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Stigmasterol stimulates transintestinal cholesterol excretion independent of liver X receptor activation in the small intestine

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Abstract

Despite advances in healthcare, cardiovascular disease (CVD) remains the leading cause of death in the United States. Elevated levels of plasma cholesterol are highly predictive of CVD and stroke and are the principal driver of atherosclerosis. Unfortunately, current cholesterol lowering agents, such as statins, are not known to reverse atherosclerotic disease once it has been established. In preclinical models, agonists of nuclear receptor, LXR, have been shown to reduce and reverse atherosclerosis. Phytosterols are bioactive non-cholesterol sterols that act as LXR agonists and regulate cholesterol metabolism and transport. We hypothesized that stigmasterol would act as an LXR agonist and alter intestinal cholesterol secretion to promote cholesterol elimination. Mice were fed a control diet, or a diet supplemented with stigmasterol (0.3% w/w) or T0901317 (0.015% w/w), a known LXR agonist. In this experiment we analyzed the sterol content of bile, intestinal perfusate, plasma, and feces. Additionally, the liver and small intestine were analyzed for relative levels of transcripts known to be regulated by LXR. We observed that T0901317 robustly promoted cholesterol elimination and acted as a strong LXR agonist. Stigmasterol promoted transintestinal cholesterol secretion through an LXR-independent pathway.

1. Introduction

Despite advances in prevention and treatment, death due to cardiovascular disease (CVD) remains among the largest causes of death, accounting for greater than one out of every three deaths in the US [1]. An estimated 635,000 new coronary events (hospitalized MI or CHD death) occur annually alongside 300,000 recurrent events [1].

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Conflicts of interest

None to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnutbio.2019.108263.

1.1. Reverse cholesterol transport

Statins and PCSK9 inhibitors reduce the risk of cardiovascular disease (CVD) by increasing the abundance of the low density lipoprotein receptor (LDLR) in the liver and accelerating hepatic clearance of LDL cholesterol. This reduces the delivery of cholesterol from the liver to peripheral tissues (forward cholesterol transport) as well as the accumulation of these particles in the artery wall where they promote the progression of atherosclerotic plaques. To promote regression of atherosclerotic plaques, cholesterol must be removed from plaque macrophages by circulating high density lipoprotein (HDL), delivered to the liver, and excreted from the body as unesterified cholesterol or as bile acid [2]. The process of "reverse cholesterol transport" (RCT) is coordinated by the liver X receptors, LXRα (NR1H3) and LXRβ (NR1H2) [3]. LXR activation upregulates genes that promote cholesterol efflux from macrophages (ABCA1, ABCG1), increases the formation of nascent HDL in liver and intestine (ABCA1), and mediates elimination of cholesterol by biliary and intestinal secretion (ABCG5, ABCG8) and metabolism to bile acid (CYP7A1). Indeed, the loss of LXRs in mice reduces RCT and accelerates atherosclerotic disease whereas LXR agonists promote RCT and reduce plaque burden [4–6].

LXRs are also regulators of energy metabolism and promote the synthesis of triglycerides, particularly in the liver where LXR agonists cause steatosis [7]. Strategies to develop selective LXR modulators that increase genes in the RCT pathway while remaining neutral towards lipogenesis have thus far failed. An alternate approach is the development of tissue-specific LXR agonists. This strategy was validated in a proof of concept experiment in which an intestinal-specific, constitutively-active LXR transgene was shown to increase HDL-C, enhance RCT, and reduce atherosclerosis [8]. Two compounds are reported to exhibit intestinal-specific LXR activity, one of which was shown to increase macrophage to feces RCT [9,10].

1.2. Phytosterols as potential LXR agonists

Phytosterols are non-cholesterol sterols present in plant-based foods that reduce plasma cholesterol when consumed as nutritional supplements. The mechanism(s) by which phytosterols reduce plasma cholesterol are not fully understood. Phytosterols are generally thought to compete with cholesterol for solubilization in bile acid micelles, thereby reducing cholesterol absorption [11]. Other studies suggest that intestinal gene expression is dysregulated by phytosterol supplementation, but genes that directly contribute to sterol flux were largely unaffected [12]. Recent studies suggest that the presence of phytosterols in the intestinal lumen mobilizes cholesterol from the brush border membrane as well as the delivery of plasma cholesterol to the intestinal lumen, a pathway known as transintestinal cholesterol elimination (TICE) [13,14]. This observation is supported by cholesterol balance studies that indicate non-biliary cholesterol excretion is increased five-fold in response to phytosterol feeding in mice [15].

Individually, phytosterols exhibit a variety of biological activities that include modulating nuclear receptor activity [16,17]. Stigmasterol is a phytosterol with reported LXR activity in cultured macrophages and adrenal cells [18,19]. Stigmasterol supplementation reduced cholesterol and phytosterol absorption, suppressed hepatic bile acid and cholesterol

synthesis, and reduced plasma cholesterol in rats [20]. However, stigmasterol is a minor phytosterol in edible oils, comprising less than 10% of total sterol content [21].

