation between ileal total FGF15 mRNA expression and these endpoints in those mice (Supplemental Fig. 3D and 3E). Correlations for the pooled genotypes are shown in Supplemental Figure 4.

4. Discussion

A major finding of this study is that interruption of the EHC of bile acids at the intestinal apical versus basolateral membrane has differential effects on the development of hypercholesterolemia and atherosclerosis in the apoE null model. Blocking ileal bile acid absorption at the apical membrane effectively decreased plasma cholesterol levels and blunted development of atherosclerosis, whereas blocking intestinal basolateral membrane transport was not protective.

The effects associated with Asbt-deficiency in apoE$^{-/-}$ or Ldlr$^{-/-}$ mice were similar to those reported for bile acid sequestrants [18], ASBT inhibitors [20–22], or ASBT mutations in humans [23] and mice [11, 12], and included decreased levels of plasma apoB containing lipoproteins and decreased aortic cholesterol deposition. The Asbt$^{-/-}$apoE$^{-/-}$ mice exhibited increased hepatic cholesterol demand for bile acid synthesis, as evidenced by the reduced hepatic total cholesterol content and increased expression of HMG-CoA reductase, HMG-CoA synthase, Cyp7a1, and Cyp8b1. In contrast, hepatic expression of these genes important for cholesterol and bile acid synthesis were unaffected or reduced in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. Previous studies demonstrated an important role for hepatic Cyp7a1 in cholesterol homeostasis, including the findings that apoB-containing lipoprotein cholesterol is reduced in Cyp7a1 transgenic mice [24, 25] but increased in Cyp7a1 null mice [26, 27] or human subjects with CYP7A1 mutations [28]. It is important

to note that total plasma and hepatic cholesterol levels were not elevated in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice, despite evidence of reduced Cyp7a1 expression. A potential explanation is that the reduced conversion of cholesterol to bile acids in Ostα$^{-/-}$apoE$^{-/-}$ mice is balanced by reduced intestinal cholesterol absorption secondary to decreases in the size and hydrophobicity of the bile acid pool [13]. Indeed, fecal neutral sterol excretion was increased in Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice after 14 weeks on diet (8.26±0.73 versus 2.11±0.21 mg/day/100 g body weight; n=10; $p<0.05$). The effect of reduced intestinal cholesterol absorption may also be balanced by increased *de novo* cholesterol synthesis in small intestine since HMG CoA reductase and HMG CoA synthase mRNA expression was increased down the length of the small intestine of Ostα$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice (data not shown). It is also important to note that the mice were fed a cholesterol (0.1%)-containing, low fat, purified diet with alpha cellulose as the only source of fiber for the 16-week dietary challenge. The low cholesterol content, and more potent bile acid and cholesterol-binding properties of rodent chow may explain the reduced plasma cholesterol levels in the chow fed Ostα$^{-/-}$apoE$^{-/-}$ mice (at the 6-week old baseline measurement) and the reduced plasma cholesterol levels previously reported for both Ostα$^{-/-}$ and Asbt$^{-/-}$ mice under chow-fed conditions [9, 10, 13].

Previous studies suggest that interruption of the EHC of bile acids using bile acid sequestrants or ASBT inhibitors decreases plasma cholesterol by increasing LDL fractional turnover (receptor-mediated clearance) [5, 29, 30]. However, introduction of Asbt deficiency (this study), administration of bile acid sequestrants to Ldlr null mice [31], or administration of an ASBT inhibitor to Ldlr-apoE double null mice [20] also reduces plasma cholesterol levels, suggesting that additional mechanisms are also operative. The most likely explanation is that interruption of the EHC of bile acids (by administration of a bile acid sequestrant or inactivation/inhibition of the Asbt) leads to a significant negative cholesterol balance, as demonstrated recently in bile acid sequestrant-fed Ldlr null mice [19].