Like other phytosterols, stigmasterol is poorly absorbed due to the activity of the ABCG5 ABCG8 sterol transporter, the body's principal defense against the accumulation of non-cholesterol sterols [22–25]. In the absence of functional ABCG5 ABCG8, phytosterol bioavailability increases significantly, an effect blocked by pharmacologic or genetic inactivation of the primary mediator of cholesterol absorption, Nieman Pick C 1-like 1 (NPC1L1), in mice and humans [26–29]. This indicates that phytosterols, including stigmasterol, cross the brush border membrane, but are returned to the intestinal lumen by ABCG5 ABCG8. We hypothesized that stigmasterol would confer intestinal-specific LXR activity and promote TICE due to its ability to access enterocytes, but not the plasma compartment, where it would gain access to the liver and promote steatosis.

We challenged C57Bl6/J mice with stigmasterol for a period of 4 days and analyzed sterol balance, biliary and intestinal cholesterol secretion, and the expression of LXR-regulated genes. While stigmasterol promoted neutral sterol excretion and increased intestinal cholesterol secretion rates, it failed to induce the expression LXR-regulated transcripts in the proximal small intestine.

2. Methods

2.1. Animals and treatments

C57BL/6 J male and female mice were purchased from Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and housed in individually ventilated cages (14:10, light: dark) with free access to feed and water. Test diets were formulated from control diet (Research Diets Inc., AIN-76A) and supplemented with either 0.3% (w/w) stigmasterol (Sigma-Aldrich, Product Number S2424) or 0.015% (w/w) T0901317 (Cayman Chemical, Product Code 71810). At 8 weeks of age, mice were singly housed with access to water and control diet for 3 days. On day four, mice were randomized to receive either control diet, stigmasterol-, or T0901317-supplemented diet (*ad libidum*) for an additional 4 days. On Day 8, mice were euthanized for plasma and tissue dissection (n=8 per sex). Mice were euthanized by carbon dioxide and exsanguination via cardiac puncture at termination. Liver and small intestine sections were dissected, snap frozen in liquid nitrogen, and stored in −80 °C. Small intestine was divided into three equal sections representing the duodenum, jejunum, and ileum. Blood was collected in EDTA-coated tubes, centrifuged at 2000×g for 15 min at 4 °C, and plasma stored at −80 °C.

2.2. Surgical assessment of biliary and intestinal cholesterol secretion

An identically treated second cohort (n=7–8 per sex) underwent surgical assessment of biliary and intestinal cholesterol secretion rates as previously described with minor modifications. [30] Mice were anesthetized using 2% isoflurane (Henry Schein, Product Number 050033) and placed on a temperature-controlled heating pad system (Protech International Inc., TC-1000 Temperature Controller) to maintain a body temperature of 37 °C. Mice were supplied with oxygen (Scott Gross, Item Number 421) and isoflurane using a

nose cone. A longitudinal cut was made along the midline from the lower abdomen to the sternum. Two forceps were then used to expose the peritoneum where an incision was made to expose the abdominal organs. Using a sterile cotton tipped applicator that was moistened with warm PBS (Sigma-Aldrich, P3813), the lobes of the liver were then carefully lifted to expose the common bile duct and gallbladder. The common bile duct was then ligated using a 8 cm silk suture, and the gallbladder cannulated with 3 cm of PE tubing (Braintree Scientific Inc., PE10 tubing). The cannula was then secured to the bile duct using another suture. Basal bile was collected for the first 30 min with collections occurring at 15 min intervals thereafter for 120 min total. Collected bile was kept on ice. After basal bile collection, the tail vein was fitted with an inflow tail vein catheter (Braintree Scientific Inc., Item Number MTV-01) and infused with 20 mM sodium taurocholate (Sigma, 86,339) at a rate of 100 nmol/min using a syringe pump (Kent Scientific, model Genie Plus) to maintain biliary cholesterol secretion for the duration of the procedure. For the intestinal perfusate collection, a perfusate inflow catheter was inserted into the proximal small intestine just below the fundus of the stomach. This catheter was connected to a peristaltic pump (Bio-Rad, Econo Pump Model EP1) and facilitated the flow of perfusate into the proximal small intestine at 15 μl/min. A collection cannula was inserted about 10 cm distal to the inflow catheter. Sutures were used to secure the inflow catheter and outflow cannula to the small intestine. Prior to collections beginning, the small intestine was flushed with warm modified Krebs–Henseleit buffer (Fisher, NC0881992) to remove the luminal contents. Collections were done over a 90 min period with fractions collected every 15 min and stored on ice. Bile and intestinal perfusate samples were centrifuges at 15,000 × g for 15 min and stored in −80 °C until analysis.

2.3. Intestinal perfusate preparation

Intestinal perfusate was composed of modified Krebs–Henseleit buffer with sodium taurocholate and L-α-phosphatidylcholine. For each surgery 20 mL of intestinal perfusate were prepared. Sodium taurocholate was dissolved in methanol (Fischer Scientific, Product Number A4564); L-α-phosphatidylcholine was dissolved in chloroform (Fischer Scientific, Product Number C6064). The solutions were combined, mixed, and dried under streaming nitrogen (g) at 45 °C. Films were lyophilized and stored at −20 °C. On the day of the surgery, taurocholate and phophatidylcholine films were reconstituted in Krebs–Henseleit buffer. The final buffer concentration is 10 mM sodium taurocholate and 2 mM L-α-phosphatidylcholine.

2.4. Lipid analysis

Total cholesterol in plasma, bile, and intestinal perfusate were measured using an enzymatic, colorimetric assay (Fisher Scientific). Absorbance was measured at 492 nm using the Biotek Synergy H1 Hybrid plate reader. Total phospholipids in the basal bile were measured by enzymatic, colorimetric assay (Sigma Aldrich, MAK122) at 570 nm. Cholesterol and other neutral sterols were extracted from feces by gas chromatography as previously described [31].

2.5. Bile acid analysis

Total bile acids were measured in the feces and bile using by colorimetric assay. Feces were dried overnight at 60 °C. Dried fecal material (200 mg) was added to 100% ethanol containing 10 mM NaOH, incubated for 2 h in a 70 °C water with vortexing every 15 min, and centrifuged for 10 min at 1300 × g. The supernatant was transferred to a new tube, sealed, and refrigerated overnight at 4 °C. Ten 10 μl of fecal extract or bile (diluted 1:10 in methanol) were added to 200 μl of assay buffer glycine (Sigma, G8898), hydrazine sulfate salt (Sigma, 216,046), disodium EDTA (Sigma, 2,726,046), βnicotinamide dinucleotide (Sigma, N1511), and hydroxysteroid dehydrogenase (Worthington Biochemical, LS004910), incubated at room temperature for 1 h, and absorbance measured at 340 nm.

2.6. Gene expression

LS174T human adenocarcinoma colon cancer cells (ATCC, 70003535) were cultured in eagle's minimal essential medium (EMEM, ATCC, 00831) with 10% fetal bovine serum (Atlanta Biologicals) and a 1% penicillin/streptomycin (Sigma, 087M4871V) at 37 °C and 5% carbon dioxide. Cells were seeded with 100,000 cells per well in 12-well plates and cultured 48 h. Media was replaced with serum-free EMEM that contained 0.2% fatty acid free bovine serum albumin (Sigma, SLBT1197) containing the indicated concentration of T0901317 (In DMSO) or stigmasterol for 24 h. The EC_{50} was calculated by non-linear regression using GraphPad Prism (Version 7.04).

RNA was isolated from duodenum, liver or LS174T cells using RNA STAT-60 (TelTest, Inc.) followed by RNeasy Mini Kit (Qiagen) and reverse transcribed to cDNA (iScript, Bio-Rad). The relative abundance of transcripts was determined using validated SYBR Green assays on Quant Studio 7 Flex Real-Time PCR System (Thermo Fischer Scientific). GAPDH was used for normalization and differences between groups calculated by the ΔΔCt method.

2.7. Statistical analysis

All data are presented as mean±S.E.M. with statistical significance defined as $P<.05$. Data were analyzed using linear regression, unpaired t-test, one-way ANOVA, and two-way ANOVA where indicated. A Dunnett's *post hoc* test was used to compare treatment groups with the control group. Statistical analyses were performed using GraphPad Prism (Version 7.04).

3. Results

3.1. LXR activity in vitro

We previously published that stigmasterol could induce the expression of the LXR target gene, ABCA1, in mouse and human macrophages [18]. To determine if this was also true in human intestinal cells, we conducted a dose–response experiment LS174T colon cancer cells (Fig. 1). The induction of ABCA1 mRNA was used as a marker of LXR transcriptional activity. The synthetic LXR agonist, T0901317, was used as a positive control. In these cells, stigmasterol displayed an EC50 of 200 mM whereas the potent, synthetic agonist calculated to 70 nM.

3.2. Effect of stigmasterol on cholesterol balance and secretion

Mice were challenged with diets containing stigmasterol (0.3%) ad mixed in the semi-synthetic AIN76A diet. T0901317 was included in the experimental design as a positive control since it is known to induce LXR-dependent gene expression in both liver and intestine, promote RCT, and elevate TICE [4–6,32]. Plasma cholesterol was modestly elevated in response to T091317, but unaffected by the inclusion of stigmasterol in the diet (Fig. 2). Fecal stigmasterol, neutral and acidic sterols were measured by GC-FID (Fig. 2b–d). Stigmasterol was detected in the feces of each group as it is present in small quantities in rodent diet formulations, but was detected at 30 mg/day/ 100 g body weight in stigmasterol fed mice. Fecal cholesterol levels were elevated 50% in response to stigmasterol feeding while T091317 increased fecal cholesterol levels almost 4-fold. Fecal acidic sterols were also increased in response to T091317. While there was a trend for increased fecal acidic sterols in stigmasterol-fed mice, this failed to reach statistical significance.