Similar to human subjects treated with bile acid sequestrants, plasma TG levels were increased in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. The underlying mechanisms by which interruption of the EHC of bile acids impairs TG homeostasis are complex and likely involve increased VLDL TG secretion as well as reducing plasma TG metabolism. An important role for FXR in this process is supported by the findings that plasma TG levels were decreased following administration of synthetic FXR agonists [32, 33] and increased in FXR null mice [34]. The mechanism appears to involve attenuating the FXR-mediated inhibition of hepatic de novo lipogenesis [35, 36], as well as decreasing expression of FXR target gene important for plasma TG metabolism expression such as apoC-II and phospholipid transfer protein [37]. However, the response can be modified significantly by diet and genetic factors and there is considerable heterogeneity in the plasma TG response to bile acids and bile acid sequestrants between different species, models, and experimental paradigms. For example, plasma TG levels were increased in the Asbt$^{-/-}$apoE$^{-/-}$ mice in this study, but plasma TG levels were variable and not reduced in apoE$^{-/-}$ mice treated with an Asbt inhibitor [21]. The increase in plasma TG levels was not observed in Asbt$^{-/-}$Ldlr$^{-/-}$ mice in our study, a result that is similar to a recent study of bile acid sequestrant-fed Ldlr$^{-/-}$ mice [19].

Another major finding is that these results suggest that repression of ileal FGF15 expression and induction of hepatic bile acid synthesis is required for the atheroprotective effects associated with the interruption of the EHC of bile acids. As previously reported [12, 17], Ileal FGF15 expression was significantly reduced in Asbt$^{-/-}$ mice. Further analysis revealed that ileal FGF15 expression was negatively correlated with hepatic Cyp7a1 expression, and positively correlated with plasma and aortic cholesterol levels. These findings are consistent with human studies where fasting circulating levels of the human ortholog of FGF15,

FGF19 are reduced in subjects treated chronically with cholestyramine [38], and analysis of a larger group of subjects found a negative correlation between serum levels of FGF19 and 7α-hydroxy-4-cholesten-3-one (C4) [39], an intermediate in the bile acid biosynthetic pathway and marker of CYP7A1 activity. Attempts to make similar measurements of mouse FGF15 protein in portal or peripheral blood using the previously generated anti-FGF15 antibody [13] or commercially available anti-mouse FGF15 antibodies have thus far been unsuccessful (data not shown). The reason is unclear, but may be due to low sensitivity or high background for the existing antibodies, rapid hepatic clearance of FGF15 resulting in very low systemic concentrations, or as yet unanswered questions regarding the secretion and circulation of FGF15. However, short-term administration of pharmacological doses of recombinant human FGF19 to *ob/ob* mice has recently been shown to increase plasma cholesterol levels [40], and the lipid raising action was thought to be secondary to activation of hepatic FGFR4 and repression of Cyp7a1 expression.

In conclusion, these results provide additional insight to the anti-hypercholesterolemic and atheroprotective mechanisms of action for interventions that interrupt the EHC of bile acids. However, as both inhibition of ileal bile acid absorption [4, 41] and administration of FGF15/19 [42, 43] have been reported to have beneficial effects with regard to glucose homeostasis and lipid metabolism in models of obesity and diabetes, further studies will be needed to understand the underlying molecular pathways and their therapeutic potential.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Janet sawyer for assistance with the statistical analysis. Sources of funding: Research reported in this publication was supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under award number DK047987 and an American Heart Association Mid-Atlantic Affiliate Grant-in-aid (to PAD).

References

1. Dawson, PA. Bile formation and the enterohepatic circulation. In: Johnson, LR., editor. Physiology of the Gastrointestinal Tract. 5. Oxford: Academic Press; 2012. p. 1461-1484.
2. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res. 1993; 34:1637–1659. [PubMed: 8245716]
3. Packard CJ, Shepherd J. The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery. J Lipid Res. 1982; 23:1081–1098. [PubMed: 6757355]
4. Out C, Groen AK, Brufau G. Bile acid sequestrants: more than simple resins. Curr Opin Lipidol. 2012; 23:43–55. [PubMed: 22186660]
5. Huff MW, Telford DE, Edwards JY, Burnett JR, Barrett PH, Rapp SR, et al. Inhibition of the apical sodium-dependent bile acid transporter reduces LDL cholesterol and apoB by enhanced plasma clearance of LDL apoB. Arterioscler Thromb Vasc Biol. 2002; 22:1884–1891. [PubMed: 12426220]
6. Root C, Smith CD, Sundseth SS, Pink HM, Wilson JG, Lewis MC. Ileal bile acid transporter inhibition, CYP7A1 induction, and antilipemic action of 264W94. J Lipid Res. 2002; 43:1320–1330. [PubMed: 12177176]
7. Buchwald H, Varco RL, Matts JP, Long JM, Fitch LL, Campbell GS, et al. Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). N Engl J Med. 1990; 323:946–955. [PubMed: 2205799]

8. Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res. 2009; 50:2340–2357. [PubMed: 19498215]
9. Rao A, Haywood J, Craddock AL, Belinsky MG, Kruh GD, Dawson PA. The organic solute transporter alpha-beta, Ostalpha-Ostbeta, is essential for intestinal bile acid transport and homeostasis. Proc Natl Acad Sci U S A. 2008; 105:3891–3896. [PubMed: 18292224]
10. Ballatori N, Fang F, Christian WV, Li N, Hammond CL. Ostalpha-Ostbeta is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. Am J Physiol Gastrointest Liver Physiol. 2008; 295:G179–G186. [PubMed: 18497332]
11. Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Kluckman K, et al. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. J Biol Chem. 2003; 278:33920–33927. [PubMed: 12819193]
12. Lundasen T, Andersson EM, Snaith M, Lindmark H, Lundberg J, Ostlund-Lindqvist AM, et al. Inhibition of intestinal bile acid transporter Slc10a2 improves triglyceride metabolism and normalizes elevated plasma glucose levels in mice. PLoS One. 2012; 7:e37787. [PubMed: 22662222]
13. Lan T, Rao A, Haywood J, Kock ND, Dawson PA. Mouse organic solute transporter alpha deficiency alters FGF15 expression and bile acid metabolism. J Hepatol. 2012; 57:359–365. [PubMed: 22542490]
14. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab. 2005; 2:217–225. [PubMed: 16213224]
15. Kong B, Wang L, Chiang JY, Zhang Y, Klaassen CD, Guo GL. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. Hepatology. 2012; 56:1034–1043. [PubMed: 22467244]
16. Lee RG, Kelley KL, Sawyer JK, Farese RV Jr, Parks JS, Rudel LL. Plasma cholesteryl esters provided by lecithin:cholesterol acyltransferase and acyl-coenzyme a:cholesterol acyltransferase 2 have opposite atherosclerotic potential. Circ Res. 2004; 95:998–1004. [PubMed: 15486318]
17. Jung D, Inagaki T, Gerard RD, Dawson PA, Kliewer SA, Mangelsdorf DJ, et al. FXR agonists and FGF15 reduce fecal bile acid excretion in a mouse model of bile acid malabsorption. J Lipid Res. 2007; 48:2693–2700. [PubMed: 17823457]
18. Terasaka N, Miyazaki A, Kasanuki N, Ito K, Ubukata N, Koieyama T, et al. ACAT inhibitor pactimibe sulfate (CS-505) reduces and stabilizes atherosclerotic lesions by cholesterol-lowering and direct effects in apolipoprotein E-deficient mice. Atherosclerosis. 2007; 190:239–247. [PubMed: 16626720]
19. Meissner M, Wolters H, de Boer RA, Havinga R, Boverhof R, Bloks VW, et al. Bile acid sequestration normalizes plasma cholesterol and reduces atherosclerosis in hypercholesterolemic mice. No additional effect of physical activity. Atherosclerosis. 2013
20. Galman C, Ostlund-Lindqvist AM, Bjorquist A, Schreyer S, Svensson L, Angelin B, et al. Pharmacological interference with intestinal bile acid transport reduces plasma cholesterol in LDL receptor/apoE deficiency. Faseb J. 2003; 17:265–267. [PubMed: 12475897]
21. Bhat BG, Rapp SR, Beaudry JA, Napawan N, Butteiger DN, Hall KA, et al. Inhibition of ileal bile acid transport and reduced atherosclerosis in apoE−/− mice by SC-435. J Lipid Res. 2003; 44:1614–1621. [PubMed: 12810816]
22. Braun A, Yesilaltay A, Acton S, Broschat KO, Krul ES, Napawan N, et al. Inhibition of intestinal absorption of cholesterol by ezetimibe or bile acids by SC-435 alters lipoprotein metabolism and extends the lifespan of SR-BI/apoE double knockout mice. Atherosclerosis. 2007
23. Oelkers P, Kirby LC, Heubi JE, Dawson PA. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J Clin Invest. 1997; 99:1880–1887. [PubMed: 9109432]
24. Li T, Matozel M, Boehme S, Kong B, Nilsson LM, Guo G, et al. Overexpression of cholesterol 7alpha-hydroxylase promotes hepatic bile acid synthesis and secretion and maintains cholesterol homeostasis. Hepatology. 2011; 53:996–1006. [PubMed: 21319191]