The gall bladder was cannulated and bile diverted into collection tubes for a period of 30 min to determine basal bile composition and flow (Fig. 3). Bile flow did not differ between treatment groups, but there was an increase in basal biliary cholesterol in mice challenged with T0901317. T0901317 treatment also resulted in a decrease in bile acid and phospholipid concentrations in basal bile. Neither bile flow nor biliary lipid composition differed between control and stigmasterol fed mice.

3.3. Biliary and intestinal cholesterol secretion rates

To determine the source of elevated fecal cholesterol in response to stigmasterol feeding, rates of taurocholate-stimulated biliary and intestinal cholesterol secretion were simultaneously measured. Biliary lipid secretion declines precipitously once the enterohepatic bile acid pool is depleted during the collection of basal bile (30 min). To maintain biliary lipid secretion, taurocholate was infused through a tail-vein catheter. During the same period, taurocholate-phosphatidylcholine micelles are perfused through the proximal small intestine. The rate of bile acid entering the proximal small intestine is equal to that secreted by the liver. Under these conditions, the relative rates of hepatic and intestinal cholesterol secretion were determined within the same mouse.

Bile and intestinal perfusates were collected at 15 min intervals for a period of 90 min (Fig. 4). The cumulative secretion of cholesterol was calculated over the 90 min procedure and the data analyzed by linear regression. Mice treated with T0901317 had increased biliary and intestinal cholesterol secretion compared to controls. Stigmasterol modestly increased biliary cholesterol secretion compared to control mice and significantly increased intestinal cholesterol secretion to levels similar to those of T0901317 treated mice. The sum of intestinal and hepatic cholesterol secretion was also calculated (Fig. 4c). Both T0901317 and stigmasterol increased overall cholesterol secretion compared to control mice consistent with the effects of both compounds on fecal cholesterol loss. Bile flow during the procedure was also calculated. In contrast to basal bile flow, T0901317 treatment increased taurocholate-dependent bile flow compared to control while stigmasterol had no effect.

3.4. Effect of stigmasterol on gene expression

We next evaluated expression of LXR target genes in liver and the duodenal segment of the intestine to determine if stigmasterol had a tissue-specific effect on the pathway (Fig. 5). As expected, T0901317 increased the expression of LXR target genes in both tissues, most notably ABCG5 and ABCG8 which are known to promote cholesterol secretion from both organs. Conversely, stigmasterol had no impact on the relative abundance of LXR target genes in either tissue.

We next measured duodenal expression of additional genes that might account for increased delivery of cholesterol to the intestinal lumen (Fig. 6). Expression of SREBP2 and its target genes 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), hydroxymethylglutaryl-CoA synthase (HMGCS) and LDLR were unaffected by stigmasterol or the synthetic LXR agonists. In response to T0901317, NPC1L1 was reduced whereas mRNA levels for ABCB1a and b were increased. However, these transcripts were not altered in stigmasterol treated mice compared to controls.

The data for gene expression are presented irrespective of sex (Fig. 5&6). However, a sex by treatment interaction was detected in the two-way ANOVA for hepatic SREBP-1c and intestinal ABCA1 (Fig. S1). Post-hoc analysis within sex indicated that the magnitude of the response to T0901317 was greater in females than in male mice for ABCA1 and SREBP-1c.

4. Discussion

4.1. LXR activity

Although relatively weak, stigmasterol increased expression of the LXR target gene, ABCA1, in cultured human intestinal cells. The weak activity in these cells may reflect that stigmasterol is less active towards human LXRs in whole cells, but may also be due to poor uptake. NPC1L1 is responsible for sterol uptake in enterocytes *in vivo*. Its mRNA levels were at the lower limit of detection by rtPCR and transcript abundance was not induced by sterol depletion in LS74T cells. We previously reported that stigmasterol induced expression of LXR target genes in culture macrophages [18]. How macrophages accumulate stigmasterolinthe absence of NPC1L1 is not known, but given their role in surveillance and scavenging in innate immunity perhaps it is not surprising that they can accumulate xenosterols more readily than intestinal enterocytes. Conversely, sitosterol and campesterol were active towards LXRα and β in a cell-free, coactivator peptide recruitment assay, yet had no impact on LXR-regulated genes in cultured macrophages [18,33]. Thus, the activity of selected phytosterols towards LXR may be cell-specific and dependent upon intracellular trafficking, metabolism, perturbation of cellular sterol metabolism, or other factors.

In vivo, stigmasterol failed to induce an LXR response in either liver or intestine. The lack of LXR activity in the intestine is presumably due to the failure to achieve intracellular concentrations that approach the EC_{50}. This may be due to a variety of factors such as poor solubility in the intestinal chyme or the high efficiency of the ABCG5 ABCG8 sterol transporter. YT32 demonstrates that xenosterols can produce intestinal-specific effects towards LXR [10]. Like ergosterol, stigmasterol has been shown to be useful scaffold to develop LXR agonists in a drug discovery effort [34]. However, the naturally occurring

compound does not possess the combination of solubility and potency towards LXR to produce the desired effect *in vivo*.

4.2. Sterol balance and TICE

Despite inactivity towards intestinal LXR, stigmasterol increased fecal sterol loss and TICE. The effect size for intestinal cholesterol secretion approached that of the small molecule agonist. T0901317 presumably promotes net sterol loss to the intestinal lumen through the combination of increased ABCG5, ABCG8, ABCB1a, and b as well as reduced NPC1L1, all of which are expected to tip the balance in favor of apical transport of cholesterol to the intestinal lumen to promote TICE [35–39]. Like stigmasterol alone, mixtures of phytosterols increased non-biliary cholesterol excretion in the absence of the induction of the LXR target gene, ABCG5 [15]. In fact, ABCG5 was modestly reduced in the small intestine in response to phytosterol feeding at levels above 1%.

Whole body cholesterol balance data and *in situ* secretion data are consistent with respect to the effect of stigmasterol promoting sterol loss. However, the mechanism by which stigmasterol and other phytosterols promote such an effect remains elusive. The expression of genes known to mediate the sterol flux across the brush border membrane was unaffected. Increased delivery of cholesterol to the intestinal lumen could also be associated with increased delivery of cholesterol to the G5G8 accessible pool. The pathway(s) responsible for such delivery have yet to be defined. We did not see upregulation of LDLR, SREBP2, HMGCR or HMGCS (Fig. 6), suggesting that stigmasterol does not deplete enterocytes of cholesterol and that basolateral uptake and *de novo* synthesis do not account for increased TICE. Mixed results for the role of HDL and SR-BI in TICE have been reported. In radiolabeled tracer studies, the delivery of HDL cholesterol to the small bowel was shown to be receptor-dependent, but modest. [40] In this and previous studies, mRNA levels for SR-BI are near the lower limits of detection by rtPCR. Consequently, a prominent role for SR-BImediated uptake of HDL cholesterol in TICE appears unlikely. Similarly, mixed results have been reported for a role of LDLR and enhanced basolateral uptake to support TICE. [37] We did not see differences at the transcriptional level for LDLR. However, it should be noted that we did not track delivery of radiolabeled cholesterol from LDL particles or other lipoproteins to the intestinal lumen.

Physiological factors such as competition with endogenous biliaryderived cholesterol for reabsorption and intestinal enterocyte turnover have been suggested as mechanisms that potentially contribute to TICE in the absence of altered expression of cholesterol transport proteins. In our surgical model, the intestine is flushed and the perfusate contains no stigmasterol. Thus, the effect of stigmasterol on intestinal cholesterol secretion rate is not due to its physical presence in the lumen during the surgical procedure. Intestinal perfusates are centrifuged to remove any shed cells over the course of our procedure. While increased enterocyte shedding may contribute to fecal sterol loss, it cannot account for increased intestinal cholesterol secretion in our surgical model. Histological analysis of sections collected from the proximal small intestine revealed no effect of the LXR agonist or stigmasterol on intestinal morphology (Fig. S2). The elucidation of the molecular

mechanisms that govern basolateral uptake and intracellular transport of cholesterol to the apical membrane will be required to advance our understanding of TICE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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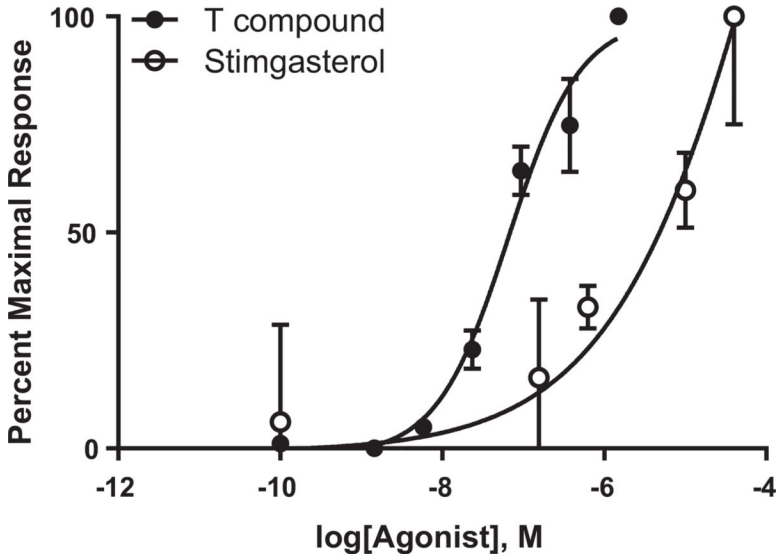

Fig. 1.

Dose response curve of stigmasterol and T0901317 in human intestinal LS174T cells. Cells were cultured to confluence and incubated in serum-free medium containing the indicated amount of agonist for 16hr. RNA was isolated and the relative abundance or transcripts encoding ABCA1 was determined by rtPCR. Data were normalized to GAPDH and expressed as percent maximal response (n=3).