25. Miyake JH, Duong-Polk XT, Taylor JM, Du EZ, Castellani LW, Lusis AJ, et al. Transgenic expression of cholesterol-7-alpha-hydroxylase prevents atherosclerosis in C57BL/6J mice. Arterioscler Thromb Vasc Biol. 2002; 22:121–126. [PubMed: 11788471]
26. Post SM, Groenendijk M, van der Hoogt CC, Fievet C, Luc G, Hoekstra M, et al. Cholesterol 7alpha-hydroxylase deficiency in mice on an APOE*3-Leiden background increases hepatic ABCA1 mRNA expression and HDL-cholesterol. Arteriosclerosis, thrombosis, and vascular biology. 2006; 26:2724–2730.
27. Erickson SK, Lear SR, Deane S, Dubrac S, Huling SL, Nguyen L, et al. Hypercholesterolemia and changes in lipid and bile acid metabolism in male and female cyp7A1-deficient mice. J Lipid Res. 2003; 44:1001–1009. [PubMed: 12588950]
28. Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, et al. Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. J Clin Invest. 2002; 110:109–117. [PubMed: 12093894]
29. Gaw A, Packard CJ, Lindsay GM, Collins SM, Lorimer AR, Shepherd J. Metabolism of apolipoprotein B in primary moderate hypercholesterolaemia: effects of acipimox and cholestyramine therapy. Eur J Med Res. 1995; 1:38–48. [PubMed: 9392692]
30. Gaw A, Packard CJ, Lindsay GM, Murray EF, Griffin BA, Caslake MJ, et al. Effects of colestipol alone and in combination with simvastatin on apolipoprotein B metabolism. Arteriosclerosis, thrombosis, and vascular biology. 1996; 16:236–249.
31. Rudling M, Angelin B. Growth hormone reduces plasma cholesterol in LDL receptor-deficient mice. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2001; 15:1350–1356. [PubMed: 11387232]
32. Evans MJ, Mahaney PE, Borges-Marcucci L, Lai K, Wang S, Krueger JA, et al. A synthetic farnesoid X receptor (FXR) agonist promotes cholesterol lowering in models of dyslipidemia. Am J Physiol Gastrointest Liver Physiol. 2009; 296:G543–552. [PubMed: 19136377]
33. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci U S A. 2006; 103:1006–1011. [PubMed: 16410358]
34. Zhang Y, Wang X, Vales C, Lee FY, Lee H, Lusis AJ, et al. FXR deficiency causes reduced atherosclerosis in Ldlr−/− mice. Arterioscler Thromb Vasc Biol. 2006; 26:2316–2321. [PubMed: 16825595]
35. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest. 2004; 113:1408–1418. [PubMed: 15146238]
36. Herrema H, Meissner M, van Dijk TH, Brufau G, Boverhof R, Oosterveer MH, et al. Bile salt sequestration induces hepatic de novo lipogenesis through farnesoid X receptor- and liver X receptor alpha-controlled metabolic pathways in mice. Hepatology. 2010; 51:806–816. [PubMed: 19998408]
37. Kast HR, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol. 2001; 15:1720–1728. [PubMed: 11579204]
38. Lundasen T, Galman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. J Intern Med. 2006; 260:530–536. [PubMed: 17116003]
39. Galman C, Angelin B, Rudling M. Pronounced variation in bile acid synthesis in humans is related to gender, hypertriglyceridaemia and circulating levels of fibroblast growth factor 19. J Intern Med. 2011; 270:580–588. [PubMed: 22003820]
40. Wu X, Ge H, Baribault H, Gupte J, Weiszmann J, Lemon B, et al. Dual actions of fibroblast growth factor 19 on lipid metabolism. Journal of lipid research. 2013; 54:325–332. [PubMed: 23204296]
41. Watanabe M, Morimoto K, Houten SM, Kaneko-Iwasaki N, Sugizaki T, Horai Y, et al. Bile acid binding resin improves metabolic control through the induction of energy expenditure. PLoS One. 2012; 7:e38286. [PubMed: 22952571]

42. Potthoff MJ, Boney-Montoya J, Choi M, He T, Sunny NE, Satapati S, et al. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1alpha pathway. Cell metabolism. 2011; 13:729–738. [PubMed: 21641554]