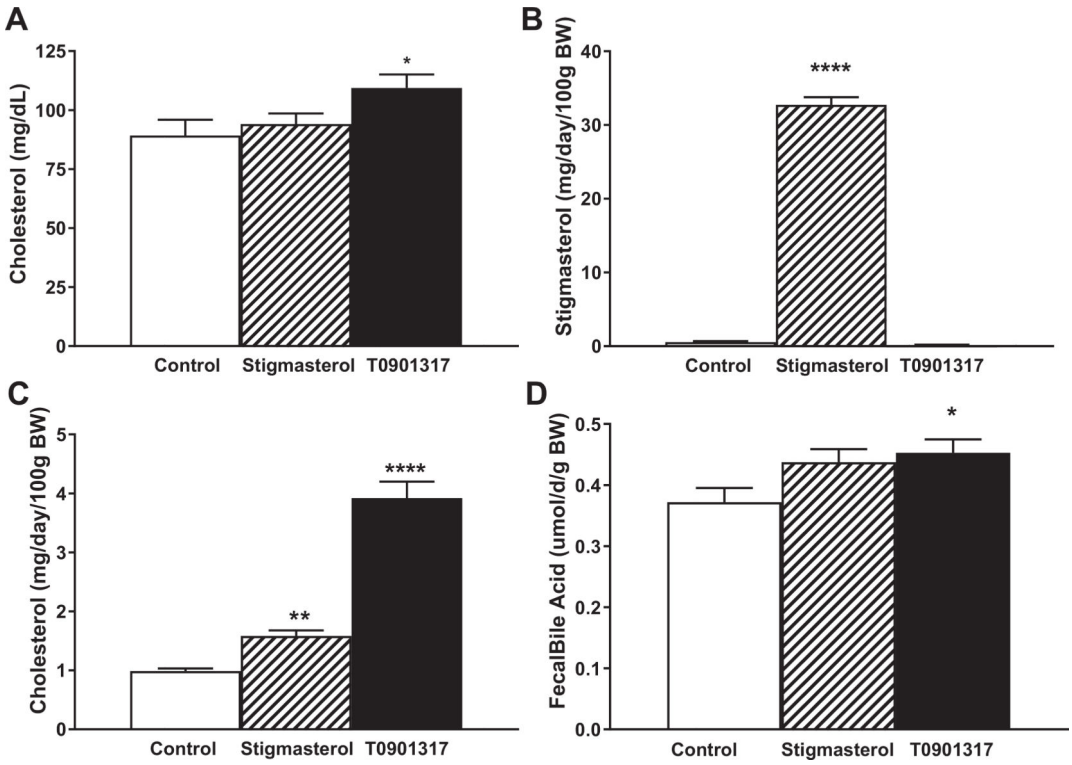

Fig. 2.

The effect of stigmasterol on plasma and fecal sterols in mice. Plasma (A) and feces (B-D) were collected and analyzed by GC-FID to quantify neutral sterols (A-C). Acidic sterols were determined by enzymatic, colorimetric assay (D). Data are mean±S.E.M. (n= 16). Data were analyzed by ANOVA. Dunnet's post-hoc tests were used to determine significant differences from control. * P<.05, ** P<.01, **** P<.0001.

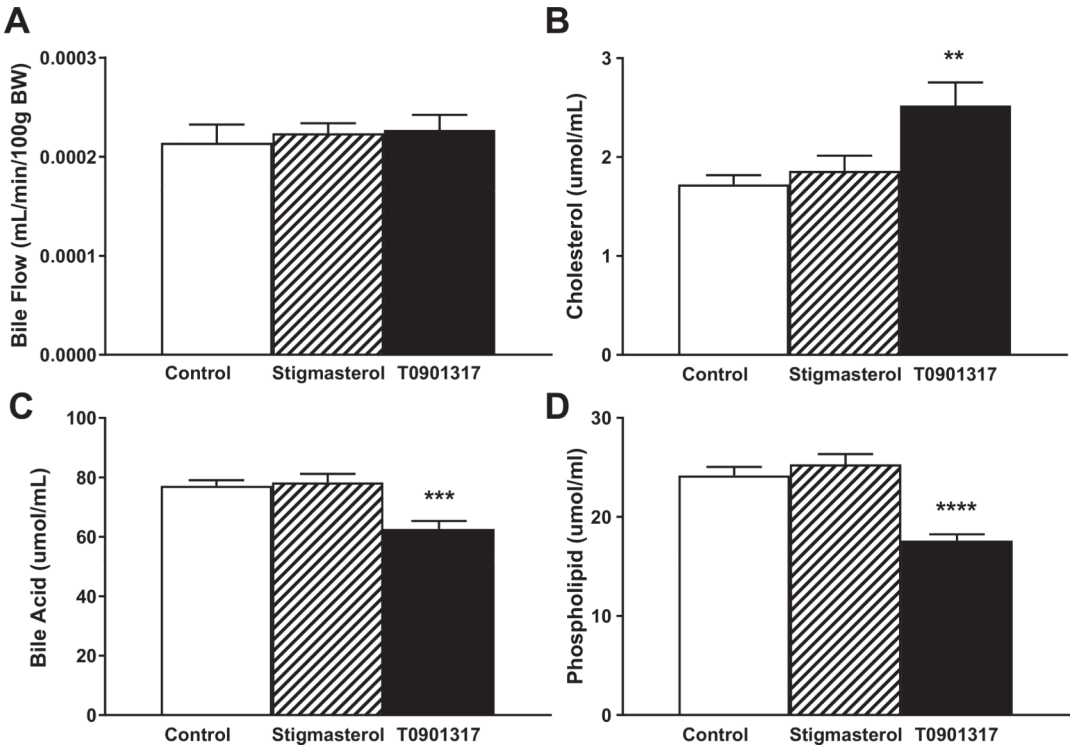

Fig. 3.

Basal bile flow (A) and composition (C-D). Basal bile was collected for 30 minutes. Total cholesterol, bile acid, and phospholipid concentrations were determined by enzymatic, colorimetric, enzymatic assay. Data are mean±S.E.M. (n = 15–17). Data were analyzed by ANOVA. Dunnet's post-hoc tests were used to determine significant differences from control *P<.01, ***P<.001, ****P<.0001

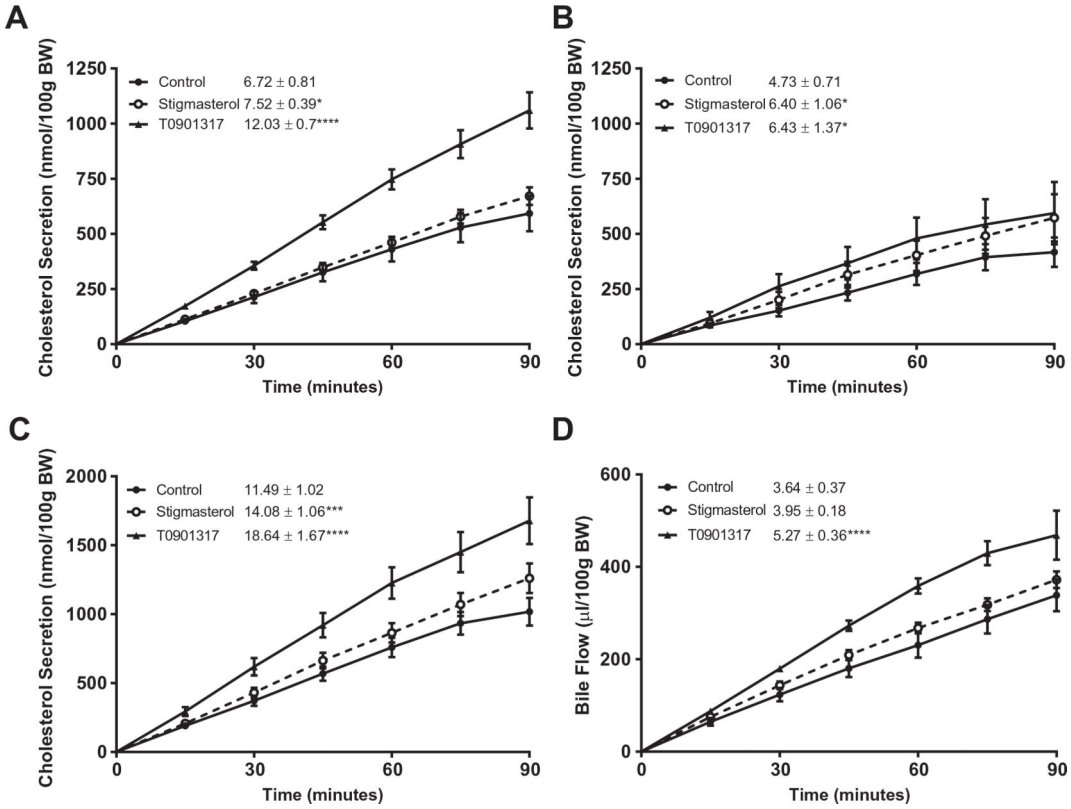

Fig. 4.

Taurocholate-dependent biliary (A), intestinal (B), and the sum of biliary and intestinal (C) cholesterol secretion in mice treated with stigmasterol or T0901317. Bile acid was infused via tail vein and taurocholate-phosphatidylcholine micelles were perfused through the proximal small intestine to determine the relative rates of biliary and intestinal cholesterol secretion. Cholesterol content in bile and intestinal perfusates were determined by enzymatic, colorimetric assay. Bile flow (D) was determined gravimetrically. Data are mean ±S.E.M. (n = 15–17). Data were analyzed by linear regression. Slopes of regression lines (nmol/min/100g bw) are indicated adjacent to legend and significantly differ from controlat **P*<.05, ****P*<.001, *****P*<.0001.