43. Wu AL, Coulter S, Liddle C, Wong A, Eastham-Anderson J, French DM, et al. FGF19 regulates cell proliferation, glucose and bile acid metabolism via FGFR4-dependent and independent pathways. PLoS One. 2011; 6:e17868. [PubMed: 21437243]

Highlights

- Inactivation of Asbt attenuates atherosclerosis in apoE$^{-/-}$ and Ldlr$^{-/-}$ mice
- Inactivation of Ostα was not atheroprotective in apoE$^{-/-}$ mice
- Ileal FGF15 expression positively correlated with atherosclerosis in apoE$^{-/-}$ mice

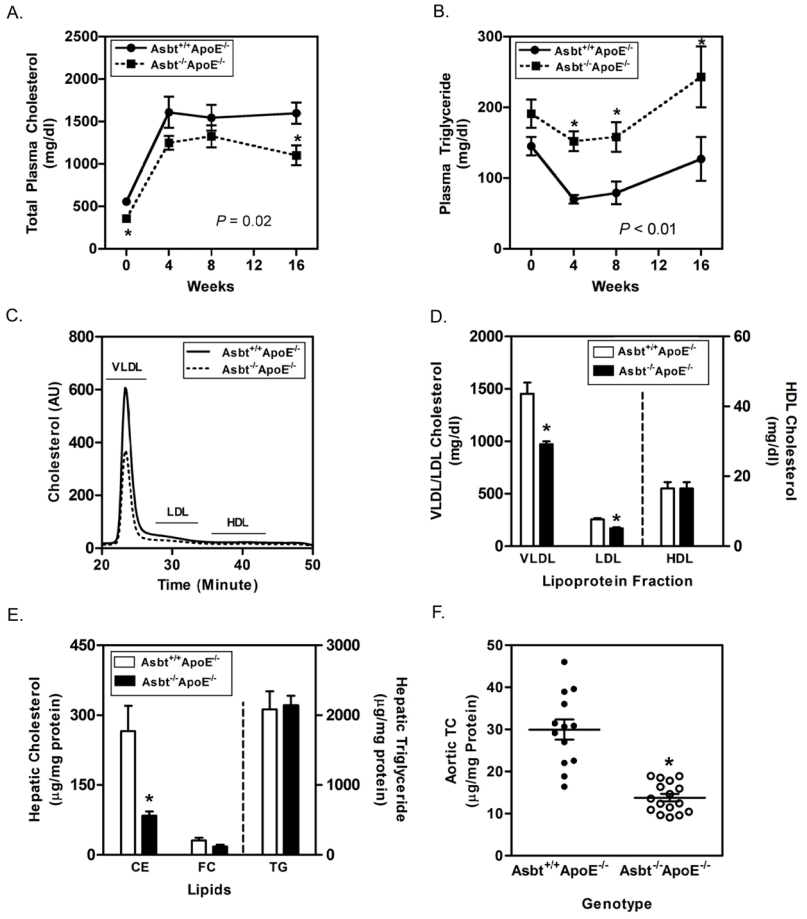

Fig. 1.

Effect of Asbt-deficiency on plasma lipids, hepatic lipids, and aortic cholesterol content in apoE$^{-/-}$ mice. Female apoE$^{-/-}$ and Asbt$^{-/-}$apoE$^{-/-}$ mice were fed an atherogenic diet for 16 weeks. (A) Plasma total cholesterol levels. (B) Plasma TG levels (n =13–16). (C) Separation of plasma lipoprotein fractions by FPLC and analysis by online cholesterol assay. Panel shows the mean profile (n=4–5). The VLDL, LDL, and HDL regions are indicated. (D) Levels of plasma cholesterol in the VLDL, LDL, and HDL fractions (n=5). (E) Hepatic lipid levels (n=5) were analyzed by enzymatic assay. (F) Measurements of total cholesterol extracted from aortas expressed as μg of total cholesterol/mg of aortic protein (n=13–16). *P* values in panels 1A and 1B were determined by 2-way repeated measures ANOVA with genotype and time as factors. Asterisks indicate a significant mean difference from Asbt$^{+/+}$apoE$^{-/-}$ mice (p<0.05).