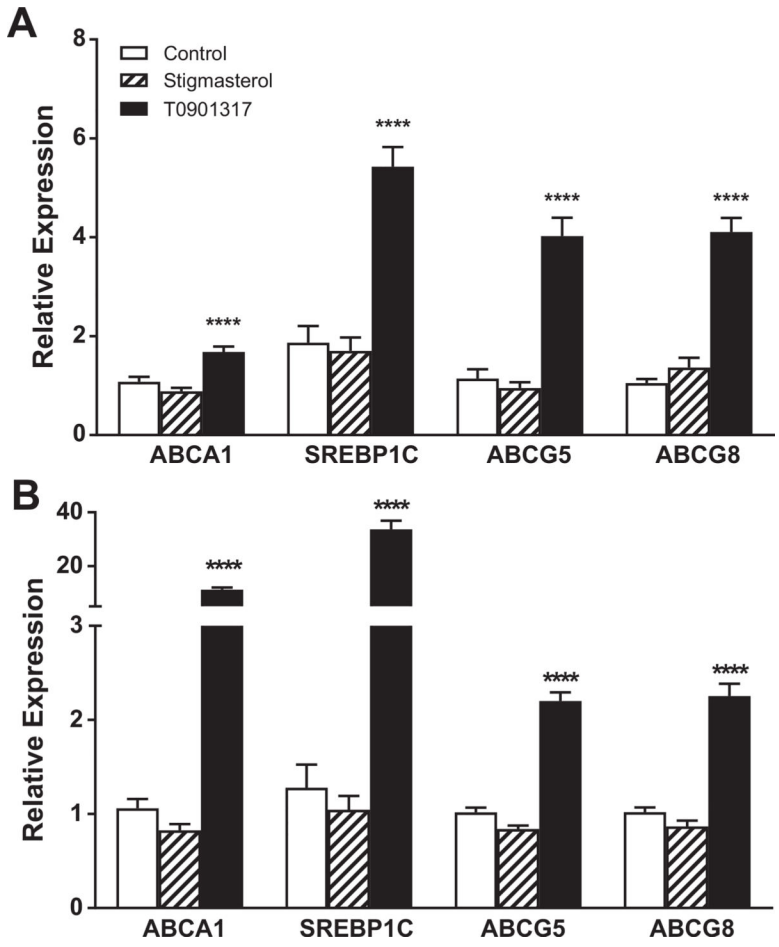

Fig. 5.

Hepatic (A) and Intestinal (B) expression of LXR-regulated and cholesterol metabolizing genes. RNA was isolated from liver and duodenal tissue and the relative abundance of transcripts determined by rtPCR. Transcript levels were normalized to GAPDH. The relative abundance of transcripts was calculated by the Ct method and are expressed relative to the control group. Data are mean±S.E.M. (n=16). Data were analyzed by ANOVA. Dunnet's post-hoc tests were used to determine significant differences from control. ** P<.01, *** P<.001, **** P<.0001

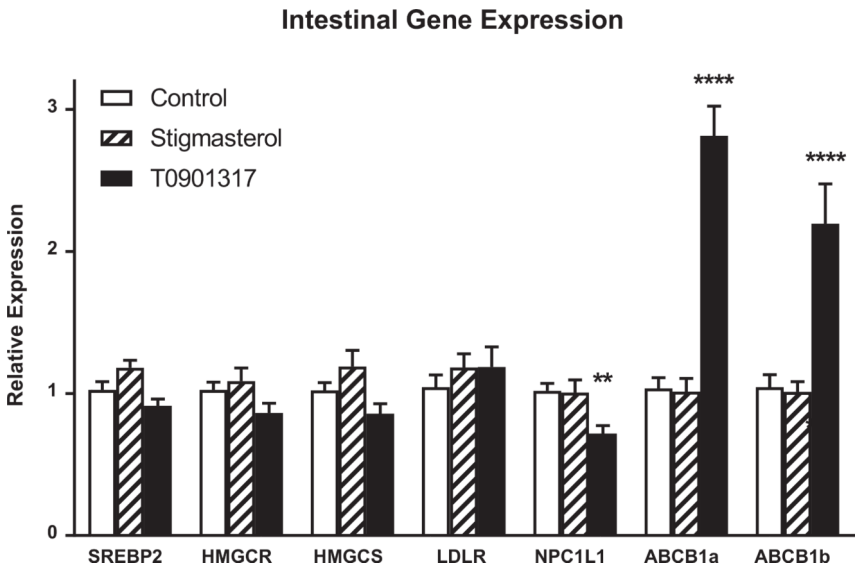

Fig. 6.

Intestinal expression of sterol regulatory and transport genes. RNA was isolated from liver and duodenal tissue and the relative abundance of transcripts determined by rtPCR. Transcript levels were normalized to GAPDH. The relative abundance of transcripts was calculated by the Ctmethod and are expressed relative to the control group. Data are mean ±S.E.M. (n=16). Data were analyzed by ANOVA. Dunnet's post-hoc tests were used to determine significant differences from control. ** P<.01, **** P<.0001.