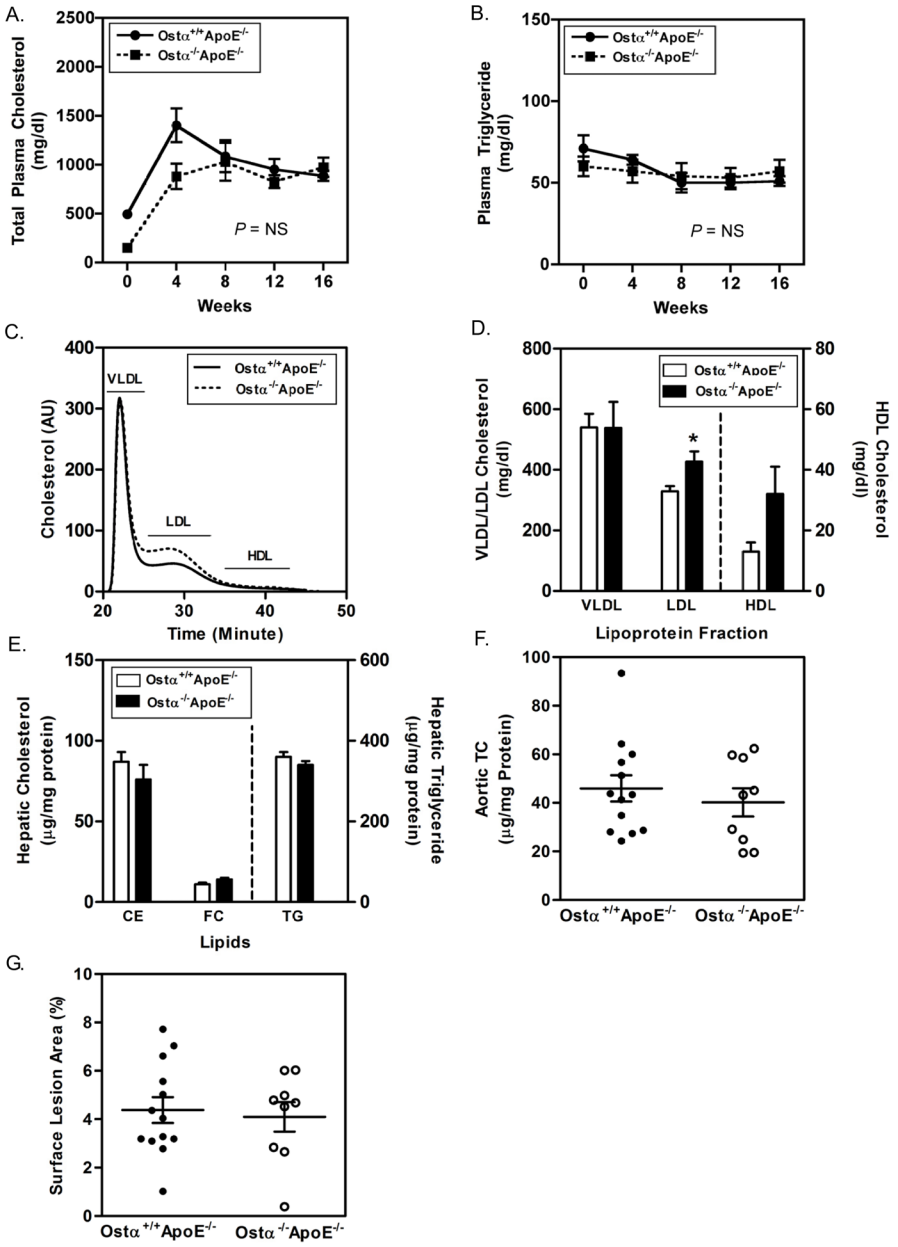

Fig. 2.

Effect of Ostα-deficiency on plasma lipids, hepatic lipids, and aortic cholesterol content in apoE$^{-/-}$ mice. Female apoE$^{-/-}$ and Ostα$^{-/-}$apoE$^{-/-}$ mice were fed an atherogenic diet for 16 weeks. (A) Plasma total cholesterol levels. (B) Plasma TG levels (n=10). (C) Separation of plasma lipoprotein fractions by HPLC and analysis by online cholesterol assay. Panel shows the mean profile (n=10). The VLDL, LDL, and HDL regions are indicated (n=10). (D) Levels of plasma cholesterol in the VLDL, LDL, and HDL fractions (n=5). (E) Hepatic lipid levels were analyzed by an enzymatic assay. (F) Measurements of total cholesterol extracted from aortas expressed as μg of total cholesterol/mg of aortic protein (n=9–13). (G) Aortic surface lesion area was normalized to percentage of total aortic surface area (n=9–13). *P* values in panels 1A and 1B were determined by 2-way repeated measures ANOVA with genotype and time as factors. NS, not significant. Asterisks indicate a significant mean difference from the Ostα$^{+/+}$apoE$^{-/-}$ mice ($p<0.05$).

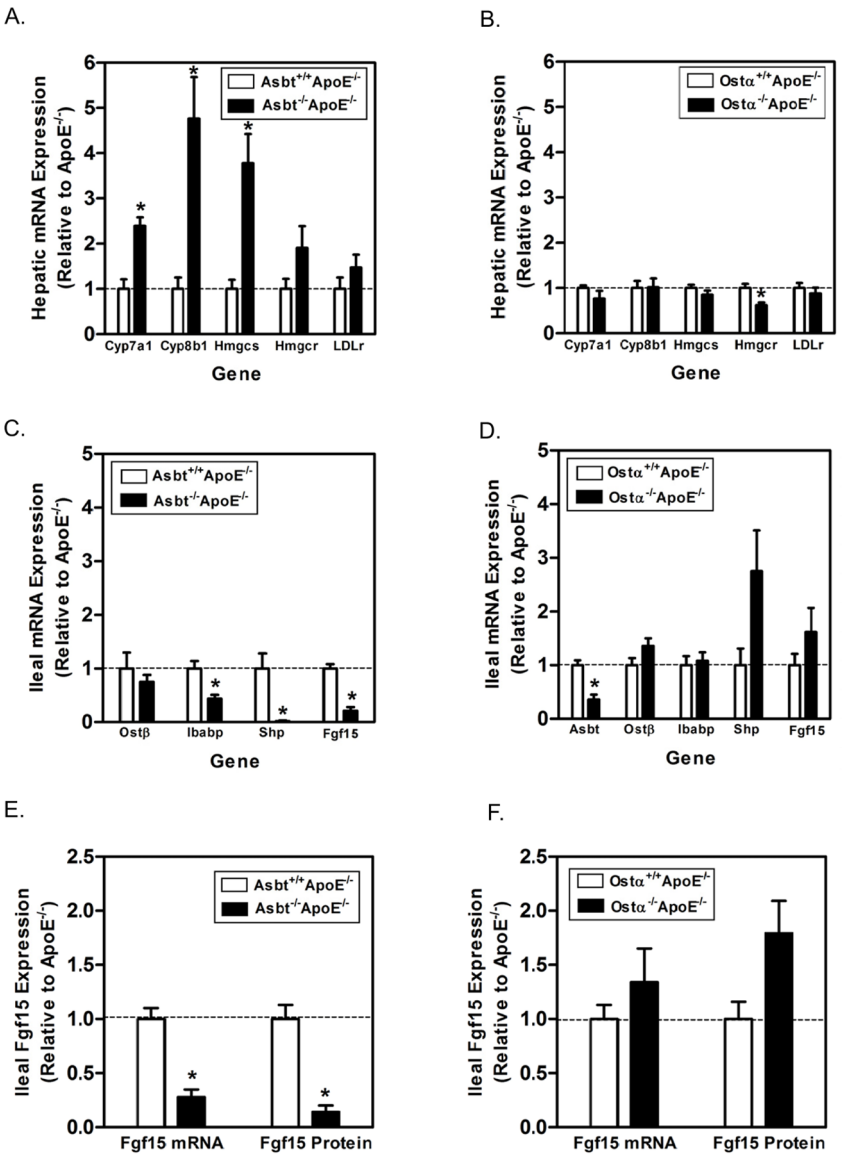

Fig. 3.

Effect of Asbt and Ostα deficiency on hepatic and ileal gene expression in apoE$^{-/-}$ mice. Mice from the indicated genotypes were fed an atherogenic diet for 16 weeks and (A, B) Hepatic and (C, D) ileal RNA was isolated from individual mice for real-time PCR analysis. The bars show the normalized threshold values (mean±SEM) plotted relative to the background-matched apoE$^{-/-}$ control mice. The asterisks indicate a significant difference (p<0.05) versus the apoE$^{-/-}$ mice. (E, F) Ileal RNA and protein was extracted from individual mice (n=9–10) and subjected to real-time PCR and immunoblotting analysis of FGF15 mRNA and protein expression. Values are expressed relative to the background-matched apoE$^{-/-}$ control mice. Significant differences (p<0.05) relative to apoE$^{-/-}$ mice are indicated by an asterisk.